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Cordia Myxa Fruit Effect on Bacterial Adhesion to Heat-Cured Acrylic Denture Bases

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Abstract:

Maintaining clean dentures is crucial for the health of the patients and the dental personnels. This study investigated the antibacterial effect of Cordia Myxa Fruit (CMF) extract on *Bacillus subtilis* adhesion to heat-cured acrylic denture bases, comparing it to Glutaraldehyde 2%. Forty disc-shaped heat-cured acrylic specimens were divided into four groups: untreated control, Glutaraldehyde 2% immersion for 10 minutes, and in CMF extract at concentrations of 100 mg/ml and 150 mg/ml for 15 minutes. *Bacillus subtilis* adhesion was assessed by spectrophotometric analysis. Surface morphology was examined using scanning electron microscopy. Statistical analysis included one-way ANOVA and Tukey HSD, statistical significance established at $p < 0.05$. CMF extract significantly reduced bacterial adhesion compared to the untreated control ($p < 0.05$). The CMF extract of the 150 mg/ml group showed antibacterial effects comparable to Glutaraldehyde 2%, with no significant difference between them ($p > 0.05$). Scanning electron microscopy revealed that Glutaraldehyde treatment caused degradation, while CMF-treated specimens maintained surface integrity similar to untreated control. CMF extract demonstrated effective antibacterial activity against *Bacillus subtilis*, suggesting its potential as a natural, safer alternative to chemical disinfectants in dental practice.

Keywords : Denture base, Bacillus subtilis, Natural disinfectant, Heat cure acrylic, Cordia Myxa.

1. Introduction:

Heat-cured acrylic resin has been the most common material utilized for the fabrication of complete and partial dentures. Due to its favourable aesthetics, physical, and cost-benefit properties(Al-Rubaie & Al-Khafaji, 2024). However, one of the main challenging aspects associated with heat-cured acrylic resin is its susceptibility to microbial colonization on the surface (Olms et al,. 2018). Rough or uneven surface topography of dentures encourages the adhesion of microbes and debris; surface imperfections increase surface area. Numerous

microbial organisms are on the palatal side of the denture were identified in higher quantities than on the palate itself, indicating that the acrylic denture works as a storage site for infection (Onwubu et al,. 2017).

The production of dentures includes a multi-phase process comprising both laboratory and clinical operations. At these phases, dentures can have pathogenic and opportunistic bacteria, presenting a danger of cross-contamination (Glass et al., 2010). Insufficient disinfection may let pathogens to infiltrate the dental laboratory environment, jeopardizing the safety of patients, dental staff, and clinicians. Consequently, the implementation of appropriate infection prevention strategies is necessary to prevent the spread of disease. (Centers for Disease Control and Prevention, 2016). The guidelines for infection management in dentistry laboratories during the COVID-19 pandemic and subsequent normalization period indicate that disinfection via immersion is recommended for items that are unsuitable for sterilization (Basmacı et al., 2021).

Traditional recommended approaches to prevent cross-contamination involve the use of Glutaraldehyde 2% with the immersion technique. Glutaraldehyde is a high-level disinfectant utilized for heat-sensitive semicritical instruments, as advised by the Centers for Disease Control and Prevention (CDC), along with the Food and Drug Administration (FDA) (Kohn et al., 2003). This powerful, sporicidal compound is highly poisonous. Despite implementing appropriate procedures, such as container closure to minimize vapor release, chemical-resistant protective outfits, googles, and face shields, Glutaraldehyde-based medications can cause negative health effects, dermatological conditions, eye irritation, respiratory complications, and skin sensitization have been recorded along with, changes in the material's properties over time. Medical disposable gloves are ineffective barriers due to their inadequate chemical protection against glutaraldehydes. (Khattab & Nada, 2015; Kohn et al., 2003). As a result, there has been a growing interest in using natural antibacterial agents that are both effective and safe.

Medicinal plants are of significant value for medical care and pharmacology (Jamshidi-Kia et al., 2017). They have served as a crucial origion of cures for human illnesses since antiquity, providing a substantial a collection of bioactive substances with healing potential. In addition to supporting the formulation of many pharmaceutical pharmaceuticals, medicinal plants provide accessible and affordable therapy alternatives, especially in places with limited access to modern medicine.

Moreover, these plants are integral to traditional medical systems globally, safeguarding cultural knowledge and practices down through generations. Their importance transcends healing; they aid in preservation of biodiversity and ecological balance. Identifying and utilizing the benefits of therapeutic plants is essential for persistent healthcare, pharmaceutical development, and the conservation of cultural knowledge (Zahra et al,. 2024), (Jacob et al,. 2024).

Cordia Myxa Fruit (CMF), identified as "Bumber" in Iraq, is a medical plant known for its antimicrobial and anti-inflammatory qualities, has shown promise for pharmacological and

medical uses, demonstrating antibacterial properties in vitro (Al-Musawi et al., 2022; Alyamani et al., 2023).

It has been found to possess highly bioactive chemicals, serves as an expectorant and demulcent for respiratory tract infections, in addition to functioning as a diuretic and anti-diarrheal agent. Compounds extracted from the genus Cordia have been recognized for their antimicrobial, cytotoxic against tumor cells, anti-inflammatory, and free radical scavenging capabilities (Al-Musawi et al., 2022).

A study evaluated the bioactive components of CMF ethanol extract using GC-MS and HPLC analyses. GC-MS identified 19 major compounds, notably α-D-glucopyranoside and O-α-D-glucopyranosyl-(1→3)-β-D-fructofuranosyl, which were present in significant amounts and have been associated with antimicrobial activity. HPLC analysis further detected gallic acid, ferulic acid, chlorogenic acid, caffeic acid, and fumaric acid in the CMF extract, with gallic acid notably abundant and recognized for its antibacterial properties (Al-Musawi, et al., 2022).

Cordia Myxa Fruit extract had antimicrobial properties against *S. aureus*, *E. coli*, *S. enterica*, *B. subtilis*, and *P. aeruginosa*, with results indicating antibacterial activity against all pathogenic microorganisms at a concentration of 100 mg/mL(Al-Musawi et al., 2022).

Antibacterial wound dressing agent was synthetized utilizing CMF extract combined with polycaprolactone/chitosan (PCL/CH) nanofibers, which exhibited notable antibacterial efficacy against *S. aureus* at a concentration of 100 mg/mL, as well as against *E. coli*, *S. enterica*, *B. subtilis*, and *P. aeruginosa*, respectively (Alyamani et al., 2023).

The current research aims to assess the antibacterial efficacy of CMF extract solution against *Bacillus subtilis* bacteria by evaluating its effect on bacterial adhesion to heat-cured acrylic surface.

Null hypothesis: Immersion in CMF has no significant antibacterial effect against *Bacillus subtilis* bacteria compared to the control group; The alternative hypothesis is that immersion in CMF has a significant antibacterial effect against *Bacillus subtilis* bacteria compared to the control group.

2. Materials and Methods:

2.1 Preparation of Disinfectants:

This research was performed with two kinds of disinfectants: Glutaraldehyde 2% (M.P.C., Baghdad, Iraq), and the alcoholic extract of CMF at two concentrations, 100 and 150 mg /ml

2.2 CMF extract preparation:

The fruit was collected from a tree in the Al-Jamea neighborhood in Baghdad, Iraq. The fruit was identified by the Iraqi National Herbarium in Baghdad, Iraq, with certificate no.1743 on 18/3/2025 as Cordia Myxa L. Only physically intact ones were selected. The fruits were cleaned, the cleaning procedure was done by removing any dirt and extra genus materials, then washing them three times with running tap water, and once with distilled water (Al-Musawi et al., 2022),

and the seeds of the fruits were removed by pressing on them, then dried in the shade for two weeks. The fruits were weighed daily using an electronic scale (Adventurer, United states) during the drying process until a consistent weight was achieved for three consecutive days. Temperature and humidity were measured three times daily by a thermohygrometer (THLMT, China), and the average was 40°C at 25 % RH (Taha & Abass, 2025). Finally the dried fruit was ground into powder by using an electric blender (Kenwood, China). The ground powder was put on a sufficient length of medical gauze and sewn into closed thimbles, defatted with petroleum ether (40:60) (El-Massry et al, 2021).

The extraction was done using the Soxhlet method until exhaustion (Larki et al., 2020). The extract has been concentrated under reduced pressure using a rotary evaporator (ISO LAB, Germany), at 40-50°C to ensure the complete elimination of solvent, resulting in a viscous dark residue (Taha & Abass, 2025). The extract was stored within an opaque glass jar in the refrigerator to prevent decomposition (Larki et al., 2020). From this extract, concentrations of 100 and 150 mg/ml were prepared at the time of the experimental tests, by reconstituting the crude extract in non-ionized distilled water (AL-Rafidain Environment Office, Iraq) (Abdul-Ameer, 2020). Each concentration was weighed in a digital electronic balance in a separate beaker, then distilled water was added and stirred with a glass stirrer until completely dissolved.

2.3 Specimen Preparation:

A disc-shaped specimen measuring 5mm in diameter and 2mm in thickness, in accordance with the measurements of test tubes, was fabricated (Shakir & Abass, 2018). Plastic patterns were fabricated according to the given dimensions. A separating medium (Imicryl, Turkey) was used to coat the metallic dental flasks (Aixin, China). Molds were fabricated utilizing type III dental stone (Zhermack, Italy) following the conventional flasking procedure for removable dentures, which involved mixing of dental stone in accordance with the manufacturer's specifications, put into the lower section of the flask, and vibrated using a vibrator machine (VIBRAMAT, China) to get rid of the air bubbles. The patterns were then inserted into the stone before it sets, for their half thickness to have access for removing them after opening the flasks. When the stone material is completely set, the stone and plastic pattern surfaces were coated with a separating medium and left to dry. After that, the upper part of the flask was placed on its counterpart, filled with stone, vibrated, the flask cover was placed on top, and left aside until completely set. When the stone was set, the flask was meticulously opened to detach the two parts, and the patterns were taken out from the mold using a wax knife carefully. The resulting molds were covered with a separating agent and left for drying .

According to the instructions of the manufacturer, which involved adding 14 ml of liquid for every 34 g of powder, heat-cured acrylic material (SMD, Turkey) had been mixed. The packaging procedure commenced with the mix approached the dough stage. The resin was taken out from the jar, shaped into rolls, and subsequently placed into the mold that had been pre-treated with a separating agent. A polyethelyene sheet was placed between the two portions of

the flask and closed together (Al-Rubaie & Al-Khafaji, 2025). The flask parts were then gathered and put under a hydraulic press, which applied a gradual pressure of up to 20 bar to make sure that the acrylic was equally distributed inside the mold space. The flask was then opened to cut excess material using a sharp blade. The trials were accomplished by applying 100 bar pressure for five minutes to the flask.

For the curing process, the flask was firmly clamped by the flask clamp to be ready for transfer to a water bath. The flask clamp was placed into a digital water bath to undergo the curing process in compliance with the guidelines provided by the manufacturer, this involved placing the flasks in the boiling water and turning the heat source off for 15 min, then boiling for 20 min. The flasks were allowed to cool down slowly in the water bath after polymerization. Specimens were removed after deflasking (Mohammed & Farhan, 2024).

Specimens were finished to remove any excess material from the margins with the use of finishing burs (china), followed by verification of the specimen measurements with a vernier and a ruler. All specimens were polished (Excluding the surface roughness specimens) using a lathe polishing machine (Bego, Germany) with a brush disc and pumice slurry with water for approximately 2 minutes to achieve a lustreous finish. The finishing and polishing were performed at 1500 rpm speed and a minimal pressure to prevent thermal deformation. The specimens were then ultrasonically cleaned for 20 minutes (Al-Rubaie & Al-Khafaji, 2025). They were kept in distilled water for 48 hours at 37°C before the tests were established (Hussien, 2018).

2.4 Test Groups:

A total of 40 Heat-cured acrylic specimens were prepared, which were distributed to the following four test groups:

- The first control group (CG): without CMF immersion (no treatment).
- The second control group (GA): Immersion of heat-cured acrylic specimens in Glutaraldehyde 2% for 10 min.
- Test group (CMF100): Immersion of heat-cured acrylic specimens in CMF extract 100 mg/ml for 15 min.
- Test group (CMF150): Immersion of heat-cured acrylic specimens in CMF extract 150 mg/ml for 15 min.

2.5 Bacillus subtilis adhesion test:

Bacillus subtilis bacteria were aquired from the Scientific Research Commission / Center for Environment, Water, and Renewable Energy / Food Contamination Research Lab/ Baghdad /Iraq. Polymerase chain reaction (PCR) was performed to identify the bacteria genetically (Ghizzawi & AL, 2024), (Chang, Shangkuan et al,. 2003; Dabire et al,. 2018). All specimens were sterilized for 15 minutes at 121˚C and 15 Psi in an autoclave and kept in a sterile container until use (Khalaf et al,. 2023). The prepared extract solutions were disinfected using a sterile syringe filter with a size 0.2 µm for each concentration (Sirakov et al,. 2018).

On the first day, a small amount of *Bacillus subtilis* colonies were separately injected into 10 milliliters of sterilized Tryptic Soy Broth (TSB) and next aerobically incubated at 37ºC for 24 hours. The next day, the turbidity of the tubes was calibrated to match that of McFarland tube No. 5, corresponding to 10^7 organisms/ml. Under sterile conditions, 100 µl from the last test tube was inoculated within 10 ml of TSB. Then, each specimen was put separately inside the inoculation tubes then incubated at 37ºC for 24 hours in an aerobic environment (Mahmood et al,. 2017).

After the specimens were washed with saline, each specimen was immersed according to test group protocol in the specific disinfectant. Then, the specimens underwent washing and were stained with 1% crystal violet for 10 min (Noori et al,. 2023). They were completely rinsed in phosphate-buffered saline at the end of the process to remove all the excess stains, dried on filter paper, followed by immersion in three ml of 96% ethanol for three min. The alcohol solution was analyzed with a spectrophotometer at 600 nm (Noori et al., 2023). The optical density (OD) measured from the alcohol solution gives an indication of the adhered bacteria on each specimen.

2.6 *Scanning electron microscope (SEM):*

The SEM was applied to investigate the surface topography and morphology of the specimens treated with CMF extract solutions of concentrations 100 and 150 mg/ml for 15 min, along with the control and glutaraldehyde 25% for 10 min. One sample was chosen from each group.The acrylic resin specimens were sputter-coated with gold alloy to a thickness of roughly 5 nm, which enables the electrons to distribute evenly into the samples due to the nonconductive properties of the acrylic resin. A magnification of 500× was used (Ayaz & Ustun, 2020).

2.7 *Statistical analysis:*

Data were entered and analyzed using the SPSS program (IBM version 27). The mean and standard deviation of each group were calculated. One-way ANOVA and Tukey HSD were utilized to compare between groups. The level of statistical significance was set at $p < 0.05$.

3.Results:

3.1 *Bacillus subtilis adhesion test:*

Figure 1 shows that *Bacillus subtilis* adhesion decreases as the concentration of CMF increases. The highest mean was for CG (M=0.059), and the lowest mean was for both group GA (M=0.015) and group CMF1500 (M=0.015).

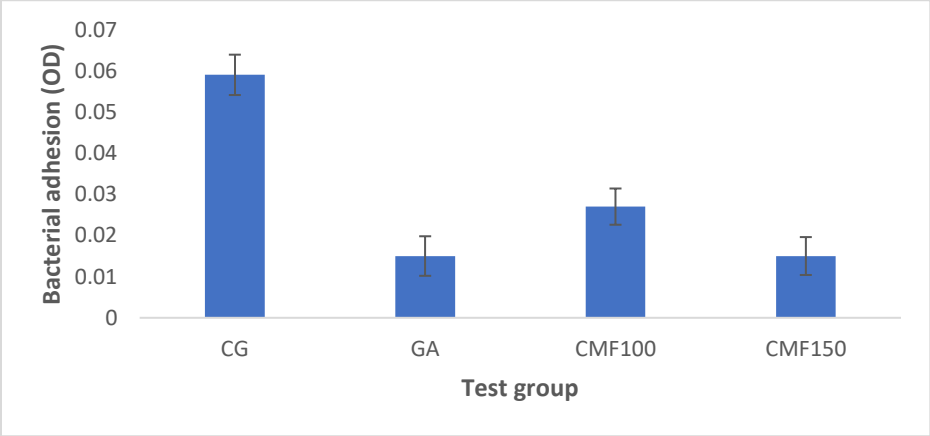

Figure 1: Means and standard deviations for *Bacillus subtilis* adhesion (OD)

One-way ANOVA analysis revealed a significant difference, $p<0.05$, between all test groups, Table 2.

Table 2: One-way ANOVA analysis for *Bacillus subtilis* adhesion (OD)

	Sum of Squares	Degree of freedom	Mean Square	F	*p*
Between Groups	.006	3	.002	96.497	.000 *
Within Groups	.000	16	.000		
Total	.007	19			

* Statistical significance is established at the 0.05 level

Tukey HSD analysis showed a significant change, $p<0.05$, between CG and all test groups; while comparing test groups with GA revealed that CMF150 had the same effect of GA, there was no significant change between them, $p>0.05$. Table 3.

Table 3: Tukey HSD post hoc comparison for the *Bacillus subtilis* adhesion (OD)

Test group		**Mean Difference**	***p*-value**
CG	**GA**	.0440	.000 *
	CMF100	.0314	.000 *
	CMF150	.0438	.000 *
GA	**CG**	-.0440	.000 *
	CMF100	-.0126	.003 *
	CMF150	-.0002	1.000

* Statistical significance is established at the 0.05 level

3.2 *Scanning electron microscopy analysis SEM*

The SEM analysis of heat-cured acrylic resin specimens after immersion in Glutaraldehyde 2% revealed a noticeable surface degradation, shown as numerous tiny scratches when compared to the first control group (without treatment). Treated specimens with Glutaraldehyde 2% exhibited an increase in surface roughness; these morphological changes suggest that exposure to Glutaraldehyde 2% may compromise the physical integrity of the material, potentially affecting its clinical durability and aesthetic performance. while after immersion in CMF extract, there was no morphological change when compared with the first control group. Figure 2.

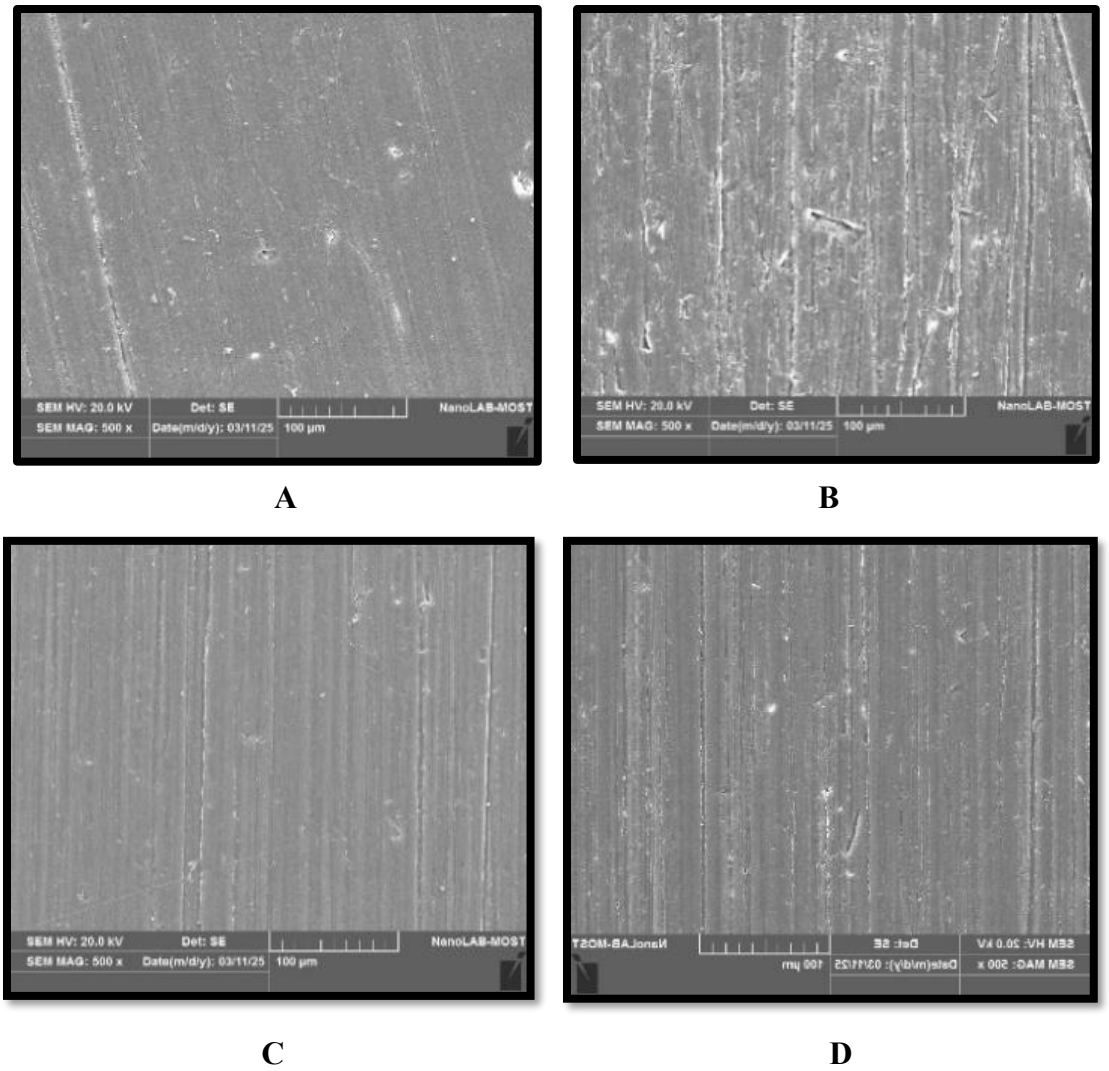

Figure 2: Scanning Electron Microscope pictures at 500x magnification. (A) For the first control group CG(-ve). (B) For the second control group GA(+ve). (C) for the CMF100 test group. (D) for the CMF150 test group

4.Discussion:

4.1 ***Bacillus subtilis adhesion test:***

According to the Centers for Disease Control and Prevention (2016), *Bacillus subtilis* was employed as a biological indicator for successful disinfection (Centers for Disease Control and Prevention, 2016). *Bacillus subtilis* are capable of converting into spores to withstand extreme circumstances. Bacterial spore formation is a survival strategy that allows bacteria to endure extreme environmental challenges such as elevated temperatures, low humidity, radiation, and chemical disinfectants. Despite remaining dormant for prolonged durations, the spores can reactivate and rapidly differentiate into actively growing cells (Zhang et al, 2020).

This test showed antibacterial efficiency for CMF extract at a concentration of 150 mg/ml for 15 min, against *Bacillus subtilis* bacteria, which had the same effect as Glutaraldehyde 2% disinfectant. So, the null hypothesis was dismissed while the alternative hypothesis was confirmed.

This may be attributed to the bioactive compounds in the CMF extract, which may inhibit bacterial adhesion on the surface of heat-cured acrylic after immersion by formation of antibacterial biofilm on acrylic surface after immersion, a thin layer of bioactive compounds may be absorbed on surface, creating a protective film (Cowan, 1999; Zahra et al,. 2024). CMF extract modulates bacterial hydrophobicity and attachment to surfaces. The reductions in hydrophobicity and adhesion have been related to the degradation of bacterial attachment structures, such as pili (which exhibit high hydrophobicity), by substances like tannins(Hui & Dykes, 2012; Voravuthikunchai & Suwalak, 2009). It was also reported that polyphenolic constituents in the extract possess anti-glucosyltransferase activities, which may hinder the development of sticky, insoluble glucan, a substance that facilitates bacteria's solid adhesion to surfaces (Nostro et al., 2004).

This agrees with (Al-Musawi et al., 2022), (Alyamani et al., 2023), and (Zahra et al,. 2024) results.

4.2 *Scanning electron microscope analysis SEM:*

Scanning Electron Microscopy (SEM) is widely employed in dental materials research. SEM provides detailed insights into surface morphology, roughness, and the effects of various treatments on these materials(115). The SEM is capable of producing highly detailed visual images of particles with outstanding detail and resolution (116).The SEM image of the control group displays a relatively smooth and intact surface, characteristic of polished heat-cured polymethyl methacrylate (PMMA) resin. No significant irregularities, cracks, or erosions are observed, confirming the inert nature of distilled water in preserving the surface morphology of PMMA. This observation was aligned with findings that water immersion alone had a negligible impact on the surface degradation of acrylics (Costa et al., 2021).

While immersion of heat-cured acrylic specimens in Glutaraldehyde 2%, this group exhibits a noticeably rougher surface with evident surface irregularities and micro-pitting. Glutaraldehyde, a potent chemical disinfectant, can interact with the PMMA matrix, potentially leading to degradation and increased surface roughness. Recent studies report that chemical disinfectants, especially aldehydes, may compromise the mechanical and surface properties of acrylic resins after repeated or prolonged exposure (Costa et al., 2021; Yarar et al,. 2023).

After immersion in CMF extract concentrations 100 and 150 mg/ml, the surface topography appears smoother than the Glutaraldehyde-treated group and relatively comparable to the control. Only minor textural changes were visible. This suggests that the CMF extract at these concentrations does not significantly affect the structural integrity of the acrylic surface reducing the chance of bacterial adhesion on surface and biofilm proliferation (Onwubu et al,. 2017). The

plant's mucilaginous and antioxidant-rich phytochemical composition may offer protective effects rather than chemical degradation (Gad et al,. 2020). While direct SEM studies on CMF were scarce, natural plant-based antimicrobials have shown promise in maintaining the surface properties of PMMA (Fathy et al,. 2023).

Conclusion: Immersion in CMF extract exhibited significant antibacterial properties, with potential for use as a natural disinfectant in dental care clinics and laboratories, instead of toxic chemical disinfectants.

Recommendations: It is recommended that future studies may involve:

1. Testing the antimicrobial efficiency against *Candida albicans, Staphylococcus aureus, and Pseudomonas aeruginosa.*
2. Evaluating the long-term immersion effect of CMF extract on heat-cured acrylic properties.
3. Examining the efficiency of spraying and incorporating CMF extract as disinfection techniques.
4. Testing the antibacterial effeciency of CMF extract on other prosthetic materials such as: soft liner, gypsum products, and impression materials.

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Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflicts of Interest: The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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