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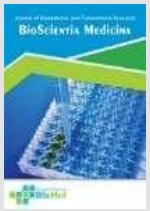

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Pomegranate Extracts as a Natural Denture Cleanser: A Promising Alternative to Sodium Bicarbonate for Inhibiting *Candida albicans*

Cindy Denhara Wijaya[1*], Sumitro[1], Annisa Rizqi Ramadhani Sitio[2], Fitriah Khairani Harahap[2]

[1]Dentistry Study Program, Faculty of Medicine, Dentistry and Health Sciences, Universitas Prima Indonesia, Medan, Indonesia

[2]Students of Dentistry Study Program, Faculty of Medicine, Dentistry and Health Sciences, Universitas Prima Indonesia, Medan, Indonesia

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***Corresponding author:**
Cindy Denhara Wijaya

E-mail address:
cindydenharawijaya@unprimdn.ac.id

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A B S T R A C T

Background: Denture stomatitis, caused primarily by *Candida albicans* overgrowth, is a common oral health issue among denture wearers. Maintaining denture hygiene is crucial for prevention. This study investigated the efficacy of red pomegranate (*Punica granatum* L.) peel and fruit extracts as natural denture cleansers compared to sodium bicarbonate, a standard denture cleansing agent. **Methods:** Heat-cured acrylic resin plates were fabricated and contaminated with *C. albicans*. Samples were divided into four groups and soaked for 8 hours in: 75% pomegranate peel extract, 75% pomegranate fruit extract, 5% sodium bicarbonate solution (positive control), and aquadest (negative control). *C. albicans* colony counts were performed using the spread plate technique and colony counter. Data were analyzed using Kruskal-Wallis and Mann-Whitney tests. **Results:** Both pomegranate peel and fruit extracts significantly reduced *C. albicans* growth compared to the negative control ($p<0.05$). The peel extract showed comparable efficacy to sodium bicarbonate ($p>0.05$), while the fruit extract demonstrated slightly lower but still substantial antifungal activity. **Conclusion:** Red pomegranate peel and fruit extracts hold promise as natural denture cleansers for inhibiting *C. albicans* and potentially preventing denture stomatitis. Further research is warranted to evaluate their long-term effects on denture materials and clinical efficacy.

1. Introduction

Edentulism, the loss of natural teeth, is a prevalent oral health concern, particularly among older adults. This condition can significantly impact an individual's quality of life, affecting their ability to eat, speak, and maintain proper nutrition. To address the functional and aesthetic challenges posed by edentulism, dentures, whether partial or complete, are commonly used as prosthetic replacements for missing teeth. Dentures play a crucial role in restoring oral function, improving facial aesthetics, and enhancing the overall well-being of edentulous individuals. While dentures offer numerous benefits, they also introduce significant changes to the oral environment. The presence of a denture can alter salivary flow, microbial colonization patterns, and the accumulation of denture plaque. These changes can create conditions conducive to the overgrowth of opportunistic microorganisms, such as *Candida albicans*, a commensal fungus that resides naturally in the oral cavity. Under favorable conditions, *C. albicans* can transition from a harmless commensal to a pathogenic state, leading to various oral infections.[1-3]

Candida albicans is a major etiological factor in denture stomatitis, a common inflammatory condition affecting the oral mucosa beneath dentures. Denture

stomatitis is characterized by redness, swelling, and discomfort in the areas where the denture contacts the oral tissues. In severe cases, it can cause pain, difficulty in eating, and even lead to systemic complications if left untreated. The prevalence of denture stomatitis is high among denture wearers, with studies reporting a range of 60% to 75%, primarily affecting elderly women. Several factors contribute to the development of denture stomatitis, including poor denture hygiene, continuous denture wear, smoking, and compromised immune status. Maintaining proper denture hygiene is therefore crucial to prevent *C. albicans* overgrowth and associated oral health issues. Current denture cleansing methods include mechanical cleaning, such as brushing, and chemical soaking. Brushing with a denture brush and specialized denture cleansers helps remove food debris, plaque, and stains from the denture surfaces. However, mechanical cleaning alone may not be sufficient to eliminate all microorganisms, especially those embedded in the denture plaque. Moreover, excessive or improper brushing can cause wear and tear on denture surfaces over time, leading to surface roughness and micro-cracks that can harbor microorganisms. Chemical soaking involves immersing the denture in a solution containing antimicrobial agents. Commercially available denture cleansers typically contain sodium bicarbonate, alkaline peroxide, or hypochlorite as their active ingredients. These agents have shown good efficacy in reducing microbial load on dentures. However, concerns remain regarding their potential long-term effects on denture materials. Prolonged exposure to these chemical agents can cause surface degradation, discoloration, and loss of denture material integrity. Additionally, some individuals may experience adverse reactions or sensitivities to certain denture cleansing agents.[4-7]

The limitations of current denture cleansing methods and the increasing demand for natural oral health solutions have led to a growing interest in exploring plant-based alternatives. Natural extracts derived from plants have been recognized for their potential health benefits, including antimicrobial properties. These extracts contain various bioactive compounds, such as flavonoids, tannins, and alkaloids, which have shown inhibitory effects on *C. albicans* growth. Pomegranate (*Punica granatum* L.), a fruit rich in antioxidants and bioactive compounds, has emerged as a promising candidate for natural denture cleansing. Both the peel and fruit of pomegranate contain a wide array of phytochemicals, including flavonoids, tannins, and alkaloids, which have demonstrated inhibitory effects on *C. albicans* growth.[8-10] This study aimed to evaluate the effectiveness of red pomegranate peel and fruit extracts as natural denture cleansers compared to sodium bicarbonate in inhibiting *C. albicans* growth on heat-cured acrylic resin, a commonly used denture base material.

2. Methods

Heat-cured acrylic resin plates, serving as the denture base material for this study, were fabricated according to ISO standards using a standardized protocol. The dimensions of each plate were 10 mm x 10 mm x 2 mm, ensuring uniformity across all samples. The fabrication process involved mixing the acrylic resin powder and liquid according to the manufacturer's instructions, followed by packing the mixture into a mold and curing it under controlled heat and pressure. After curing, the plates were carefully polished to achieve a smooth surface, free from porosities. This meticulous polishing process was essential to minimize the potential for microbial adhesion and to ensure that the surface properties of the plates did not influence the experimental results. To ensure sterility and eliminate any potential contamination that could interfere with the experimental results, the fabricated acrylic resin plates were sterilized in an autoclave at 121°C for 15 minutes. This sterilization process effectively eliminated any microorganisms that may have been present on the plates, ensuring that the subsequent experiments were conducted under controlled conditions. A pure culture of *Candida albicans* (ATCC

10231) was obtained from a certified laboratory to ensure the use of a standardized and authenticated strain. The culture was prepared for inoculation by transferring a small amount of the *C. albicans* inoculum from the stock culture to a fresh Sabouraud Dextrose Agar (SDA) plate. The SDA plate was then incubated at 37°C for 24 hours to allow the *C. albicans* to grow and form colonies.

Red pomegranates were obtained from a local market, ensuring they were ripe and free from any signs of spoilage. The selection of ripe and healthy pomegranates was crucial to ensure the optimal quality and concentration of bioactive compounds in the extracts. The peel and fruit were separated, and the fruit was dried in an oven at 50°C for 6 days until the moisture content was less than 10%. Drying the fruit reduced the water content, preventing microbial growth and facilitating the extraction process. The dried fruit was then ground into a fine powder using a blender, increasing the surface area for efficient extraction of bioactive compounds. The peel was dried separately in an oven at 60°C for 24 hours and similarly ground into powder. The higher drying temperature for the peel was chosen based on its thicker and tougher texture compared to the fruit. Grinding the peel into powder ensured consistency and maximized the extraction of bioactive compounds from the peel. Each powder (peel and fruit) was macerated in 96% ethanol for 72 hours in a closed container, with manual stirring every 24 hours. Maceration is a common extraction technique that involves soaking the plant material in a solvent to dissolve the desired compounds. Ethanol was chosen as the solvent due to its ability to extract a wide range of bioactive compounds from pomegranate. The closed container prevented the evaporation of ethanol and maintained a consistent extraction environment. Manual stirring every 24 hours ensured thorough mixing and maximized the contact between the plant material and the solvent. After maceration, the mixture was filtered using filter paper to remove the solid plant material from the liquid extract. The filtrate, containing the extracted bioactive compounds, was then concentrated using a rotary evaporator at 50°C for 45 minutes to remove the ethanol. This process yielded a thick, concentrated extract that was rich in bioactive compounds. The concentrated pomegranate peel and fruit extracts were stored in sealed containers in a refrigerator until further use. This storage condition ensured the stability and preserved the bioactivity of the extracts, preventing degradation or contamination.

The study followed a posttest-only control group design, a robust experimental design that allows for the comparison of different treatment groups to a control group. This design minimizes the influence of extraneous variables and increases the internal validity of the study. A total of 24 acrylic resin plates were divided into four groups (n=6 per group); Group 1: Soaked in 75% pomegranate peel extract; Group 2: Soaked in 75% pomegranate fruit extract; Group 3: Soaked in 5% sodium bicarbonate solution (positive control); Group 4: Soaked in aquadest (negative control). Each acrylic resin plate was immersed in 10 ml of the respective solution for 8 hours, simulating the typical overnight soaking duration for denture cleansing. This standardized soaking procedure ensured that all plates were exposed to the treatment solutions for the same amount of time, minimizing variability and allowing for a fair comparison between the groups.

Prior to soaking, the acrylic resin plates were pre-treated with sterile aquadest for 24 hours to reduce residual monomer, a substance that can inhibit microbial adhesion. This pre-treatment step ensured that the plates were free from any substances that could interfere with the adherence of *C. albicans*. After pre-treatment, the plates were pre-conditioned with artificial saliva for 1 hour to mimic the oral environment and facilitate *C. albicans* adherence. Artificial saliva provides a more realistic simulation of the oral cavity, as it contains components that promote microbial attachment and growth. Each pre-conditioned plate was then contaminated with a standardized suspension of *C. albicans* (1.5 x 10^8 CFU/ml) and incubated for 24 hours at 37°C to allow

for fungal adherence. The standardized suspension ensured that all plates were exposed to the same concentration of *C. albicans*, minimizing variability. Incubation at 37°C, the normal human body temperature, provided optimal conditions for *C. albicans* growth and adherence to the acrylic resin plates.

After the 8-hour soaking period, the plates were removed from the solutions and rinsed with Phosphate Buffered Saline (PBS) to remove non-adherent *C. albicans* cells. PBS is a physiological buffer solution that helps maintain the pH and osmotic balance of cells, preventing damage to the *C. albicans* cells during rinsing. The plates were then vortexed for 30 seconds to detach the adherent *C. albicans* cells from the acrylic resin surface. Vortexing ensured that the majority of the adherent cells were dislodged from the plates, allowing for accurate colony counting. Serial dilutions (10^-1 and 10^-2) were prepared using 0.9% NaCl solution to reduce the concentration of *C. albicans* cells to a countable range. 0.1 ml of the 10^-2 dilution was then spread uniformly onto Sabouraud Dextrose Agar (SDA) plates using a sterile spreader. SDA is a selective medium that promotes the growth of fungi while inhibiting the growth of bacteria, ensuring that the colonies counted were predominantly *C. albicans*. The SDA plates were incubated for 48 hours at 37°C to allow the *C. albicans* colonies to grow and become visible. After incubation, the number of *C. albicans* colonies on each plate was counted using a digital colony counter (Interscience Scan® 300). The digital colony counter provided an accurate and efficient method for counting colonies, minimizing human error.

Data obtained from the colony counting were entered into SPSS software version 24 for statistical analysis. Before analysis, the data were checked for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. These tests assessed whether the data met the assumptions required for parametric statistical tests. Since the sample size was small (n=6 per group), the non-parametric Kruskal-Wallis test was used to compare the effectiveness of the different treatments. The Kruskal-Wallis test is a rank-based test that does not require the data to be normally distributed, making it suitable for small sample sizes. Mann-Whitney tests were performed for pairwise comparisons between groups to determine specific differences between the treatment groups. The Mann-Whitney test is a non-parametric test used to compare two independent groups. The level of statistical significance was set at $p<0.05$. This means that a p-value less than 0.05 was considered statistically significant, indicating that the observed differences between groups were unlikely to have occurred by chance.

3. Results

Table 1 presents the results of the phytochemical analysis conducted on both the peel and fruit extracts of pomegranate. This analysis aimed to identify the presence of various secondary metabolites, which are bioactive compounds that contribute to the medicinal properties of plants; Alkaloids: Alkaloids were detected in the peel extract but not in the fruit extract. Alkaloids are a diverse group of compounds with various pharmacological activities, including antimicrobial properties. Their presence in the peel extract suggests its potential as an antifungal agent; Flavonoids: Both the peel and fruit extracts tested positive for flavonoids. Flavonoids are potent antioxidants with anti-inflammatory and antimicrobial effects. Their presence in both extracts indicates their potential contribution to the overall antifungal activity; Glycosides: Glycosides were also found in both the peel and fruit extracts. Glycosides are compounds consisting of a sugar molecule attached to a non-sugar molecule. They have various biological activities, including antimicrobial effects; Saponins: Saponins were detected in both the peel and fruit extracts. Saponins are amphipathic compounds with soap-like properties. They have been shown to possess antifungal activity by disrupting fungal cell membranes; Tannins: Tannins were present in both the peel and fruit extracts. Tannins are polyphenolic compounds with astringent properties. They have

been reported to have antimicrobial activity, including antifungal effects; Triterpenes/Steroids: Both the peel and fruit extracts tested positive for triterpenes/steroids. These compounds are a class of lipids with diverse biological activities, including antifungal properties.

Table 2 presents the results of the study investigating the effects of different treatment groups on *Candida albicans* colony counts. The table provides the raw colony counts for each replicate within each treatment group, as well as the mean and standard deviation of the colony counts for each group; Pomegranate Peel Extract (75%): The mean *C. albicans* colony count for this group was 7.50 x 10^-2 CFU/ml, with a standard deviation of 6.285. This indicates a significant reduction in *C. albicans* growth compared to the Aquadest (Control) group; Pomegranate Fruit Extract (75%): The mean *C. albicans* colony count for this group was 13.17 x 10^-2 CFU/ml, with a standard deviation of 6.853. This also demonstrates a substantial reduction in *C. albicans* growth compared to the control group, although the reduction was slightly less pronounced than that observed with the Pomegranate Peel Extract; Sodium Bicarbonate (5%): The mean *C. albicans* colony count for this group was 6.17 x 10^-2 CFU/ml, with a standard deviation of 3.601. This shows a significant reduction in *C. albicans* growth compared to the control group, and the reduction was comparable to that observed with the Pomegranate Peel Extract; Aquadest (Control): The mean *C. albicans* colony count for this group was 201.33 x 10^-2 CFU/ml, with a standard deviation of 49.423. This high colony count represents the baseline growth of *C. albicans* without any treatment.

Table 1. Phytochemical analysis of pomegranate peel and fruit extracts.

No.	Secondary metabolite	Reagent	Peel	Fruit
1	Alkaloid	Dragendroff, Bouchardat, Mayer	+	-
2	Flavonoid	Mg powder + amyl alcohol	+	+
3	Glycoside	Molisch + H_2SO_4 + HCl	+	+
4	Saponin	Hot water/shaking	+	+
5	Tannin	$FeCl_3$	+	+
6	Triterpene/Steroid	Lieberman-Burchard	+	+

Table 2. Effect of different treatment groups on *Candida albicans* colony counts.

Treatment group	Replicate	*C. albicans* colony count (10^-2 CFU/ml)	Mean (10^-2 CFU/ml)	Standard Deviation
Pomegranate Peel Extract (75%)	1	20		
	2	7		
	3	7		
	4	3		
	5	6	7.50	6.285
	6	5		
Pomegranate Fruit Extract (75%)	1	17		
	2	10		
	3	11		
	4	20		
	5	2	13.17	6.853
	6	19		
Sodium Bicarbonate (5%)	1	10		
	2	2		
	3	3		
	4	10		
	5	4	6.17	3.601
	6	8		
Aquadest (Control)	1	207		
	2	161		
	3	281		
	4	224		
	5	194	201.33	49.423
	6	141		

Table 3 presents the results of the Kruskal-Wallis test, which was used to compare the *Candida albicans* colony counts among the different treatment groups. The Kruskal-Wallis test is a non-parametric test that compares the medians of two or more groups. It is used when the data do not meet the assumptions of normality required for parametric tests like ANOVA; Peel Extract (75%) vs. Sodium Bicarbonate (5%) vs. Aquadest: The chi-square value of 11.415 with 2 degrees of freedom resulted in a p-value of 0.003. This p-value is less than the significance level of 0.05, indicating a statistically significant difference in *C. albicans* colony counts between these three groups. This suggests that both the Pomegranate Peel Extract and Sodium Bicarbonate were effective in reducing *C. albicans* growth compared to the Aquadest (Control); Fruit Extract (75%) vs. Sodium Bicarbonate (5%) vs. Aquadest: The chi-square value of 13.223 with 2 degrees of freedom resulted in a p-value of 0.001. This p-value is also less than 0.05, indicating a statistically significant difference in *C. albicans* colony counts between these three groups. This suggests that both the Pomegranate Fruit Extract and Sodium Bicarbonate were effective in reducing *C. albicans* growth compared to the control; Peel Extract (75%) vs. Fruit Extract (75%) vs. Sodium Bicarbonate (5%) vs. Aquadest: The chi-square value of 15.348 with 3 degrees of freedom resulted in a p-value of 0.002. This p-value is less than 0.05, indicating a statistically significant difference in *C. albicans* colony counts among all four treatment groups. This suggests that there were differences in the effectiveness of the different treatments in reducing *C. albicans* growth.

Table 3. Kruskal-Wallis test results for *Candida albicans* colony counts.

Treatment group comparison	Chi-Square	df	p-value	Significant Difference?
Peel Extract (75%) vs. Sodium Bicarbonate (5%) vs. Aquadest	11.415	2	0.003	Yes
Fruit Extract (75%) vs. Sodium Bicarbonate (5%) vs. Aquadest	13.223	2	0.001	Yes
Peel Extract (75%) vs. Fruit Extract (75%) vs. Sodium Bicarbonate (5%) vs. Aquadest	15.348	3	0.002	Yes

Table 4 presents the results of the Mann-Whitney U test, which was used to conduct pairwise comparisons between the different treatment groups. The Mann-Whitney U test is a non-parametric test that compares the ranks of two independent groups. It is used when the data do not meet the assumptions of normality required for parametric tests like the t-test; Peel Extract (75%) vs. Fruit Extract (75%): The Mann-Whitney U value of 10.500 and the Wilcoxon W value of 31.500 resulted in a Z score of -1.203 and a p-value of 0.229. This p-value is greater than 0.05, indicating no statistically significant difference in *C. albicans* colony counts between the Pomegranate Peel Extract and the Pomegranate Fruit Extract. This suggests that the peel and fruit extracts had comparable antifungal activity; Peel Extract (75%) vs. Sodium Bicarbonate (5%): The Mann-Whitney U value of 17.000 and the Wilcoxon W value of 38.000 resulted in a Z score of -0.161 and a p-value of 0.872. This p-value is greater than 0.05, indicating no statistically significant difference in *C. albicans* colony counts between the Pomegranate Peel Extract and Sodium Bicarbonate. This suggests that the peel extract was as effective as sodium bicarbonate in reducing *C. albicans* growth; Peel Extract (75%) vs. Aquadest: The Mann-Whitney U value of 0.000 and the Wilcoxon W value of 21.000 resulted in a Z score of -2.882 and a p-value of 0.004. This p-value is less than 0.05, indicating a statistically significant difference in *C. albicans* colony counts between the Pomegranate Peel Extract and the Aquadest (Control). This confirms that the peel extract was effective in reducing *C. albicans* growth compared to the control; Fruit Extract (75%) vs. Sodium Bicarbonate (5%): The Mann-Whitney U

value of 5.500 and the Wilcoxon W value of 26.500 resulted in a Z score of -2.009 and a p-value of 0.045. This p-value is less than 0.05, indicating a statistically significant difference in *C. albicans* colony counts between the Pomegranate Fruit Extract and Sodium Bicarbonate. This suggests that although the fruit extract was effective in reducing *C. albicans* growth, it was slightly less effective than sodium bicarbonate; Fruit Extract (75%) vs. Aquadest: The Mann-Whitney U value of 0.000 and the Wilcoxon W value of 21.000 resulted in a Z score of -2.882 and a p-value of 0.004. This p-value is less than 0.05, indicating a statistically significant difference in *C. albicans* colony counts between the Pomegranate Fruit Extract and the Aquadest (Control). This confirms that the fruit extract was effective in reducing *C. albicans* growth compared to the control; Sodium Bicarbonate (5%) vs. Aquadest: The Mann-Whitney U value of 0.000 and the Wilcoxon W value of 21.000 resulted in a Z score of -2.887 and a p-value of 0.004. This p-value is less than 0.05, indicating a statistically significant difference in *C. albicans* colony counts between Sodium Bicarbonate and the Aquadest (Control). This confirms that sodium bicarbonate was effective in reducing *C. albicans* growth compared to the control.

Table 4. Mann-Whitney test results for Pairwise comparisons of *Candida albicans* colony counts.

Treatment group comparison	Mann-Whitney U	Wilcoxon W	Z	p-value	Significant Difference?
Peel Extract (75%) vs. Fruit Extract (75%)	10.500	31.500	-1.203	0.229	No
Peel Extract (75%) vs. Sodium Bicarbonate (5%)	17.000	38.000	-161	0.872	No
Peel Extract (75%) vs. Aquadest	0.000	21.000	-2.882	0.004	Yes
Fruit Extract (75%) vs. Sodium Bicarbonate (5%)	5.500	26.500	-2.009	0.045	Yes
Fruit Extract (75%) vs. Aquadest	0.000	21.000	-2.882	0.004	Yes
Sodium Bicarbonate (5%) vs. Aquadest	0.000	21.000	-2.887	0.004	Yes

4. Discussion

The potent antifungal activity observed in pomegranate extracts against *Candida albicans* can be primarily attributed to their rich and diverse profile of bioactive compounds. These compounds, including punicalagins, flavonoids, tannins, and other secondary metabolites, act individually and synergistically to disrupt various aspects of fungal cell structure, metabolism, and growth. Punicalagins are the most abundant polyphenols found in pomegranate, particularly concentrated in the peel. They are ellagitannins, a type of hydrolyzable tannin, known for their strong antioxidant and antimicrobial properties. The fungal cell wall is a rigid structure composed mainly of chitin, glucans, and proteins, providing structural integrity and protection to the fungal cell. Punicalagins have been shown to disrupt the fungal cell wall by interfering with the synthesis and assembly of its components. This disruption compromises the cell wall's integrity, leading to cell leakage and ultimately cell death. Chitin is a major structural component of the fungal cell wall, providing rigidity and strength. Punicalagins have been shown to inhibit chitin synthesis by targeting chitin synthase, a key enzyme involved in chitin production. This inhibition weakens the cell wall, making the fungal cell more susceptible to osmotic stress and lysis. Glucans are another major component of the fungal cell wall, contributing to its structural integrity. Punicalagins can interfere with glucan synthesis by inhibiting glucan synthase, an enzyme responsible for glucan production. This disruption further weakens the cell wall, compromising its ability to protect the fungal cell. In addition to inhibiting chitin and glucan synthesis, punicalagins can also disrupt the assembly of cell wall components. This disruption prevents the proper formation of the cell wall, leading to structural defects and increased susceptibility to environmental

stress. Ergosterol is a sterol that plays a crucial role in maintaining the fluidity and integrity of fungal cell membranes. Punicalagins have been found to inhibit ergosterol biosynthesis by targeting key enzymes involved in the sterol biosynthetic pathway. This inhibition disrupts the fungal cell membrane, leading to increased permeability, leakage of cellular contents, and ultimately cell death. Squalene epoxidase is a key enzyme in the ergosterol biosynthetic pathway, catalyzing the conversion of squalene to squalene epoxide. Punicalagins have been shown to inhibit squalene epoxidase, leading to the accumulation of squalene and a decrease in ergosterol production. Lanosterol 14α-demethylase is another crucial enzyme in the ergosterol biosynthetic pathway, responsible for the removal of a methyl group from lanosterol. Punicalagins can inhibit lanosterol 14α-demethylase, leading to the accumulation of lanosterol and a decrease in ergosterol production. The inhibition of ergosterol biosynthesis disrupts the fungal cell membrane, leading to increased permeability and leakage of cellular contents. This disruption can trigger a cascade of events, including oxidative stress, mitochondrial dysfunction, and ultimately cell death. Pomegranate contains a variety of flavonoids, including quercetin, kaempferol, and anthocyanins. These compounds are known for their potent antioxidant, anti-inflammatory, and antimicrobial properties. Flavonoids have been shown to interfere with the formation of the fungal cell wall by inhibiting the enzymes involved in chitin and glucan synthesis. This disruption weakens the cell wall, making the fungal cell more susceptible to osmotic stress and lysis. Flavonoids can inhibit chitin synthase, a key enzyme involved in chitin production, leading to a decrease in chitin content in the fungal cell wall. Flavonoids can also inhibit glucan synthase, an enzyme responsible for glucan production, leading to a decrease in glucan content in the fungal cell wall. Flavonoids may act synergistically with punicalagins to disrupt the fungal cell wall by targeting different components and pathways involved in cell wall synthesis and assembly. Biofilms are complex communities of microorganisms, including *C. albicans*, that adhere to surfaces and are encased in a protective matrix. Biofilms are notoriously difficult to eradicate and contribute to the persistence of fungal infections. Flavonoids have demonstrated the ability to inhibit biofilm development by interfering with fungal adhesion, quorum sensing, and matrix production. Flavonoids can inhibit fungal adhesion by binding to fungal cell wall proteins, preventing their interaction with host cell receptors. Quorum sensing is a cell-to-cell communication system that allows bacteria to coordinate their behavior based on population density. Flavonoids can interfere with quorum sensing, disrupting the communication between fungal cells and inhibiting biofilm formation. The extracellular matrix is a key component of biofilms, providing structural integrity and protection to the microbial community. Flavonoids can inhibit matrix production by interfering with the synthesis and secretion of matrix components. Flavonoids are potent antioxidants that scavenge free radicals, protecting cells from oxidative damage. In the context of fungal infections, flavonoids can help reduce the inflammatory response and protect host tissues from damage caused by reactive oxygen species produced by both the fungus and the host immune system. Flavonoids can directly scavenge free radicals, neutralizing their harmful effects and preventing oxidative damage to cells and tissues. Flavonoids can also inhibit the activity of oxidative enzymes, such as NADPH oxidase and xanthine oxidase, which are involved in the production of reactive oxygen species. Flavonoids may also modulate host immune responses, enhancing the ability of the immune system to fight fungal infections. Tannins, particularly abundant in pomegranate peel, are polyphenolic compounds with astringent properties. Adhesion to host tissues or denture surfaces is a crucial step in the pathogenesis of *C. albicans* infections. Tannins have been shown to inhibit fungal adhesion by binding to fungal cell wall proteins, preventing their interaction with host cell receptors. Tannins can bind to various cell wall proteins, including adhesins,

which are responsible for fungal adhesion to host cells. This binding prevents the adhesins from interacting with their receptors, inhibiting fungal attachment. Fungal adhesion is also influenced by the hydrophobicity of the fungal cell surface. Tannins can alter the hydrophobicity of the fungal cell wall, making it less favorable for adhesion. Hyphal formation is a key virulence factor of *C. albicans*, allowing the fungus to invade tissues and evade host immune responses. Tannins have demonstrated the ability to inhibit hyphal formation by interfering with the signaling pathways that regulate hyphal development. The cAMP-PKA pathway is a key signaling pathway involved in hyphal formation. Tannins can inhibit this pathway by interfering with the activity of adenylate cyclase, an enzyme that produces cAMP. The MAPK pathway is another important signaling pathway involved in hyphal formation. Tannins can inhibit this pathway by interfering with the activity of MAP kinases, which are enzymes that phosphorylate and activate downstream targets involved in hyphal development. Tannins can also inhibit biofilm production by interfering with the formation of the extracellular matrix, a key component of biofilms that provides structural integrity and protection to the microbial community. The extracellular matrix is composed mainly of polysaccharides, such as glucans and mannans. Tannins can inhibit the synthesis and secretion of these polysaccharides, disrupting the formation of the matrix. The extracellular matrix also contains proteins that contribute to its structural integrity and function. Tannins can inhibit the production and activity of these proteins, further disrupting matrix formation. In addition to punicalagins, flavonoids, and tannins, pomegranate also contains other bioactive compounds that may contribute to its antifungal activity. Alkaloids are a diverse group of nitrogen-containing compounds with various pharmacological activities, including antimicrobial properties. Some alkaloids found in pomegranate have demonstrated antifungal activity against *C. albicans* by disrupting cell membrane integrity and inhibiting enzyme activity. Alkaloids can disrupt the integrity of the fungal cell membrane by interacting with its lipid bilayer. This disruption can lead to leakage of cellular contents and ultimately cell death. Alkaloids can also inhibit the activity of various enzymes essential for fungal growth and survival. For example, some alkaloids have been shown to inhibit the activity of chitin synthase and glucan synthase, further contributing to the disruption of the fungal cell wall. Terpenoids are a large class of organic compounds with diverse biological activities, including antimicrobial properties. Some terpenoids found in pomegranate have shown antifungal activity against *C. albicans* by disrupting cell membrane integrity and inhibiting ergosterol biosynthesis. Terpenoids can disrupt the integrity of the fungal cell membrane by interacting with its lipid bilayer, similar to alkaloids. This disruption can lead to leakage of cellular contents and ultimately cell death. Some terpenoids have been shown to inhibit ergosterol biosynthesis by targeting key enzymes involved in the sterol biosynthetic pathway. This inhibition disrupts the fungal cell membrane, leading to increased permeability, leakage of cellular contents, and ultimately cell death. Pomegranate contains various organic acids, such as citric acid, malic acid, and ascorbic acid. These acids can contribute to the antifungal activity of pomegranate extracts by lowering the pH of the environment, creating unfavorable conditions for fungal growth. Organic acids can lower the pH of the environment, making it less favorable for fungal growth. *C. albicans* thrives in a neutral to slightly alkaline pH range. Acidification of the environment can inhibit fungal growth and metabolism. Organic acids may also act synergistically with other bioactive compounds in pomegranate extracts to enhance their antifungal activity. For example, acidification of the environment can increase the permeability of the fungal cell wall, making it more susceptible to the action of punicalagins, flavonoids, and tannins. The various bioactive compounds present in pomegranate extracts likely act synergistically to exert their potent antifungal activity. This means that the combined effect of these compounds is greater than the sum of

their individual effects. Punicalagins and flavonoids may act synergistically to disrupt the fungal cell wall by targeting different components and pathways involved in cell wall synthesis and assembly. Punicalagins and flavonoids can both inhibit chitin and glucan synthesis, leading to a more pronounced disruption of the fungal cell wall. Punicalagins and flavonoids can also disrupt the assembly of cell wall components, further compromising the integrity of the cell wall. The different bioactive compounds may target multiple cellular processes and pathways essential for fungal growth and survival. This multi-target inhibition can overwhelm the fungal cell's defense mechanisms and lead to cell death. Punicalagins and terpenoids can both inhibit ergosterol biosynthesis, while punicalagins and flavonoids can both inhibit cell wall synthesis. This multi-target inhibition disrupts both the cell membrane and the cell wall, leading to a more effective antifungal action. Alkaloids can inhibit the activity of various enzymes essential for fungal growth and survival, while flavonoids can inhibit oxidative enzymes involved in the production of reactive oxygen species. This multi-target inhibition further disrupts fungal metabolism and growth. Some bioactive compounds in pomegranate extracts may also modulate host immune responses, enhancing the ability of the immune system to fight fungal infections. Some flavonoids and other bioactive compounds have been shown to stimulate the activity of immune cells, such as macrophages and neutrophils, which play a crucial role in eliminating fungal pathogens. Phagocytosis is the process by which immune cells engulf and destroy foreign particles, including fungal cells. Some bioactive compounds in pomegranate extracts may enhance phagocytosis, increasing the efficiency of fungal clearance. Cytokines are signaling molecules that regulate immune responses. Some bioactive compounds in pomegranate extracts may modulate cytokine production, promoting a more effective immune response against fungal infections. The peel extract, with its higher concentration of punicalagins and tannins, exhibited stronger antifungal activity compared to the fruit extract. This difference highlights the importance of considering the specific parts of the plant used for extraction when evaluating the therapeutic potential of natural products. However, both extracts demonstrated significant potential for inhibiting *C. albicans* growth and preventing denture stomatitis. The peel extract is particularly rich in punicalagins and tannins, which are potent antifungal agents. This makes the peel extract a promising candidate for the development of natural denture cleansers and other antifungal applications. Although the fruit extract has a lower concentration of punicalagins and tannins compared to the peel extract, it still contains a variety of bioactive compounds with antifungal activity. The fruit extract may be a more palatable option for oral applications, as it is less astringent than the peel extract.[11-16]

Sodium bicarbonate, commonly known as baking soda, has been a mainstay in denture cleansing solutions for decades due to its well-established antimicrobial properties. Its widespread use stems from its accessibility, affordability, and its ability to create an alkaline environment that is inhospitable to many microorganisms, including *C. albicans*. However, despite its popularity and efficacy, concerns linger regarding the potential long-term effects of sodium bicarbonate on both denture materials and oral tissues. This has led to a growing interest in exploring alternative denture cleansing agents, particularly those derived from natural sources, that offer comparable antifungal benefits without the potential drawbacks associated with sodium bicarbonate. The antimicrobial activity of sodium bicarbonate primarily stems from its ability to alkalize the environment. When dissolved in water, sodium bicarbonate dissociates into sodium ions (Na^+) and bicarbonate ions (HCO_3^-), leading to an increase in pH. This alkaline environment is unfavorable for the growth of many microorganisms, including *C. albicans*, which thrive in a neutral to slightly acidic pH range. The alkaline environment created by sodium bicarbonate can disrupt various cellular processes in microorganisms, including enzyme activity, nutrient

uptake, and cellular respiration. This disruption can lead to growth inhibition and ultimately cell death. Sodium bicarbonate has also been shown to inhibit the adhesion of *C. albicans* to denture surfaces. This inhibition can prevent biofilm formation, which is a major contributor to denture stomatitis and other oral infections. While sodium bicarbonate is generally safe and effective for denture cleansing, there are concerns regarding its potential long-term effects on denture materials and oral tissues. Prolonged exposure to sodium bicarbonate can increase the surface roughness of denture materials, particularly acrylic resins. This increased roughness can provide more sites for microbial adhesion and biofilm formation, potentially increasing the risk of denture stomatitis and other infections. Sodium bicarbonate can also affect the color stability of denture materials, causing discoloration and yellowing over time. This discoloration can be aesthetically unappealing and may require more frequent denture replacements. In some cases, prolonged exposure to sodium bicarbonate can lead to the degradation of denture materials, compromising their strength and durability. This degradation can shorten the lifespan of dentures and increase the need for repairs or replacements. Sodium bicarbonate can cause irritation and burning sensation in some individuals, particularly those with sensitive oral tissues. This discomfort can make denture wearing unpleasant and may discourage proper denture hygiene practices. The alkaline environment created by sodium bicarbonate can disrupt the natural balance of oral microflora, potentially leading to the overgrowth of opportunistic pathogens. This disruption can increase the risk of oral infections and other oral health problems. Although rare, excessive ingestion of sodium bicarbonate can lead to systemic effects, such as metabolic alkalosis and electrolyte imbalances. This is particularly concerning for individuals with kidney or heart conditions. In this study, the pomegranate peel extract exhibited comparable efficacy to sodium bicarbonate in reducing *C. albicans* colony counts. This finding suggests that pomegranate peel extract could be a promising natural alternative for denture hygiene maintenance, offering similar antifungal benefits without the potential drawbacks associated with sodium bicarbonate. Pomegranate peel extract is a natural product derived from a plant with a long history of safe use in traditional medicine and culinary practices. This natural origin makes it a more appealing option for individuals seeking natural oral health care solutions. Compared to sodium bicarbonate, pomegranate peel extract is likely to have lower toxicity and fewer side effects on oral tissues. This is particularly important for individuals with sensitive oral tissues or those who prefer natural oral health care products. Pomegranate peel extract has demonstrated activity against a wide range of microorganisms, including bacteria, fungi, and viruses. This broad-spectrum activity makes it potentially effective against various oral pathogens, not just *C. albicans*. Pomegranate peel extract is rich in antioxidants, which can help protect oral tissues from oxidative damage caused by free radicals. It also possesses anti-inflammatory properties, which may help reduce inflammation associated with denture stomatitis. Pomegranate peel extract is biodegradable and environmentally friendly, unlike sodium bicarbonate, which is a synthetic chemical.[17-20]

5. Conclusion

This study investigated the efficacy of red pomegranate (*Punica granatum* L.) peel and fruit extracts as natural denture cleansers compared to sodium bicarbonate in inhibiting *Candida albicans* growth on heat-cured acrylic resin. The results demonstrated that both pomegranate peel and fruit extracts significantly reduced *C. albicans* growth compared to the negative control ($p<0.05$). The peel extract showed comparable efficacy to sodium bicarbonate ($p>0.05$), while the fruit extract demonstrated slightly lower but still substantial antifungal activity. The potent antifungal activity of pomegranate extracts can be attributed to their rich phytochemical profile, including punicalagins, flavonoids, and tannins, which disrupt fungal cell wall

synthesis, inhibit ergosterol biosynthesis, and interfere with biofilm formation. The peel extract, with its higher concentration of punicalagins and tannins, exhibited stronger antifungal activity compared to the fruit extract. This study suggests that pomegranate peel and fruit extracts hold promise as natural denture cleansers for inhibiting *C. albicans* and potentially preventing denture stomatitis. Further research is warranted to evaluate their long-term effects on denture materials and clinical efficacy.

6. References

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