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RESEARCH ARTICLE
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ANTI-FUNGAL EFFICACY OF MISWAK EXTRACT (SALVADORA PERSICA) AND COMMERCIAL CLEANER AGAINST CANDIDA ALBICANS ON HEATCURED PMMA DENTURE

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Abstract

Denture-induced stomatitis is the most common pathogenic reaction of denture-bearing mucosa, caused by *Candida albicans*. Miswak is naturally available oral hygiene tool and have been showed to have antifungal activity against *Candida albicans* in oral cavity.

Aim: The aim of the study was to compare the antifungal efficacy between commercial cleaner (Corega) and Miswak extract (*Salvadora persica)* against *Candida albicans* on heat cured Polymethyl methacrylate (PMMA) acrylic denture base resin.

Materials and methods: Forty-eight samples of heat cured PMMA acrylic denture base resin were fabircated in the study. The sterile acrylic resin specimens were immersed in standardized cell suspension of *Candida albicans* and incubated for 60 minutes at 37^0C for cell adhesion and 2 hours at 37^0C for biofilm formation. After 24 hours biofilm was evaluated by cell viability (CFUs) on SDA and cell counting of *Candida albicans* under light microscope at x400 magnification. The fungicidal effect of commercial cleaner and Miswak extract on *Candida albicans* biofilm was then evaluated by colony-forming units on SDA and cell counting under light microscope at x 400 magnification.

Results: Screening test agar disk-diffusion assay showed mean inhibitory zone of 3mm for commercial cleaner as compared to Miswak extract, which showed mean inhibitory zone of 2mm and 1mm for different concentrations. Broth microdilution method showed 31mg/ml MIC and 62.5mg/ml Minimal Fungicidal Concentration (MFC) values for commercial cleaner as compared to Miswak extract that showed 125mg/ml MIC and 250mg/ml MFC values against *Candida albicans*. A significant difference ($p<0.05$) was observed between pre and post treatment of both commercial cleaner and Miswak extract, for CFUs and cell count for *Candida albicans*.

Conclusion: Commercial denture cleaner (Corega) showed better antifungal (*C albicans)* activity than Miswak extract (*Salvadora persica)* on heat cured PMMA acrylic denture base resin.

Keywords: Candida albicans, Commercial cleaners, Miswak, Acrylic resin

Introduction

Candida albicans is one of the common yeasts of normal micro-flora of the oral cavity. However, when biological equilibrium is lost, these microorganisms may cause oral candidiasis leading to white patches, pain, swelling, redness and cracked mouth corners (1). Candida albicans, Candida Tropicalis and Candida Glabrata are most prevalent species in causing oral candidiasis, with 80% of all infections caused by Candida species. Candida albicans account for 50% of oral candidiasis cases (1,2).

The pathogenic reaction of denture-bearing mucosa caused by Candida is Candida-associated denture stomatitis, which occurs in 65% of denture wearers. There are four forms of oral candidiasis: Pseudomembranous candidiasis, Acute erythematous candidiasis, Chronic hyperplastic candidiasis, and Chronic erythematous candidiasis. Chronic erythematous candidiasis is associated with denture stomatitis. Candida albicans are considered as most common among Candida species to cause denture stomatitis, therefore treatment of tissue bearing area of the denture is critical (1,3).

Different precautions are taken to reduce the chances of oral candidiasis, some of the proposed precautions are cleaning dentures mechanically, chemically or using both methods (4). Mechanical cleaning methods like toothbrush are efficient but may abrade polymethyl methacrylate (PMMA) surface. In addition, chemical denture cleaners are divided into five groups: Alkaline peroxides, alkaline hypochlorites, acids, disinfectants and enzymes. Chemical agents like alkaline hypochlorites, alkaline peroxides, enzymes, acids, drugs and mouthwashes are easy to use and efficient in reducing Candida biofilm. Alkaline peroxides like Corega are commonly used to clean denture surfaces (1,3,5,6).

Interestingly, household cleaners including acetic acid, are preferred denture cleaning agents by patients due to ease of availability and cost effectiveness. Comparison of commercial chemical cleaners with household cleaners have shown significantly better antifungal activity (6). Ultrasonic devices also remove Candida biofilms from PMMA surface, with the simultaneous use of a chemical agents.7Irradiation of dentures by using photodynamic therapy (PDT) or microwave has been effectively employed as an efficient method for disinfection of PMMA denture resins (8,9).

Miswak is a naturally available oral hygiene herb.Different types of miswaks, which are available include Peelu (Salvadora persica), Neem (Azadirechtaindica), Zaitoon (Oleaeuropaea), Kikar (Accaciaarabica), Ban (Glycosmicpentaphylla) and Khiran (Capparisaphylla) (10,11).The most common Miswak used is Salvadora persica, which is obtained from twigs, stems or roots of variety of plant species (11). Chemical compounds like sodium chloride, calcium oxalate, silica, fluoride, sulfated compounds, vitamin C and tannic acid are found in Salvadora persica. S persica also contains saponins, flavonoids, alkaloid named salvadorini, trimethylamine, an herbal steroid (beta-sitosterol) and benzyl Isothiocyanate. Miswak has previously demonstrated antifungal activity against Candida albicans in in-vitro studies (12).

Antifungal efficiency of Miswak against Candida albicans in-vitro has been reported(12), however, studies comparing commercial denture cleaner (Corega) and Miswak extract on PMMA resin polymer (heat cured) are limited. There have been studies showing the role of toothbrush, commercial chemical cleaners, household cleaners, microwave radiation and photodynamic therapy against Candida albicans (13-16). These agents show variable antifungal (Candida albicans) effectiveness in disinfecting PMMA based resin polymer. Use of Miswak as an antifungal drug and its antimicrobial activity on oral pathogens including C. albicans has been shown (17). However, the antifungal activity of Miswak extract (Salvadora persica) on heat cured PMMA is unclear in

indexed literature. It is hypothesized that Miswak extract will show comparable antifungal efficacy to commercially available (Corega) denture cleaners in disinfection of heat polymerized denture PMMA. Therefore, the aim of the present study was to compare the antifungal efficiency between commercial cleaner (Corega) and Miswak extract (Salvadora persica) against Candida albicans on heat cured PMMA denture base resin.

Methodology

Study design, sampling, and ethical approval

The specimens for this in-vitro analytical study were fabricated using heat cured acrylic (PMMA) denture base resin (Stellon®,UK) at Prosthodontics laboratory of Dow Dental College, Dow university of Health sciences, Karachi, Pakistan. Experimental testing was performed at Department of Biochemistry and Dow Research Institute of Biotechnology and Biomedical sciences (DRIBBS).

Sample size estimation

Sample size was calculated using OpenEpi software, by comparing means and standard deviations of denture cleaning agents, Clinsodent (0.2771 ± 0.319) and vinegar (1.0235 ±.849) with 95% CI and 80% of power (6). A total of 12 samples were required in each group, with the total sample size of 48. However, twenty-four samples were prepared for each group, resulting in a total of 48 specimens.

Forty-eight cured acrylic denture base resin specimens were prepared. Samples were divided into two groups with 24 acrylic resin specimens in each group based on type of treatments performed. Samples were treated with commercial cleaner, Corega and Miswak extract (Salvadora persica). Each group underwent a set of experiments CFUs, cellular evaluation in microscope and cell viability (microscope) with 4 control groups i.e without Candida albicans.

Inclusion and Exclusion Criteria

Acrylic resin PMMA rectangular specimens measuring 10 x10 x 2 mm, microbial strain of C.albicans ATCC, commercial cleaner Corega and Miswak extract (Salvadora persica) were used in the study. PMMA resin specimens with defected shape and fractures on visual observation were excluded.

Fabrication of Acrylic resin specimens

Heat cured PMMA acrylic denture base resin specimens were fabricated using stainless-steel plate with ten molds (10 x10 x 2 mm). The PMMA denture base resin (Stellon®,UK) was mixed with a ratio of 23.4gm of powder and 10ml of liquid (manufacturer's instructions) in a silicone bowl with a plastic instrument. At dough stage it was packed into the mold with a polythene sheet, for trial packing. The plates were placed under hydraulic press (Mestra®, Spain) at 20 kN force for removal of excessive material (18). After removal of the excess material and plastic sheet, plates were secured with screws and then placed in a polymerizing unit (Acrydig, Italy) to cure with a short curing cycle i.e. 74 ºC for 1 hour & 30 minutes and 100ºC for 30 minutes. Curing plates were allowed to bench cool for 140 minutes and then the specimens were removed (19).
Specimens were trimmed with carbide bur for exact dimension (10 x10 x 2 mm) and immersed in water at room temperature for 12 hours for release of residual monomer and stored in water at room temperature for 72 hours to hydration (20) (Figure. 1).

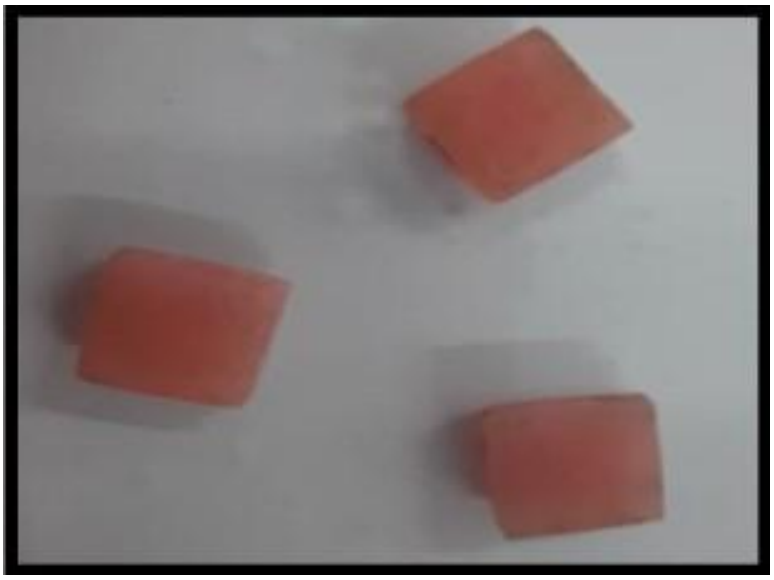


Figure 1: Acrylic specimens with (10 x 10 x 2 mm) dimensions.

Preparation of Miswak (S. Persica) Extract and denture cleanser solutions

Miswak (S. Persica) sticks were placed on aluminum foil and placed at 55◦C in an incubator for three days to dry (Figure 2). Miswak extract was prepared according to method described by Abhary et al, Mohammed et al, Jayanti et al, Al-Bayaty et al, Al-Ayed et al and Darmani et al (21-25). The dried sticks were cut into smaller pieces using a twig cutter and were pulverized to fine powder using electric grinder for 15 minutes. The pulverized powder was sieved repeatedly to obtain fine powder. The powder was transferred to sterile, airtight plastic containers with lids and each container was labeled. The 40 gm, S. Persica powder was added to 200 ml of solvent (distill water) and was soaked at room temperature for 48 hours. The soaked Miswak was transferred to six falcon tubes and kept in centrifuge for 15 minutes. Whatman (No.1) filter paper was placed in a glass funnel and the centrifuged solution was filtered in a bottle and allowed to evaporate in incubator at 40 ◦C for 72 hours. The evaporated and dried extract was considered 100% pure (26) (Figure 2).

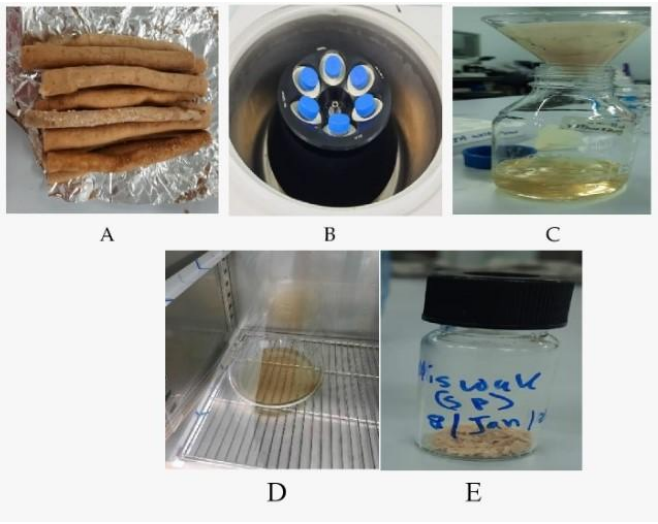
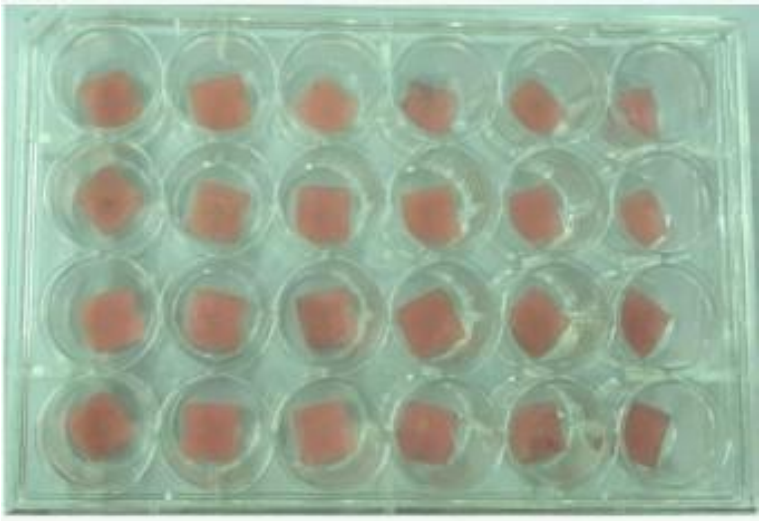


Figure 2. Steps for Miswak extract preparation. A) Miswak sticks, B) Falcon tubes with soaked Miswak S. persica in Centrifuge machine. (C) Filtration of soaked Miswak S. persica with Whatman No. 1 filtration paper. (D) Miswak S. persica evaporating in incubator at 40^0C. (E) Pure Miswak extract S. persica obtained after evaporation.

Different concentrations of Miswak S. persica were prepared by using same extract. The first S. persica solution with concentration of 200mg/ml was prepared by adding 20gm of Miswak extract in 100ml of sterile distilled water. The second solution had a concentration of 2gm/ml by adding 40gm in 20ml of sterile distilled water. Denture cleaner solution was prepared by addition of one tablet of 2gm of Corega to 100ml of sterile distill water in a media bottle, to make a 20mg/ml concentration solution using manufacturer's instructions.



The acrylic resin specimens were sterilized in ultraviolet light (wavelength: 250nm) in BSL-II safety cabinet for 10 minutes and were kept in a sterilization pouch, and specimens were overturned and were sterilized for 10 more minutes (6).**Candida Albicans growth conditions**Candida Albicans strain ATCC 10231 was purchased and revived on Mueller Hinton agar according to manual instructions. Three colonies of Candida albicans grown on Mueller Hinton agar were taken and inoculated in 10ml of Sabouraud dextrose broth. This was incubated at 37^0C at 120 rpm for 24 hours. After 24 hours cells were harvested. The density of the cells was standardized to 0.5 McFarland (1-5x10^7CFU/ml) with help of Spectrophotometer (6). 9ml of SDB was taken in 15ml falcon tube and 1 ml of inoculated broth added and mixed through pipette to dilute (1000µl) the inoculated broth. 1 ml from the diluted inoculated broth was added to curette and placed for air blanking in spectrophotometer.Antifungal activity of Corega and Miswak extract (S. persica) was determined by using standard disk-diffusion assay following the protocols of Clinical and Laboratory Standards Institute guideline (Sheehan & National Committee for Clinical Laboratory Standards, 2004), with slight modifications. The Minimum inhibitory concentration (MIC) was determined by the broth microdilution method as described in the protocols of M27-S4 (Clinical, and Laboratory Standards Institute [CLSI, 2012]). All experiments were performed in duplicates. The MIC and MFC was determined by plating 10µl from each well on SDA that was incubated at 37°C for 24 hours. The MIC was considered as no growth cultivated on SDA and MFC was considered as killing of Candida albicans on SDA (20). Twenty heat cured acrylic denture base resin specimens were immersed in 500µl standardized cell suspension of Candida albicans in each well of 24-well tissue culture plate. Four heat cured acrylic denture base resin specimens were immersed in 500µl uninoculated SDB broth as a control group. (Figure 3) Acrylic specimens were incubated for 60 minutes at 37 °C and 75 rpm for cell adhesion (26).

Figure 3. Specimens were immersed in 500µl standardized cell suspension of Candida albicans and 4 acrylic resin specimens were immersed in 500µl uninoculated SDB broth as a control in 24 well tissue culture plate.

After 2 hours incubation, non-adherent Candida albicans were aspirated with pipette and washed with 500µl sterile PBS (phosphate-buffered saline). Acrylic resin specimens were then transferred to a new 24-well tissue culture plate with 500µl SDB and incubated for 24 hours at 37 °C under 75rpm to allow biofilm development. After 24 hours of biofilm development, specimens were placed into a

new 24-well tissue culture plate containing 500µl sterilized PBS and placed on plate shaker at 250rpm for 30 minutes for detachment of Candida biofilm. The plates were incubated at 37^{O}C in aerobic conditions for 24 hours. The plates with serial dilution 10^{-2} were counted for colonies-forming units. The colony forming units (CFU) were counted and converted into colony-forming unit/ml by using the following scientific formula (26).

$$CFU = \frac{\text{No of colonies x dilution factor}}{\text{Volume plated}}$$

Ten wells containing detached Candida albicans and 2 wells of control group were evaluated through light microscope at x400 magnification by 0.4% Trypan Blue staining. Hemocytometer (Neubauer chamber) contains 25 main squares in the middle field, and each is divided into 16 squares. Counting of Candida albicans was done in 5 main squares in middle field which was then multiplied by 5 to get the total number of Candida albicans in the slide and was counted under x400 magnification, cell density was calculated by following formula (4).

Cell density = no of cells x dilution x 10,000

The fungicidal effect of commercial cleaner Corega and Miswak extract S. persica was evaluated by immersing 12 acrylic resin specimens in 500µl commercial cleaner Corega (20mg/ml concentration) and 12 acrylic resin specimens in 500µl Miswak extract S. persica solution (200mg/ml concentration) in a new 24-well tissue culture plate. Candida albicans in 10 wells (5 for Corega and 5 for Miswak extract S. persica) with 2 wells of control group were evaluated through light microscope at x400 magnification by adding 0.4% Trypan Blue stain Counting of Candida albicans was performed in 5 main squares and was multiplied by 5 to get the total number of Candida albicans in hemocytometer (4).

Cell viability is calculated by formula.

$$\text{Cell viability} = 100 \times \frac{\text{live cells}}{\text{live cells x dead cells}}$$

Detached Candida albicans in other 10 wells (5 for commercial cleaner Corega and 5 for Miswak extract S. persica) with 2 wells of control group were evaluated through cell viability (CFUs). The colony-forming units on plates at 10^{-2} dilutions were counted and converted into colony forming unit/ml by using the following scientific formula (26).

$$CFU = \frac{\text{No of colonies x dilution factor}}{\text{Volume plated}}$$

Statistical Analysis

The results were statistically analyzed by using SPSS (Statistical Package for the Social Sciences) (SPSS Inc, New York, USA). Mean and standard deviation of each variable was reported. Equality of variances and normal distribution were checked through Shapiro-Wilk test. Data concerning pretreatment and post treatment CFUs and cells in microscope was analyzed by paired sample *t*-test. The post treatment CFUs and cell viability was analyzed by independent sample *t*-test. The significance level was set to $p \leq 0.05$.

Results

The aqueous crude extracts of Miswak *Salvadora persica* showed antimicrobial activity against *Candida albicans* at 200mg/ml and 2g/ml. *Candida albicans* showed a mean inhibition zone of 3 mm against commercial cleaner Corega, 1mm against 200mg/ml of Miswak extract and 2mm for 2gm/ml Miswak extract. A mean inhibitory zone of 10mm was observed against fluconazole (Table 1).

Table 1. Mean inhibitory zones (mm) of Commercial cleaner Corega, Miswak extract and Fluconazole

Denture cleaning agents	Mean Inhibitory zone (mm)
Commercial cleaner Corega	3mm
Miswak extract *Salvadora persica* 200mg/ml	1mm
Miswak extract *Salvadora persica 2gm/ml*	2mm
Fluconazole	10mm

mm: millimeter, gm: gram, ml: milliliters, mg: milligram

MIC and MFC (broth micrdilution method)

Anticandidal effect of Miswak extract against *Candida albicans* was tested using broth microdilution assay. Various dilutions of aqueous extract of Miswak *S. persica*, commercial cleaner Corega and Fluconazole (1000 mg/ml-0.4 mg/ml) were used to determine MIC. The dilution at which no growth of *Candida albicans* was observed was taken as the minimal inhibitory concentration (MIC). The dilution at which killing of *Candida albicans* was observed was taken as minimal fungicidal concentration (MFC) (Figure 4).

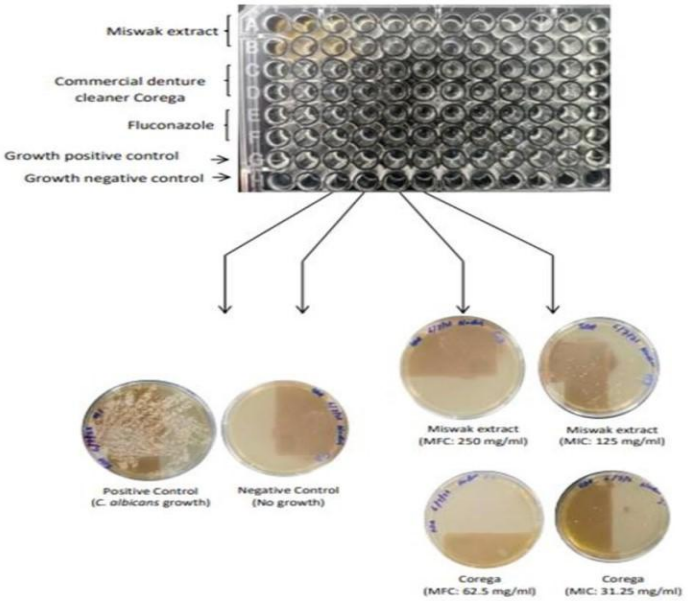


Figure 4. Anticandidal effect of *Salvadora persica*, commercial cleaner Corega and Fluconazole against *C. albicans* was tested using broth microdilution assay.

The MIC and MFC values of aqueous Miswak extract and commercial cleaner (Corega) are presented in (Table 2). MIC of Miswak extract required to inhibit the growth of *Candida albicans* was noted to be 125 mg/ml whereas, the concentration required for killing (MFC) *Candida albicans* was 250 mg/ml. Corega showed a value of 31.5mg/ml and 62.5 mg/ml for MIC and MFC, respectively. Fluconazole showed a value of 0.0078 mg/ml and 0.0156 mg/ml for MIC and MFC, respectively.

Table 2. MIC and MFC values of Commercial cleaner Corega, Miswak extract and Fluconazole

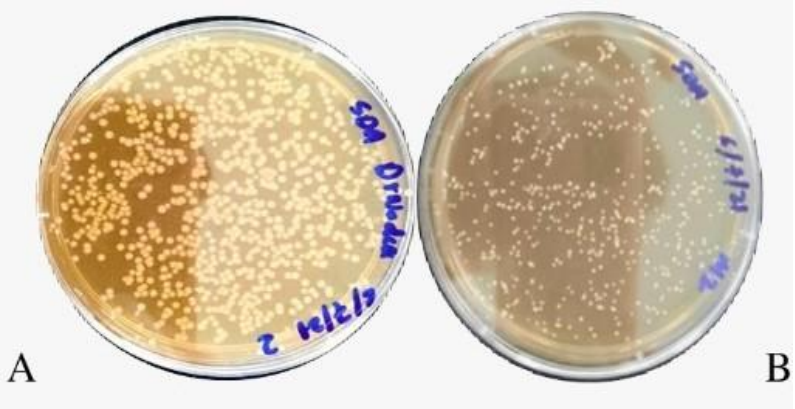
Antifungals	MIC values(mg/mL)	MFC Values(mg/mL)
Miswak extract	125	250
Commercial cleaner Corega	31.25	62.5
Fluconazole	0.0078	0.0156

Mg: milligram, ml: milliliter, MIC: minimal inhibitory concentration

Effect of Miswak extract and commercial cleaner on Candida Albicans biofilm

Results of cell viability assay (CFUs) are presented in Table 3. Resin specimens before and after treatment with Miswak extract showed mean of $5.92x10^5$ ± 8368.80 cfu/ml and $3.92x10^5$±8366.60 cfu/ml (Figure 5) with a ($p<0.05$). This represents a significant decrease in the number of *Candida albicans* cells after treating with Miswak extract.

Figure 5. Before (A) and after (B) treatment with Miswak extract *S. persica* respectively on SDA.



Results of *Candida albicans* cells assessed through microscopy before treating with Miswak extract showed1.39x10[7]±8318.60 cells and after treatment showed of 2.35x10[7] ±223606.97 with a $p<0.05$, (Figure 6).

Figure 6. Before (A) and after (B) treatment with Miswak extract *S. persica* under microscope at x400 magnification.

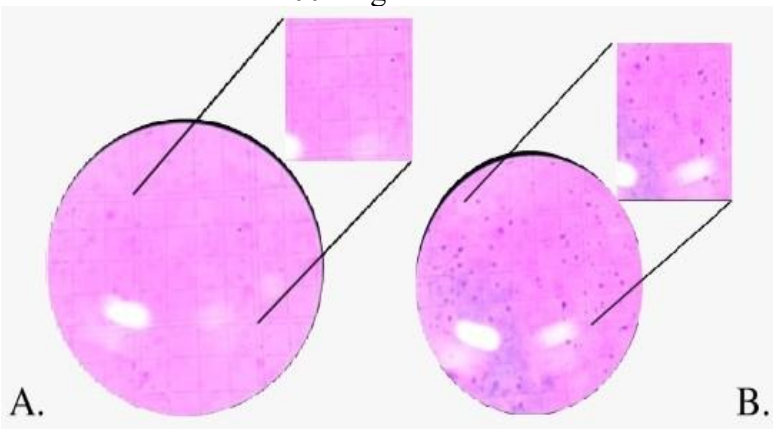


Table 3. Cell viability CFUs and Microscopy for Miswak and Corega

		Mean ± SD (Pre-treatment)	Mean ± SD (Post-treatment)	p-value
Miswak	CFUs	$5.92x10^5$± 8368.80	$3.92x10^5$± 8366.60	<0.05*
	Cells in Microscope	$1.39x10^7$± 8318.60	$2.35x10^7$± 223606.97	<0.05*
Corega	CFUs	5.92x105±	NA	NA
	Cells in Microscope	$1.39x10^7$± 135092.561	$1.42x10^8$± 32403.703	<0.05*

*NA: Not applicable. SD: Standard deviation, NA: not applicable, *p-value was calculated by using paired sample t-test.*

Acrylic resin specimens assessed for cell viability (CFUs) before and after commercial cleaner treatment showed mean of 5.92x10[5] ±8368.80 cfu/ml and 0 (Figure 7 & table 3). This showed that there was no growth on agar plates after treatment with commercial cleaner (Corega).

Figure 7. *Candida albicans* treatment with commercial cleaner (Corega) (before (A) and after (B)) respectively on SDA.

Candida albicans cells assessed through microscopy before and after treatment with commercial cleaner Corega showed mean of cells 1.42x10[8] ±32403.703 and 1.39x10[8] ±135092.561 (Figure 8 & table 3) with a *(p*<0.05). These results showed significant decrease in the number of *Candida albicans* live cells.

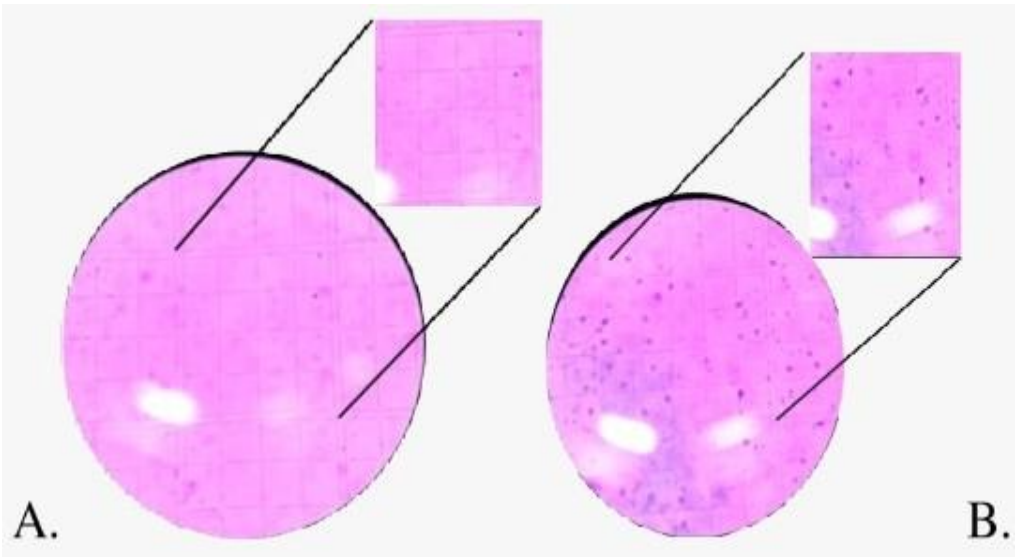
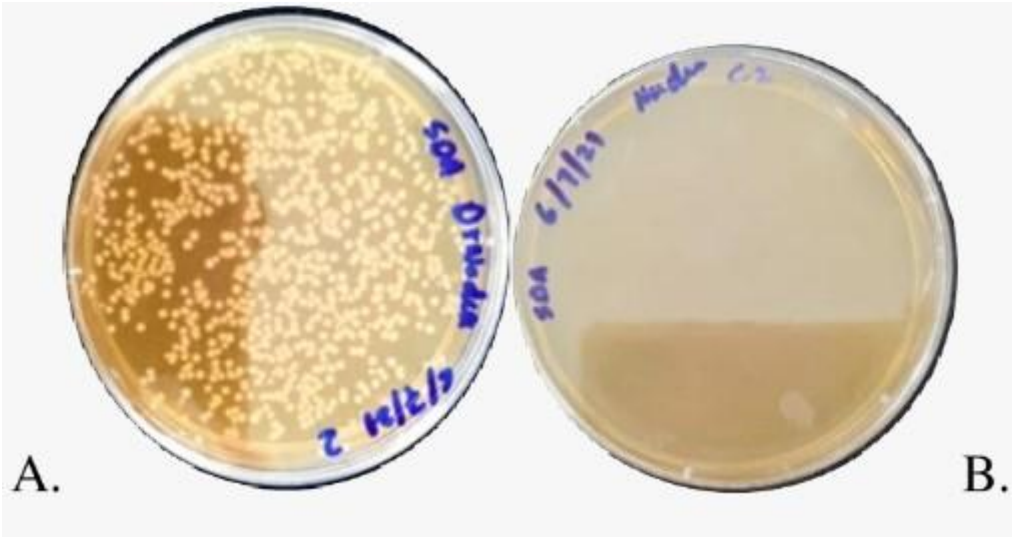


Figure 8. Before (A) and after (B) treatment with commercial cleaner Corega under microscope at x400 magnification.

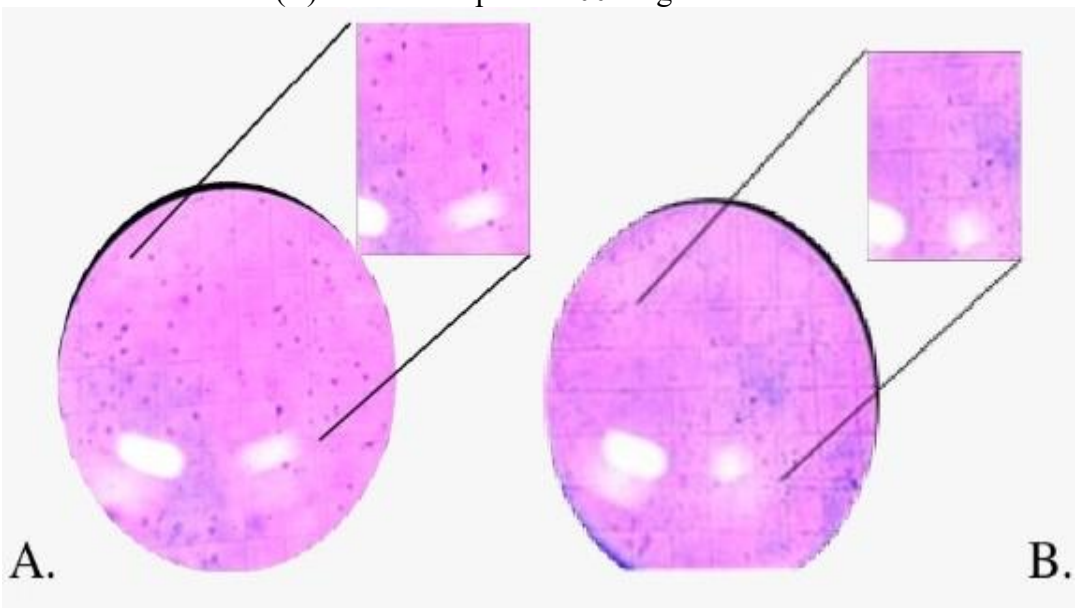


Acrylic resin specimens treated with Miswak extract showed mean CFU of 3.92x10[5] ±836680 per ml as compared to Corega with mean CFU of 0. The results showed no colonies on commercial cleaner Corega plate with prominent colonies on Miswak extract plates (p < 0.05) (Figure 9).

Figure 9. Comparison between commercial cleaner Corega (A) and Miswak (B) extract *S. persica* antifungal activity after treatment on SDA.

Treatment with Miswak extract showed 2.35x10[7] ±2236067.97 (mean ± SD) cells as compared to commercial cleaner, which showed $1.42x10^6$±32403.703 (mean ± SD) cells with ($p< 0.05$). This showed a significant decrease in *Candida albicans* with more prominent decrease in commercial cleaner group (Figure 10).

Figure 10. Comparison of antifungal activity between commercial cleaner (A) and Miswak extract (B) in microscope at x400 magnification.



Mean cell viability for Miswak extract (87.60 ± 1.67 %) compared to commercial cleaner (32.60 ± 1.51 %) were significantly different ($p<0.05$).

Discussion

The present study was based on the hypothesis, that Miswak extract will show comparable antifungal efficacy to commercially available (Corega) denture cleaners in disinfection of heat polymerized denture PMMA. It was observed that the anti-fungal efficacy of commercial cleaner Corega was better than Miswak extract (*S. persica)* on PMMA denture resin, therefore rejecting the null hypothesis. *Candida* is a communal yeast and common microbial flora of the oral cavity, which does not cause infection in physiologic state. The pathogenic reaction of denture-bearing mucosa caused by *Candida albicans* is commonly known as denture-induced stomatitis. *Candida albicans* is considered, as most common among *Candida* species to cause denture stomatitis, therefore the

treatment of tissue bearing denture is important (3).*Candida albicans* can be removed from heat cured acrylic denture base resin surfaces by toothbrushes, commercial chemical cleaners, household cleaners, plant materials microwave radiation, and photodynamic therapy (1,3-8).

Chemically Miswak is composed of octacosanol, 1-triacantanol, β-sitosterol, and β-sitosterol-3-O-β-D-glucopyranoside (27).Miswak is available as different types including Peelu (*Salvadora persica*), Neem (*Azadirechtaindica*), Zaitoon (*Oleaeuropaea*), Kikar (*Accaciaarabica*), Ban (*Glycosmicpentaphylla*) and Khiran (*Capparisaphylla*) (10,11).The most common Miswak used in is *Salvadora persica*. As it is an organic cleaning tool for teeth and is also known to be antifungal(10,12). Miswak (*S. persica)* has been proposed as a denture cleaner including in patients with denture stomatitis, wearing removable acrylic resin dentures (3).In addition, comparison of antifungal activity of commercial cleaner (Corega) and Miswak extract *S. persica* against *Candida albicans* was observed by CFUs and light microscopy cell assessments on heat cured acrylic denture base resins.

In a study conducted by Al-Bagieh (28) et al and Al-Obaida (29) et al., aqueous extracts of Miswak had shown antifungal activity against *Candida albicans* throughagar disc-diffusion and MIC. Similarly, Al-Bayati and Sulaiman (30) evaluated the mean inhibitory zone of aqueous Miswak extract through agar disc-diffusion assay. The mean inhibitory zone was 12.4mm for 200mg/ml aqueous Miswak extract against *Candida albicans*, which was higher in comparison to the present study. This could be attributed to the fact that, inhibition zone of 1mm for 200mg/ml aqueous Miswak extract against *Candida albicans* may be because of using incubator for evaporation of Miswak extract instead of employing vacuum and freeze drying.

Interestingly, Gouveia et al, (31) compared Corega tablets with other cleaners through agar disc-diffusion assay and the mean inhibitory zone was 0 which was less than the observations in the present study. The present study showed a mean inhibitory zone of 3mm for 20mg/ml Corega solution, this increase in antifungal activity is possibly due to a different *Candida albicans* strain used in the present study.

In the present study, MIC values for aqueous Miswak extract were 125mg/ml, by contrast, in a study by Al-Bayati and Sulaiman et al (30), the MIC values through microdilution method against *Candida albicans* for aqueous extract of Miswak were 6.25mg/ml. The higher inhibition of *Candida albicans* in the present study could be due to use of incubator for evaporation of Miswak extract. In addition, deFreitasfernandeset al (32), Cintia Lima Gouveia (31) and Sousa et al (33) concluded that there was statistically significant reduction in CFU on acrylic denture base resins when treated with Corega tablets. In comparison, the present study showed complete reduction of Candida albicans with no CFUs with Corega. This difference may be due to different strain of *Candida albicans* used in the present study.

Nalbant et al (34) and Kumar et al (6) concluded that when cell counting of *Candida albicans* was done under light microscope at x40 magnification, it showed statistically significant reduction in *Candida cells* with *(p*<0.05) after treating with alkaline peroxide cleaners on acrylic base resin. These findings are in line with the present study, which showed significant reduction in candida cells using a commercial cleaner Corega under light microscope at x 400 magnifications.

The present study suggests the antifungal efficacy of Miswak extract (*S. persica)* andcommercial cleaner (Corega) for denture base resins, with improved efficacy of Corega over the use of Miswak extract. However, only heat cured acrylic resin was used to prepare the samples, therefore, chemically activated, microwave activated, light activated, or reinforced resins can be assessed in future studies. In addition, a single strain of *Candida Albicans* ATCC 10231 was used in the present study. Moreover, Miswak extract of *S. persica*, was the only type used in the present experiment, however other Miswak extracts like Neem (*Azadirechtaindica*), Zaitoon (*Oleaeuropaea*), Kikar (*Accaciaarabica*), Ban (*Glycosmicpentaphylla*), Khiran (*Capparisaphylla*) and chitosan salt is available (35,36,37). Therefore, further studies assessing the antifungal efficacy of different Miswak extracts on multiple Candida albicans strains on heat cured acrylic denture base resins.

Conclusion

Within the study limitations, both Miswak extract and Corega exhibited antifungal activity on the surface of denture base materials. However, the highest inhibition zone was seen with Corega.

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Conceptualization: NW, KA, RA, SK, NA, AL;
Methodology: SAL, EA, FV, TA;
Validation: RA, SK, NA, EA, FV, AH, TA;
Formal analysis: NW, KA, NA, AL, SAL, EA, AH;
Investigation: NW, KA, RA, SK, NA, AL;
Data curation: NW, NA, AL, SAL, EA, FV, TA;
Writing—original draft preparation: SAL, FV, EA;
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