Original Article

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Evaluation of Antifungal and Antibacterial Effects of *Capparis spinosa* Root Extract on *Streptococcus Mutans* and *Candida albicans*

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ABSTRACT

Background & Objective: *Capparis spinosa* is a plant belonging to the genus Capari which is important for medicinal purposes. It is also known as a multipurpose plant in Persian medicine. In this study, we searched for natural active ingredients in mouthwash for immunocompromised patients and examined the antifungal plus antibacterial properties of the hydroalcoholic extract of the root of the Capparis plant against *Streptococcus mutans (S. mutans*) PTCC 1683 and *Candida albicans* (*C. albicans*) ATCC-10231 was investigated.

Materials & Methods: Ethanol extracts of plant roots were extracted by the percolation method. The diameter of the growth inhibition zone of *C. albicans* and *S. mutans* extracts was measured using the well plate method. Minimum inhibitory concentrations (MICs) were calculated for bacterial and fungal strains. In addition, the minimum sterilization concentration (MBC) and minimum sterilization concentration (MFC) of the extract were investigated using the broth microdilution method. The results were compared with an antibacterial and antifungal drug of 0.2% chlorhexidine nystatin.

Results: The results for both pathogens revealed that the diameter of the growth inhibition zone increased with elevation of the extract concentration. The MIC and MBC of *S. mutans* extract were 62.5 and 62.5 mg/ml, compared to 0.39 and 0.39 mg/ml for chlorhexidine. The MIC and MFC of the plant against the fungus *C. albicans* were 7.812 and 31.25 mg/ml, compared to 0.25 and 1.25 µg/ml for nystatin.

Conclusion: The ethanol extract of the Caparis plant had an antifungal effect on *C. albicans* and an antibacterial effect on *S. mutans*. However, the inhibitory and lethal effects against bacterial and fungal strains were lower than those of nystatin and chlorhexidine.

Keywords: Persian Medicine, Mouthwash, Immunosuppress, Antifungal agents, Antimicrobial agents, *Candida albicans Capparis spinosa, Streptococcus mutans*

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Introduction

Oral diseases can be of bacterial or fungal origin **(1)**. Subgingival dental plaque forms acidic and acidophilic bacteria on tooth surfaces, which cause tooth decay **(2, 3)**. *S. mutans* is the predominant bacterial species in mouth and the main caries-causing organism. With the activity of this bacterium and formation of stable biofilm, the environmental balance changes. As a result, bacteria penetrate into deeper tissues and cavities around the gum line, ultimately resulting in the disintegration of hydroxyapatite crystals in enamel and dentin as well as tooth decay **(3-5)**.
C. albicans is the main cause of oral candidiasis and is known as an opportunistic pathogen. This fungus has a high ability to grow in different anatomical parts of the body, especially the mouth. In this infection, the mucosa is involved, which causes systemic infection and even death if left untreated **(6,7, 8)**.

Antibiotic-resistant infections are becoming more common, and the side effects of certain chemical antibiotics have raised demand for medicinal plants. Several studies have been conducted with extracts of different plants to screen for antimicrobial activity and discover new antimicrobial compounds. Compared to antimicrobial drugs, antifungal drugs are limited. Also, the treatment in fungal diseases is long and ineffective, where various drug interactions and resistance have been observed. Thus, use of other treatment methods such as the use of medicinal plants is of great importance **(9,10, 11)**.

Capparis spinosa, also called *Flinders Rose*, is a perennial plant with fleshy, round leaves as well as white to pink flowers. The plant belongs to the genus *Caparis* and Caparidaceae family **(12)**. *Capparis* has a high nutritional value and is traditionally used to treat many ailments. *Caparis spinosa* in Persian medicine as a multi-functional plant is comparable to the new findings and applications of caper **(13)**. This plant grows in dry and cooler areas of western as well as central Asia and contains important bioactive substances **(14, 15).** The root and bud of this plant contain plenty of pectin, saponin, alkaloids, and terpenoid. In traditional medicine, this plant is used in the treatment of gout, rheumatism, anemia, and liver diseases. **(15, 16)**. Various studies have shown that this plant is effective on numerous and common bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, etc. **(17, 18)**. Few studies have been performed about antifungal effects of this plant on *C. albicans*. These limited studies have also been associated with contradictory results **(14)**.

The main goal of this study is to find a substance that can be used as a main ingredient in the production of mouthwash with immunosuppressive properties (allowing for known antibacterial, antibacterial, and anti-inflammatory effects). Considering the importance of medicinal plants to control fungal and bacterial infections of the mouth, this study was conducted with the purpose of investigating the antifungal and antibacterial effects of the Capparis root plant ethanol extract against *C. albicans* and *S. mutans*. and indeed, their ability to eliminate these pathogens.

Materials and Methods

Plant and microbial isolates

Capparis spinosa roots were collected from Moghan, Ardabil, Iran and registered under the scientific name *Cappari spinosa* and herbarium code 3970 at Shahid Beheshti University of Medical Sciences, Tehran.
The plant material was dried at room temperature and ground into a powder using a mechanical grinder. S. mutans standard strain PTCC 1683 was purchased from Tehran Scientific Research Group and Microbial Collection and grown on blood agar medium at 37°C for 18 to 48 hours.
The standard strain of C. albicans ATCC 10231 was prepared at the Department of Mycology, Zanjan University of Medical Sciences and cultured on Sabouraud dextrose agar (SDA) medium at 35 ± 2°C for 18–48 h.

Standard drugs

Nystatin (Sigma, Germany) was used as a positive control for antifungal tests and chlorhexidine 0.2% for antibacterial tests. The nystatin was in the form of powder, from which 100,000 units of nystatin suspension were prepared. Then, different dilutions of the drug were prepared via serial dilution.

Preparation of plant extracts and isolate pure culture

Since the ethanolic (hydroalcoholic) extract of the plant root has the most antimicrobial effects, the ethanolic extract was used in this study. Extraction was performed by the method of percolation with ethanol/water (70:30, v/v), as described further. Briefly, 317.74 g of grounded plant roots was placed inside the percolator tank and then 750 ml of 70% ethanol was passed through the sample.
The device was placed in a closed chamber under the hood and after 72 hours, the resultant solution was drained into the bottle. The procedure was repeated for other two 72-hours times with 675 ml and 550 ml of 70% ethanol each.
To eliminate the solvent and create solid extracts, the three-step extracts were mixed and concentrated under pressure in a rotary evaporator. By evaporating the solvent, the dry weight was obtained which was used to calculate the concentration in mg/ml.
Specifically, 13.28% w/w of the extract was yielded. The extract was kept at 2 to 4°C and was utilized for evaluating the potential of antimicrobial and antifungal properties. Before use, the extract was resuspended in a 1:3 mixture of distilled water and DMSO and kept at 4°C. Other concentrations of each extract were made

from the stock solutions in order to assess their antibacterial and antifungal properties.

Antibacterial susceptibility testing of the plant extracts

Antibacterial evaluation of plant extract was performed via agar well diffusion method. Pure bacterial strain was cultured on blood agar and used for the determination of growth inhibitory zone. Sets of three dilutions (250, 500, and 1000 mg/ml) of the extract and standard drugs were obtained in DMSO/water in a 3:1 ratio. Each well was filled with extract or a positive control (chlorhexidine 0.2%) and a negative/solvent control (DMSO) after being bored in the inoculation medium. Following incubation, the antimicrobial activity was determined by formation of a clear zone around the well which was recorded as millimeters. The zones of growth inhibition surrounding the discs were assessed after 18 to 24 hours of incubation at 37°C. The MIC was calculated using the broth microdilution method, in accordance with CLSI 2022 (Performance Standards for Antimicrobial Susceptibility Testing, M100, 32nd Edition). Two-fold serial dilutions of extract (1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.8 and 3.9 mg/ml) were prepared in a microtiter plate containing 150 µl of BHI broth. Then, *S. mutans* suspension was inoculated with a final concentration of 1.5×10^5 CFU/mL in each well. As a standard drug and positive control, chlorhexidine was utilized. Then, the plates were covered and kept at 37°C for 18-24 hours. The lowest extract concentration that totally prevented bacterial growth was identified as the MIC **(17).** Through sub-culturing the contents of microtitre plate wells on agar, the MBC which exceeded or was equal to the lowest MIC, was obtained. The lowest concentration of extracts with no apparent growth was considered as MBC after the plate had been incubated for 18–24 hours. The MBC for chlorhexidine was calculated similarly. The experiments were done in triplicate.

Antifungal susceptibility testing of the plant extracts

Antifungal evaluation of plant extract was performed through agar well diffusion method. The pure fungal strain was cultured on Sabouraud Dextrose Agar and used for determining the zone of inhibition. The dilutions for the fungal strain were 5, 10, 15, 20, 25, 30, 35, and 40 mg/ml. Each well was filled with extract or a positive control (1 mg/ml of Nystatin) and a negative/solvent control (DMSO) after being bored in the inoculation medium.

After incubation, the antifungal activity was determined based on formation of a clear zone around the well which was recorded as millimeters. The zones of growth inhibition surrounding the discs were assessed after 48 to 96 hours at 35±2°C,

The MIC was calculated using the broth microdilution technique in accordance with CLSI M27-A3 and M27-S4. To obtain different concentrations, extracts were prepared in two-fold serial dilutions in a microtiter plate containing RMMI 1640. A final dosage of $0.5\text{-}2.5 \times 10^3$ CFU/mL of the fungal strain was applied to each well. As a standard drug, nystatin, was utilized as the positive control. The plate, after being covered, was incubated for 24±2 h at 35±2°C.

The MIC was regarded as the lowest concentration of the extract that completely inhibited the fungal growth. To determine minimum fungicidal concentration (MFC), the wells in which the growth of fungus was not observed were selected and 10 µL of the well contents was transferred to the SDA culture medium. The cultures were incubated again for 48 hours at 37°C. After this time, the plates were checked and the plate on which no fungus had grown and which had the lowest concentration of antifungal agent was considered as MFC. The experiments were performed in triplicate.

Results

Antibacterial susceptibility testing of the plant extracts

To investigate the antibacterial effect of the extract, the diameters of zone of inhibition for different concentrations of the extract were investigated. For this purpose, the concentrations of 250, 500, and 1000 mg/ml of extract were used.
The plates were examined after 24 hours (Figure 1 A).
The diameter of inhibition zone was calculated for each of the samples (Table 1).
In addition, the diameter of the diameter of inhibition zone for 0.2% chlorhexidine (as a positive control) was also measured.

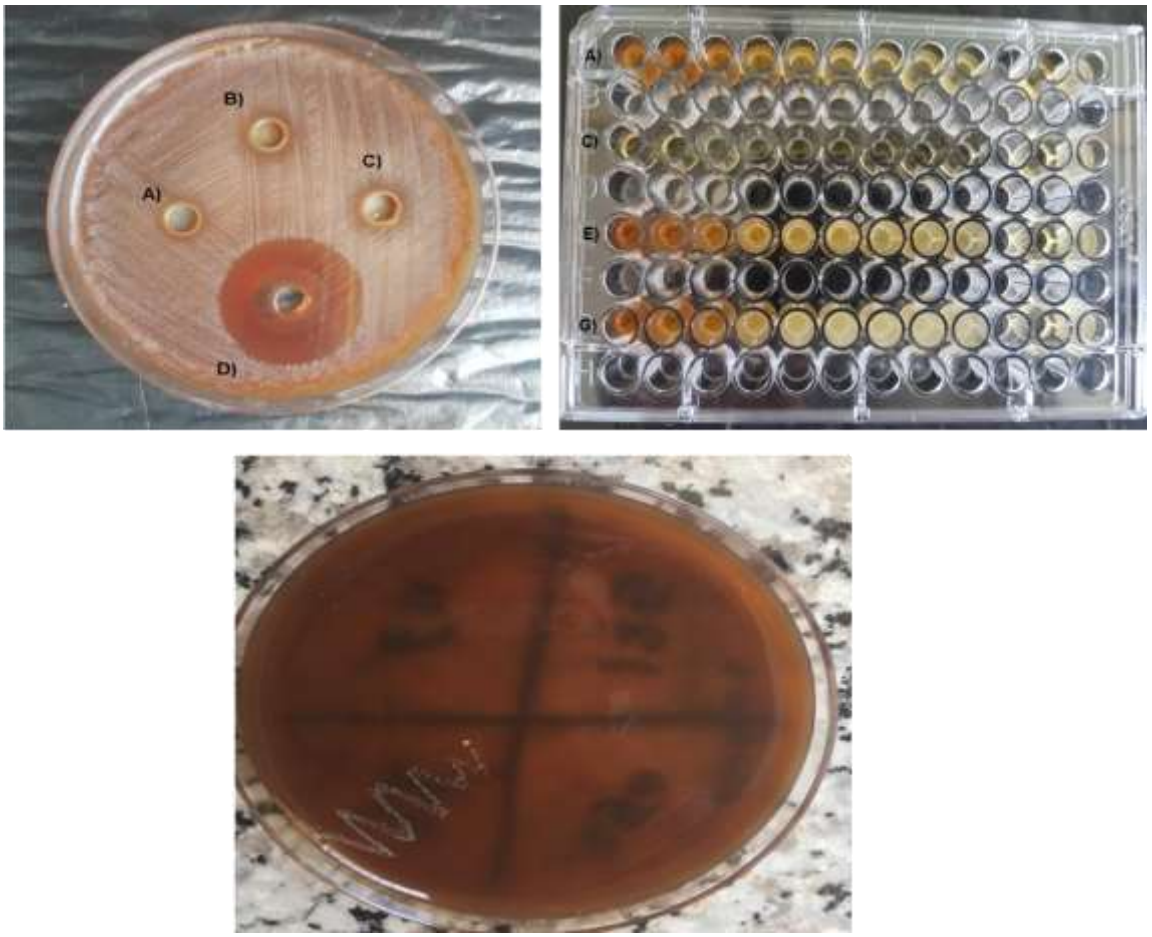


Figure 1. Antibacterial susceptibility testing of the plant extracts

A) The diameter of growth inhibition zone for concentrations of 250 mg/ml (A), 500 mg/ml (B), 1000 mg/ml (C) of plant extract and chlorhexidine 0.2% (D) against *S. mutans*. **B)** MIC evaluation of extract (A) and chlorhexidine (C). The MIC values of the extract were compared on two strains of *Staphylococcus aureus* (E) and *Pseudomonas aeruginosa* (G). **C)** The MBC assay in plate for plant extract against *S. mutans*.

Table 1. Diameter of inhibition zones together with values of MIC and MBC of plant extracts against S. *mutans*.

The test material	Different concentrations of extracts(mg/ml)			chlorhexidine 0.2%
	250	500	1000	0.2
The diameter of inhibition zone	6.5	9	11	27
MIC			62.5	0.39
MBC			62.5	0.39

As indicated in Table 1, with the increase in the concentration of the extract in each well, the antimicrobial effects have improved. This demonstrates that the antimicrobial effect of the extract is dose-dependent.

The antibacterial effect of the extract was also investigated as MIC for different concentrations of the extract and 0.2% chlorhexidine. For this purpose, the extract concentration of 250 mg/ml was used as the highest concentration for serial dilution (Figures 1B).

MBC assay plates were also examined after 24 hours for bacterial colony growth (Figure 1C). The values for MIC and MBC are reported in Table 1. MIC and MBC for plant extract were 62.5 mg/ml (for both) and 0.39 mg/ml for chlorhexidine.

In addition, the results were compared with the MIC of the extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which were 125 mg/ml for and 250 mg/ml, respectively.

Antifungal susceptibility testing of the plant extracts

To examine the antifungal effect of the extract, the diameter of the growth inhibition zone of the extract was investigated. For this purpose, concentrations of 5 to 40 mg of extract were used (Figures 2). The diameter of the inhibition zone for each sample is presented in Table 2.

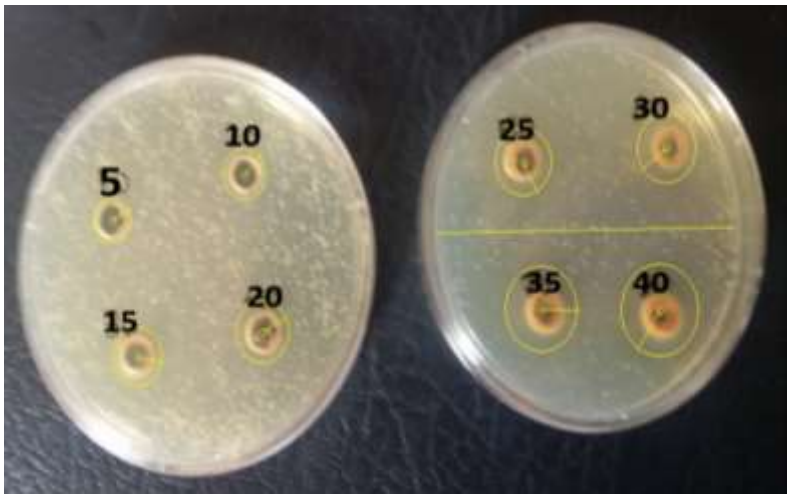


Figure 2. The growth inhibition zones for concentrations 5, 10, 15, 20, 25,30, 35, and 40 mg/ml of plant extract and nystatin against *C. albicans.*

Table 2. Diameter of inhibition zones plus values of MIC and MFC of plant extracts against *C. albicans.*

The test material	Different concentrations of extracts(mg/ml)								Nystatin
	5	10	15	20	25	30	35	40	
The diameter of inhibition zone(cm)	0.79	0.902	1.022	1.096	1.172	1/224	1.440	1.622	
MIC	7.812 µg/ml								0.25 µg/ml
MFC	31/25 µg/ml								1.25 µg/ml

As observed in Table 2, by increasing the amount of extract, the antifungal effects have improved. This shows that the antifungal effect of the extract is dose-dependent.

The antifungal effect of the extract was also investigated in the form of MIC for different concentrations of the extract and nystatin. For this purpose, concentrations of 0.25 to 16 µg/ml nystatin and 125 mg/ml to lower dilutions of the extract were prepared and used in the well as serial dilution. The results are outlined in Table 2. As presented in Table 2, the MIC values for extract and nystatin were obtained 7.812 mg/ml and 0.25 µg/ml, respectively. Also, the values of MFC for the extract and antifungal drug nystatin were obtained 31.25 mg/ml and 1.25 µg/ml, respectively.

Discussion

Capparis spinosa L., with its noteworthy antioxidant and anti-inflammatory characterizations, is known to have pivotal functions in the prevention and treatment of different diseases **(19, 20)**. Numerous *in vivo* and *in vitro* investigations are presently being conducted globally to appraise the clinical and pharmacological utilities of *C. spinosa*, in an effort to discover a fresh natural medication that brings about diminished toxic and unfavorable side effects. Additionally, numerous other investigations have been conducted, demonstrating the noteworthy impact of *Capparis spinosa* root extract on *S. mutans* and *C. albicans*, with regards to its antifungal and antibacterial properties. In the currents study, we evaluated the antifungal and antibacterial effects of *Capparis spinosa* root extract on *S. mutans* and *C. albicans*. Our result showed that at high concentrations of plant root extract, the antifungal and anti-bacterial effect increased.

According to in vitro evaluation, the antibacterial activity of *Capparis spinosa* extract fractions against a wide range of Gram-positive and Gram-negative bacteria was confirmed using diffusion well agar. The butanol fraction revealed the broadest antibacterial activity, while the hexane fraction presented the least effect. The essential oil was also tested for antibacterial activity and showed antibacterial properties, with the highest activity against Micrococcus luteus **(21)**. Oil ether, methanol, hexane, butanol, and aqueous extracts of aerial parts of *Capparis spinosa* show different degrees of antibacterial activity. The extract was found to have little to moderate activity against four types of bacteria, namely *E. coli*, *B. cereus* and *Staph. aureus*, and *S. Tipirnurium* **(22)**.

Previous studies have demonstrated the antioxidant, anti-inflammatory, and renoprotective properties of *Capparis spinosa*.**(19, 23)**.

A study used the disk diffusion method to evaluate the antimicrobial properties of *Capparis spinosa* against Gram-positive and Gram-negative bacteria using ethanol and essential oil extracts. Both extracts showed significant antibacterial effects against Gram-positive bacteria, including *Bacillus cerus* and *Staphylococcus aureus*, as well as Gram-negative bacteria, including Pseudomonas aeruginosa and E. coli **(24)**. Benahour et al. performed an in vitro evaluation of the antibacterial activity of Capparis oil against nine bacterial species. They found that the oil was inactive against *E. coli* and had little activity against the eight bacterial species evaluated. However, preference tests indicated that the oils used were not effective against the bacterial strains tested **(25)**.

Our results showed that the MIC and MBC values for S. mutans extract were 62.5 and 62.5 mg/ml, while for 0.2% chlorhexidine they were 0.39 and 0.39 mg/ml . The most potent antibacterial activity against B. subtilis was observed by Gull et al. For the methanol extract of C Bark and shoot, the growth inhibition zones were 26.8 mm and 24.6 mm, respectively. In addition, the methanol extract of C. spinosa fruit showed zones of maximum growth inhibition of 24.9 mm for Pasteurella multocida as well as 23.9 mm, 20.9 mm, and 17.7 mm for *B. subtilis*, *E coli*, and *S. aureus*, respectively**(26)**.

On the other hand, the strongest antibacterial activity of *C. spinosa* on *E. coli* was associated with methanol extracts of flower and root, with growth inhibition zones of 26.5 mm and 23.9 mm, respectively **(26)**. In addition, polysaccharides derived from *C. spinosa* leaves are suspected to have antibacterial properties. According to Mazarei et al., the aforementioned polysaccharides are used in the treatment of *E. coli*, *S. dysenteriae* and *S. typhoid* fever **(27)**. According to our findings, the MIC and MFC of the plant against the fungus *C. albicans* were 7.812 and 31.25 mg/ml, while for nystatin they were 0.25 and 1.25 µg/ml. Mahasneh also showed that butanol extracts of *C. spinosa* were effective against *C. albicans* and *A. flavus*. In addition, the ethanol extract of C. spinosa showed a cytotoxic effect against Helicobacter pylori (*H. pylori*) strains **(28, 29)**. The presence of H. pylori infection is associated with many gastroduodenal diseases, including stomach cancer. While hypothesizing that the way the extract was prepared and the specific type of solvent used could affect the microbial activity of *C. spinosa* **(30)**. The antibacterial activity of *C. spinosa* was investigated in a study by Boga et al. Studies have shown that aqueous extracts of *C. spinosa* roots significantly slowed the growth rate of Deinococcus radiophilus (D. radiophilus) compared to controls. On the other hand, *E. coli* did not respond to aqueous extracts of *C. spinosa* roots **(31)**.

Mahboubi et al. examined the effects of various extracts of the roots and fruits of *C. spinosa*, including water, ethanol, ethyl acetate, and methanol. They found that the antibacterial activity of the aqueous extract of the roots of *C. spinosa* was higher than that of C. spinosa. Spinos fruit juice extract against a wide variety of microorganisms **(32)**. Studies have reported that aqueous extracts of *C. spinosa* roots have an inhibitory effect on several bacteria and fungi, including *Staphylococcus saprophyticus, Streptococcus pyogenes, Staphylococcus aureus, E. coli, Staphylococcus epidermidis, Sella typhimurium and Salmonhimurium, Shigella dysenteriae, Bacillus subtilis, C. albicans, Candida glabrata, Shigella flexneri, Aspergillus flavus, Klebsiella pneumoniae, Bacillus cereus, Aspergillus Niger* and *Aspergillus parasiticus* **(32)**.

Conclusion

The study revealed that the ethanolic extract from *Caparis* plant had an antifungal effect on *C. albicans* and an antimicrobial effect on *S. mutans*. Although these effects have been less intense than those of

standard antibacterial and antifungal drugs, efforts are being made to prepare strategies to control human pathology using natural extracts and bioactive metabolites of medicinal plants. In addition, phytochemical research aims to reveal the chemical composition and properties of biological compounds, especially those contained in the flowers and branches of this type of plant.

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Authors' Contribution

Study Design:NKH,SS,MM.Drug Selection:NKH.Plant Extraction:MT.Microbial Lab:FH.Fungal Lab:SA.Lab Exams:AR,MD.Statistical Analysis:KM.Plant Preparation:LGH,AR

Conflict of Interest

The authors declare that they have no conflict of interest.

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Ethics Approval and consent to participate

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