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Original article

Antibacterial properties of herbal denture cleanser based on *Graptophyllum pictum L. Griff.* leaves

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ABSTRACT

Background: *Removable partial dentures (RPDs) are one therapeutic option for tooth loss replacement. Despite its high absorption and porous qualities, acrylic resin—which is frequently used as the base material for RPDs—may encourage the growth of microorganisms and the development of plaque. Graptophyllum pictum L. Griff (G. pictum) has been commonly used in herbal medicine due to its pharmacological effects, which include immunomodulatory, antioxidant, anti-inflammatory, antibacterial, and antifungal activities.* ***Purpose:*** *This study aims to assess the antibacterial properties of an herbal denture cleanser based on G. pictum in effervescent granule against Porphyromonas gingivalis (Pg) and Aggregatibacter actinomycetemcomitans (Aa).* ***Methods:*** *The antibacterial activity was examined using the spread plate method at different time points. The number of Aa and Pg bacteria was determined using colony-forming units (CFUs) and bactericidal ratio (BR%). The statistical analysis employed the Kruskal–Wallis and post-hoc Mann–Whitney U tests ($P < 0.05$).* ***Results:*** *The results indicate that the herbal cleanser's antibacterial effectiveness against Aa and Pg increased significantly over time, reaching its optimal level after a 15-minute soaking period, as evidenced by the CFUs and BR%.* ***Conclusion:*** *The herbal denture cleanser based on G. pictum demonstrated antibacterial properties against Pg and Aa, which are significant pathogens in oral infections. These results indicate that this herbal formulation has potential as an effective alternative for future denture cleaning applications.*

Keywords: *antibacterial; denture cleanser; Graptophyllum pictum L. Griff; herbal; medicine*
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INTRODUCTION

Dentures are a viable treatment option for individuals with partial or complete edentulism. They effectively restore both functionality and aesthetics for those affected by tooth loss.[1] When individuals have a limited number of remaining teeth or encounter financial constraints, the most suitable treatment is removable partial dentures (RPDs). Polymethylmethacrylate (acrylic) has been the most widely used material for the base of RPDs due to its excellent mechanical, physical, and aesthetic characteristics, as well as its ease of manipulation and low-cost production.[2] However, due to its high absorption and porous nature, acrylic can facilitate the accumulation of microorganisms and plaque formation, potentially leading to oral health complications.[3] This issue is exacerbated when denture wearers do not adhere to recommended hygiene practices and ignore denture cleanliness.[1]

In a healthy individual, *Actinomyces* was the most prevalent bacterial genus found on both dentures and remaining teeth, followed by *Streptococcus*, *Veillonella*, *Capnocytophaga*, *Neisseria*, *Prevotella*, and *Corynebacterium*.[4] In contrast, phylotype-level analysis indicated that *Porphyromonas* predominated in the microbiome of dentures from patients with stomatitis.[5] This bacterial accumulation in a denture is associated with several microbial diseases, including denture-related mucosal tissue inflammation, also known as denture stomatitis, and oral malodor.[1,5] Additionally, dentures may increase the risk of dental disease such as caries, periodontal disease, and pulmonary infections.[4]

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Proper denture cleaning is vital for maintaining oral health and prolonging the lifespan of prostheses. Cleaning methods generally fall into three categories: mechanical, chemical, or combination. Mechanical cleaning, primarily through brushing, is the most common method due to its cost-effectiveness and simplicity. However, brushing technique is crucial to this method's long-term efficacy. Abrasive dentifrices used in brushing can potentially damage dentures, underscoring the importance of selecting a suitable denture cleanser.[6] An ideal denture cleanser should exhibit several key characteristics: it should have effective disinfection, be non-toxic and non-irritating, remain stable during storage, be easy to use, and be compatible with all denture materials. Additionally, it should possess bactericidal and fungicidal properties and avoid causing changes in the denture's physical and mechanical properties, such as color or dimension. Cost-effectiveness is another significant consideration.[3,6,7] Commercial denture cleansers are predominantly chemical-based and include categories such as alkaline peroxides (e.g., perborate or percarbonate), dilute organic or inorganic acids, alkaline hypochlorite, and enzymatic solutions. Chemical-based denture cleansers, especially in effervescent form, are recognized for their high efficacy in maintaining denture hygiene and ease of use. Despite their effectiveness, these chemical agents can sometimes increase surface roughness and acrylic resin denture base discoloration.[8] Consequently, herbal-based denture cleansers have gained considerable interest as a possible alternative, given their accessibility and potentially safer profile compared to chemical-based denture cleanser solutions.[3]

Graptophyllum pictum L. Griff (G. pictum) is a member of the Acanthaceae family. It is widely recognized for its medicinal properties and is commonly referred to as "purple leaves" in Indonesia.[3,9] The bioactive compounds in *G. pictum*, including alkaloids, flavonoids, saponins, and tannins, contribute to its antibacterial efficacy.[10] Studies have demonstrated its antibacterial activity against pathogens such *as Streptococcus mutans, Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa,* and *Porphyromonas gingivalis* (Pg).[3,10–13] However, the potential application of *G. pictum* in effervescent granule formulations as a denture cleanser has not been thoroughly explored. This study aims to assess the antibacterial properties of an herbal denture cleanser based on *G. pictum* in effervescent granules against Pg and *Aggregatibacter actinomycetemcomitans* (Aa).

MATERIALS AND METHODS

This study was an experimental laboratory design using a "post-test only" control group approach. The Ethics Committee of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia, granted ethical approval for this research under reference number 063/HRECC/FODM/VII/2024. The samples of this experimental study were divided into five groups: a negative group, a positive group, and three intervention groups. Each group contained three samples arranged into a group with a random allocation technique to minimize bias.

The *G. pictum* leaves were acquired from the UPT Herbal Materia Medica Laboratory in Batu, Malang, Indonesia. The extraction process entailed immersing 150 grams of simplicia powder in 1,500 mL of 70% ethanol for a duration of 24 hours. Subsequently, the extract underwent filtration using Whatman No. 41 filter paper, and the remaining material was subjected to re-maceration until a clear solution was found. Evaporation was carried out at 60°C using a rotary vacuum evaporator. A 50% extract of *G. pictum* leaves was used in this study, in line with preliminary results.[14]

Effervescent granule formulations were prepared using a 50% *G. pictum* leaf extract via the wet granulation method. The alkaline granules were produced by combining 300 g of sodium bicarbonate, 17.5 mL of alcohol, 2 g of menthol, 1.8 g of lactose, 5 g of polyvinylpyrrolidone K-30 (PVP K-30), and 35 g of the 50% *G. pictum* leaf extract. The acidic granules were formulated by mixing 5 g of PVP K-30, 100 g of citric acid, 200 g of tartaric acid, 2 g of menthol, 1.8 g of lactose, and 35 g of the 50% *G. pictum* extract. Both alkaline and acidic granules were dried in an oven. Once dried, the two granules were mixed to form the final effervescent granule preparation.[15]

The acrylic resin plate was prepared using heat-cured QC-20 (Dentsply®, England). The resin was molded into rectangular specimens with dimensions of 8 × 8 × 2 mm, following standard procedures for material preparation. These samples were then subjected to the appropriate curing process to ensure the material's properties were fully developed for further testing and analysis. This acrylic resin plate was used to imitate the acrylic base for RPDs. [15]

Porphyromonas gingivalis (ATCC 33277) was grown in tryptic soy broth medium under anaerobic conditions at 37°C for 18 to 24 hours. Bacterial colonies were collected using a sterilized loop, transferred into 3 mL of brain heart infusion (BHI) liquid medium, and incubated at 37°C for an additional 18 hours. The bacterial suspension was subsequently standardized to match the 0.5 McFarland standard (1.5×10^8 CFU/mL). Likewise, Aa (ATCC 43718) was incubated at 37°C for 24 hours under anaerobic conditions in BHI medium. The bacterial sample was retrieved from stock cultures using a sterile loop. The bacterial concentration was then adjusted to the 0.5 McFarland standard, equating to roughly 1.5×10^8 CFU/mL.[16]

The acrylic resin plate was treated in five groups: a negative control group, where samples were immersed in aquadest for 5 minutes; a positive control group, where samples were immersed in a sodium bicarbonate solution (Polident effervescent tablet) for 5 minutes according to manufacturer instructions; and three experimental groups, where the acrylic samples were immersed in solutions of

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effervescent granules solution based on *G. pictum* (3 g of effervescent granules and 100 ml water) for 5, 10, and 15 minutes, respectively. After the immersion, 0.1 mL of bacterial suspension was spread onto Muller–Hinton agar plates. The plates were incubated for 24 hours at 37°C. The antibacterial activity was assessed using the spread plate method, and bacterial colonies were counted as colony-forming units (CFUs). The bactericidal ratio (BR%) was calculated using the following equation (BR% equation):[17]

$$BR\% = \frac{CFUs_{(1)} - CFUs_{(2)}}{CFUs_{(1)}} \times 100$$

The bactericidal ratio (BR%) was calculated to evaluate the antibacterial activity of the samples. In this calculation, $CFUs_{(1)}$ represents the number of colonies in the control group (CFUs/mL), while $CFUs_{(2)}$ refers to the number of colonies in the analyzed sample (CFUs/mL). All experiments were performed in triplicate to ensure reproducibility, and the results are presented as means ± standard deviations. This approach ensures accuracy and reliability in assessing the antibacterial efficacy of the different treatment groups.[17]

Statistical analysis was conducted on data that had been collected and processed using SPSS software (IBM, USA). The Shapiro–Wilk test ($P > 0.05$) was employed to assess the normality of the data, while the Levene test ($P > 0.05$) was used to evaluate the homogeneity of variances. As the data did not meet the assumptions of normality and homogeneity, the Kruskal–Wallis test ($P < 0.05$), a non-parametric alternative, was applied to compare the groups. Post-hoc analyses were then performed using the Mann–Whitney U test, with statistical significance at $P < 0.05$.

RESULTS

Graptophyllum pictum, commonly known for its distinct purple pigmentation in its stems and leaves, exhibits a strong natural coloration (Figure 1A). However, after the preparation of effervescent granules from *G. pictum*, the characteristic purple color is no longer observed. Instead, the granules undergo a significant color shift, transitioning to a brownish-gold (Figure 1B). Upon dissolution in water, the effervescent granules maintained a light brown coloration, with no indication of the original purple pigment (Figure 1C). This color change suggests that the formulation process may affect the stability or preservation of the plant's natural pigments, which could have important implications for the visual consistency and potential efficacy of phytopharmaceutical products.

In the antibacterial test, bacterial growth on the medium was visually identified by white-yellowish colonies. To ensure the accuracy and reliability of the antibacterial test results, CFUs were counted independently by three observers. This multi-observer approach was implemented to minimize subjectivity and enhance the precision of the CFU quantification. Furthermore, each sample was tested in triplicate, allowing for statistical validation and reproducibility of the findings.

In the antibacterial test against Pg, the positive control group demonstrated complete inhibition of bacterial growth, as evidenced by the absence of detectable CFUs. Conversely, the negative control group exhibited the highest CFUs, reflecting unimpeded bacterial proliferation. Post-hoc analysis using the Mann–Whitney test further indicated no statistically significant difference between the negative control and the samples immersed in the *G. pictum* effervescent solution for 5 and 10 minutes ($P > 0.05$), suggesting limited antibacterial effect at these time intervals. However, the 15-minute immersion in the *G. pictum* effervescent solution showed no significant difference compared to the positive control ($P > 0.05$), indicating that extended exposure to the solution produces a comparable antibacterial effect to the standard positive control (Figure 2A–B). The bactericidal ratio indicated that the potency of the antibacterial activity increased with prolonged immersion time in *G. pictum* effervescence solution. Moreover, the highest antibacterial activity was observed in the 15-minute immersion of acrylic in the *G. pictum* effervescent solution ($P < 0.05$), which showed no



Figure 1. Visual representation of *Graptophyllum pictum L. Griff (G. pictum)* and its effervescent granule formulation. The natural form of *G. pictum* showcases its characteristic vibrant purple pigmentation in the leaves and stems (A). Effervescent granules derived from *G. pictum* exhibit a brownish-gold color after preparation (B). Effervescent granules dissolved in warm water result in a light brown solution (C).

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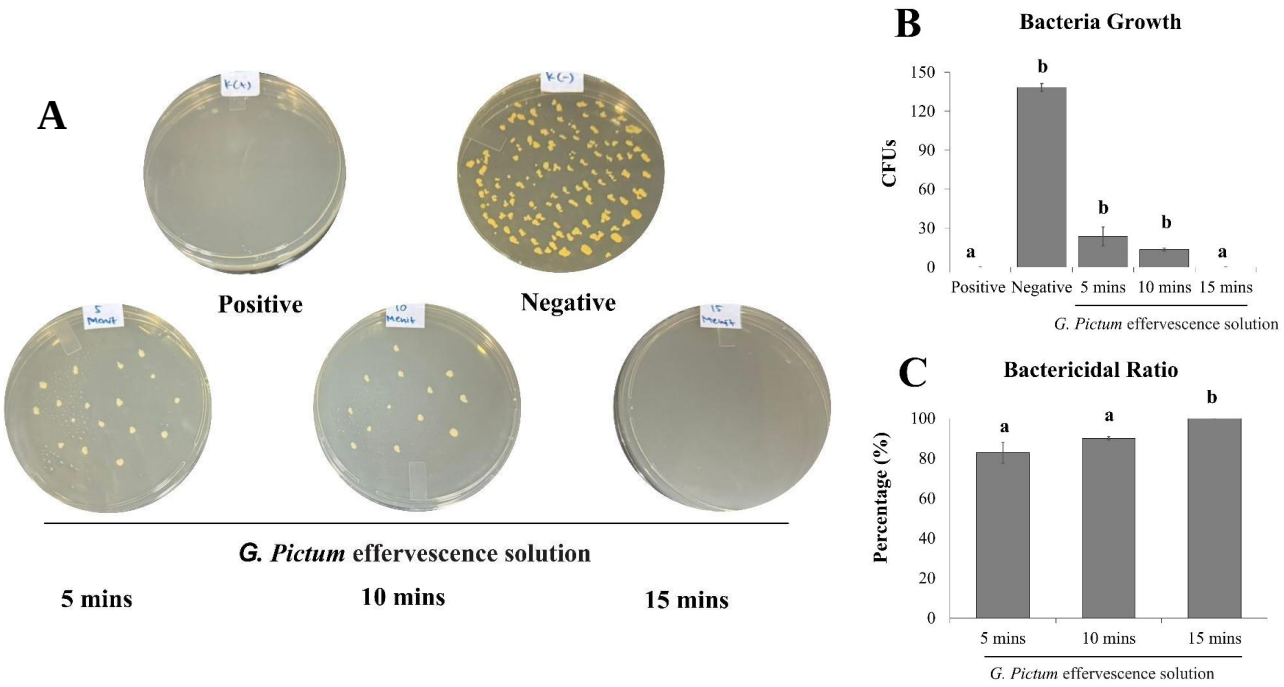


Figure 2. Antibacterial efficacy of herbal-based denture cleanser based on *Graptophyllum pictum L. Griff (G. pictum)* against *Porphyromonas gingivalis*. Visual representation of bacterial growth on the media across different experimental groups, with bacterial colonies identified by their whitish-yellow coloration (A). Quantification of bacterial growth in terms of colony-forming units (CFUs) (B). Bactericidal ratio for groups immersed in *G. pictum* effervescent solution for 5, 10, and 15 minutes (C). Different letters denote statistically significant differences ($P < 0.05$, post-hoc Mann–Whitney test).

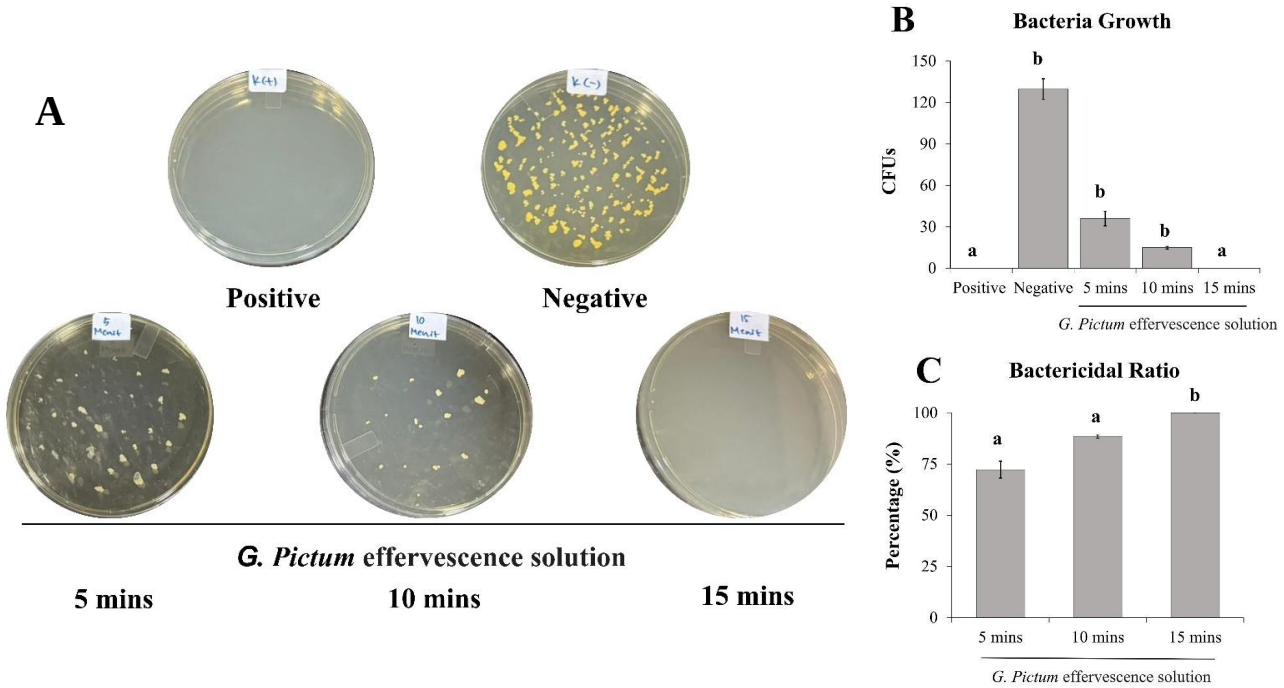


Figure 3. Antibacterial efficacy of herbal-based denture cleanser based on *Graptophyllum pictum L. Griff (G. pictum)* against *Aggregatibacter actinomycetemcomitans*. Visual representation of bacterial growth on the media across different experimental groups, with bacterial colonies identified by their white-yellowish coloration (A). Quantification of bacterial growth in terms of colony-forming units (CFUs) (B). Bactericidal ratio for groups immersed in *G. pictum* effervescent solution for 5, 10, and 15 minutes. Different letters denote statistically significant differences ($P < 0.05$, post-hoc Mann–Whitney test).

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significant difference from the positive control, suggesting comparable antibacterial effectiveness (Figure 2C).

The antibacterial testing using Aa yielded results similar to those observed with Pg. Analysis revealed no significant difference between the negative control and acrylic samples immersed in the effervescent solution based on *G. pictum* for 5 and 10 minutes ($P > 0.05$). However, immersion for 15 minutes produced results comparable to those of the positive control, demonstrating a notable increase in antibacterial efficacy ($P > 0.05$) (Figure 3A–B). The bactericidal ratio further supported this observation, indicating that the highest level of antibacterial activity was achieved with the 15-minute immersion ($P < 0.05$) (Figure 3C). These findings suggest that extended exposure to the *G. pictum* effervescent granule enhances its antibacterial potency, aligning with the results in the Pg.

DISCUSSION

Many denture wearers struggle with maintaining proper hygiene due to a lack of understanding about effective cleaning techniques and the absence of standardized denture hygiene assessments. This gap in knowledge and evaluation can result in inadequate cleaning, leading to increased bacterial accumulation. Furthermore, dentists often do not regularly assess or document the condition of patients' dentures, which can result in undetected hygiene issues. Poor denture hygiene negatively impacts the clinical longevity of dentures and promotes plaque aggregation. Effective denture cleansers with strong antibacterial properties are essential for preventing the development of dental caries, periodontal disease, denture stomatitis, and halitosis by ensuring that harmful bacteria are adequately removed.[18]

This study showed the antibacterial effectiveness of an herbal denture cleanser based on *G. pictum* formulated as effervescent granules against Pg and Aa. Effervescent granules contain oxidants, such as sodium bicarbonate, which release carbon dioxide bubbles upon dissociation in water. Additionally, sodium lauryl sulfate, a commonly added detergent, helps disrupt biofilms and enhance the cleaning efficacy of the oxidants as shown in our positive control.[18] Our study incorporated sodium bicarbonate with a 50% extract of *G. pictum* due to its antibacterial potency. The immersion time for acrylic in the *G. pictum* herbal-based denture cleanser was determined based on the optimal bactericidal activity observed with the positive control: Polident effervescent tablets, which effectively eliminated all bacteria within 5 minutes.[19] In contrast, previous studies have shown that herbal-based denture cleansers require longer exposure (15 minutes) to achieve similar antibacterial effects.[20] Therefore, the extended immersion period for the herbal cleanser was considered necessary to ensure its efficacy.

The effectiveness of *G. pictum* antibacterial properties is closely linked to its phytochemical composition. It has been reported that *G. pictum* contains numerous bioactive compounds with significant antibacterial activity, including flavonoids, alkaloids, polyphenol, triterpenoids, tannins, and saponins.[3,12,14,21] These compounds contribute to *G. pictum*'s potential as a natural alternative for denture cleansers, offering a broad spectrum of antibacterial activity against various pathogens. Alkaloids, polyphenols, triterpenoids, tannins, and saponins in *G. pictum* exhibit antibacterial properties through various mechanisms. Alkaloids damage the peptidoglycan layer of bacterial cell walls, interfere with nucleic acid and protein synthesis, alter membrane permeability, and inhibit efflux pumps, leading to bacterial cell death. Polyphenols disrupt bacterial membranes, causing cell lysis, denaturing proteins, and inhibiting essential cellular processes. Triterpenoids, as lipophilic compounds, bind to bacterial membranes, reducing permeability and leading to leakage of vital components. Tannins disrupt bacterial membranes, contract cell walls, and coagulate protoplasm, inhibiting bacterial growth. Saponins contribute by forming foam-like structures that disrupt bacterial cell walls. The combined action of these bioactive compounds enhances the antibacterial efficacy of *G. pictum*, making it a promising natural antibacterial agent for clinical and pharmaceutical applications.[22,23]

Our study demonstrated that the antibacterial efficacy of a denture cleanser based on *G. pictum* improved with prolonged immersion time, reinforcing the importance of contact duration in achieving optimal bacterial reduction. Specifically, the 5- and 10-minute immersion periods were notably less effective in reducing bacterial load compared to the 15-minute immersion, which emerged as the optimal duration. At 15 minutes, the denture cleanser exhibited bactericidal activity comparable to the positive control.[20] This study suggests that a longer exposure period enhances the antimicrobial action of *G. pictum*. This aligns with previous literature indicating that herbal-based cleansers generally require extended immersion times to achieve effectiveness due to their natural bioactive compounds, which act more gradually than synthetic chemical agents. Our findings further confirm that the denture cleanser based on *G. pictum* similarly required an extended immersion period compared to chemical denture cleansers, such as effervescent tablets, to reach comparable levels of bacterial reduction. This highlights the potential of *G. pictum* as a natural antibacterial agent while emphasizing the need for sufficient immersion time to maximize its efficacy. Future studies could explore whether further extending the immersion period beyond 15 minutes provides additional benefits or if saturation of antibacterial effects occurs at this threshold.

The antibacterial efficacy of herbal materials often varies between gram-negative and gram-positive bacteria due to their distinct cell wall structures, which influence their susceptibility to antimicrobial agents. Gram-negative bacteria have a more complex outer structure, consisting of an additional outer membrane, a thin peptidoglycan layer, and an inner membrane, which serves as a protective

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barrier against many antibacterial agents. In contrast, gram-positive bacteria lack this outer membrane but possess a thicker peptidoglycan layer, which makes them more susceptible to certain antibacterial compounds. Previous studies have suggested that *G. pictum* extract exhibits greater antibacterial activity against gram-positive bacteria, likely due to its ability to disrupt peptidoglycan synthesis and cellular integrity more effectively in these strains.[13] However, in contrast to these findings, our study demonstrated that the denture cleanser based on *G. pictum* is also highly effective against Pg and Aa, both of which are gram-negative bacteria. Notably, the antibacterial activity of the *G. pictum* denture cleanser was comparable to that of the positive control, highlighting its strong efficacy against gram-negative pathogens. This suggests that *G. pictum* may have broader antimicrobial potential beyond previously reported gram-positive specificity. The ability of *G. pictum* to effectively target gram-negative bacteria could be attributed to multiple bioactive compounds within the extract, which may disrupt membrane permeability, interfere with essential bacterial metabolic processes, or inhibit efflux pumps that typically confer resistance. These findings expand the potential applications of G. *pictum*-based denture cleansers and highlight their promise as a natural alternative to chemical disinfectants in maintaining oral hygiene, particularly for individuals at risk of bacterial infections associated with dental prostheses. Further research is warranted to explore the specific mechanisms underlying *G. pictum's* broad-spectrum antibacterial activity and to determine whether formulation modification could enhance its efficacy against an even wider range of bacterial strains.

Although this study successfully demonstrated the antibacterial potential of a denture cleanser based on *G. pictum*, it did not fully elucidate the cellular mechanisms underlying its antibacterial efficacy. A more detailed investigation into the molecular pathways and interactions between *G. pictum* components and bacterial cells is needed to provide a comprehensive understanding of its mechanism of action. Additionally, the scope of this study was limited to acrylic denture materials, excluding other commonly used dental materials. Future research should explore the efficacy of this herbal cleanser across a broader range of prosthetic materials. Moreover, this study focused on a single bacterial species, leaving the effectiveness of *G. pictum* against other relevant oral pathogens unexplored such as *Treponema denticola, Prevotella intermedia*, and *Fusobacterium Nucleatum*. Despite these limitations, this study represents a significant advancement, as it is the first to report the antibacterial properties of a denture cleanser based on *G. pictum* formulated as an effervescent granule. Denture cleanser based on *G. pictum* has demonstrated antibacterial properties against Pg and Aa, which are significant pathogens in oral infections. These results suggest that this herbal formulation has the potential to serve as an effective alternative for future denture cleaning applications.

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