

Physicochemical Characterization and Antibacterial Efficacy of Toothpaste Formulations Containing Arabica Coffee Husk Ethanol Extract Against *Streptococcus mutans* and *Staphylococcus aureus*

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Abstract

Background: Coffee husk, a major by-product of the coffee industry, is rich in phenolics, chlorogenic acid, and caffeine with known antioxidant and antimicrobial activities. This study evaluated Arabica coffee husk extract as an active ingredient in herbal toothpaste formulations, focusing on stability and antibacterial efficacy. Oral health problems, particularly dental caries, remain a global concern, affecting 2.3 billion people in permanent teeth and over 530 million children in primary teeth. *Streptococcus mutans* is the main cariogenic bacterium, while *Staphylococcus aureus* also contributes to oral infections. Safe, effective, and natural antimicrobial agents are urgently needed for prevention. **Methodology:** Arabica coffee husk powder was characterized Physicochemical according to the Indonesian Materia Medica (MMI). Toothpaste formulations containing 1%, 3%, and 5% w/w ethanol extract were prepared and tested for organoleptic properties, homogeneity, and stability over three weeks at room temperature. Stability was assessed weekly by monitoring pH, viscosity, temperature, phase separation, and color changes. Antibacterial activity against *S. mutans* and *S. aureus* was evaluated using the agar well diffusion method. All treatment were conducted in triplicate ($n = 3$). **Findings:** Phytochemical evaluation confirmed compliance with MMI standards. All formulations remained stable in color, odor, and consistency, with no phase separation or microbial contamination. Antibacterial assays showed concentration-dependent inhibition: the 5% extract (F3) produced inhibition zones of 18.2 ± 0.5 mm against *S. mutans* and 15.6 ± 0.7 mm against *S. aureus*. The negative control (without extract) showed no inhibition, while the positive control (chlorhexidine) produced clear zones. **Contribution:** Arabica coffee husk extract can be formulated into stable toothpaste with significant antibacterial activity, particularly against *S. mutans*, supporting its potential as a sustainable natural oral care agent.

Keywords: Antibacterial Activity; Coffee Husk Extract; Herbal Toothpaste; Stability Study; *Streptococcus mutans*



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INTRODUCTION

Indonesia is one of the world's largest producers of coffee, particularly Arabica, which generates substantial agricultural by-products in the form of coffee husks. In parallel, domestic coffee consumption has shown an increasing trend, from approximately 4.45 million bags (267,000 tons) in 2020/2021 to 4.79 million bags (287,100 tons) in 2023/2024 ([International Coffee Organization \[ICO\], 2024](#)). This continuous growth not only reflects rising demand but also underscores the increasing volume of waste produced, thereby emphasizing the urgency of developing innovative and sustainable strategies for its utilization. Coffee husks contain a wide range of bioactive compounds including polyphenols, flavonoids, tannins, and caffeine, all of which have demonstrated antibacterial and antioxidant activities. Previous studies have reported their effectiveness against oral pathogens such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum* associated with halitosis, as well as *Streptococcus mutans*, the primary cariogenic bacterium responsible for dental caries ([Frascareli et al., 2011](#); [Kim et al., 2024](#)). These findings provide a strong rationale for considering coffee husk extract as a natural active ingredient for oral health formulations.

Conventional toothpaste formulations, however, frequently include synthetic chemical agents such as triclosan and sodium lauryl sulfate (SLS). While effective, long-term exposure to these compounds has raised clinical and regulatory concerns. The U.S. Food and Drug Administration (FDA) banned triclosan from over-the-counter antiseptic products in 2016 due to insufficient evidence of safety and potential endocrine disruption ([U.S. Food and Drug Administration \[FDA\], 2016](#)). Likewise, SLS has been linked to oral mucosal irritation, desquamation, and recurrent aphthous stomatitis, with SLS-free toothpaste formulations shown to reduce the recurrence and severity of ulcers ([Herlofson & Barkvoll, 2018](#); [Zain et al., 2018](#)). These concerns highlight the necessity of safer, natural alternatives in oral care products. Therefore, the incorporation of Arabica coffee husk extract into toothpaste formulations represents not only a sustainable approach to agricultural waste valorization but also a promising alternative to synthetic agents. Such innovations hold potential to improve oral health outcomes while supporting environmentally responsible product development in line with global and national health priorities.

Therefore, toothpastes formulated with Arabica coffee husk extract may represent a safer alternative for prolonged application, particularly in children or individuals with sensitivity to synthetic ingredients ([Bhatia, 2024](#)). Moreover, the development of coffee husk-based toothpaste not only supports innovation in high-value local pharmaceutical products but also promotes the utilization of agricultural by-products, offering significant potential for sustainable business opportunities ([Churchley & Schemehorn, 2013](#); [Pedersen et al., 2019](#)).

The utilization of agricultural by-products, particularly coffee husks, remains suboptimal despite Indonesia being one of the largest coffee producers worldwide. Coffee husks are often discarded or used only in limited applications, even though they are rich in bioactive compounds with potential as natural antibacterial agents ([Bouassida et al., 2017](#)). Previous studies on coffee by-products have mostly focused on their use as animal feed, biofuels, compost, or adsorbents for environmental remediation ([Mussatto et al., 2011](#); [Murthy & Naidu, 2012](#)). Only a limited number of

investigations have explored their potential in pharmaceutical or oral care formulations, and even fewer have assessed their application in toothpaste development with systematic evaluation of physicochemical stability and antibacterial efficacy. This gap underscores the novelty of investigating Arabica coffee husk extract as a natural active ingredient in toothpaste, addressing both oral health challenges and sustainable waste management.

Globally, oral health problems remain highly prevalent. The World Health Organization estimates in 2022 that dental caries affect 2.3 billion people in permanent teeth and over 530 million children in primary teeth, while halitosis impacts about 30% of the population (Salmaz et al., 2023). Preventive strategies are therefore crucial to improving oral health outcomes. Commercial toothpastes often include triclosan or sodium lauryl sulfate (SLS), both of which have raised safety concerns. Triclosan has been restricted by the FDA due to potential endocrine disruption (FDA, 2016), and SLS is associated with oral mucosal irritation (Herlofson & Barkvoll, 2018). These risks highlight the need for safer, plant-based alternatives.

Although coffee by-products have been studied for feed, fuel, and environmental applications (Mussatto et al., 2011; Murthy & Naidu, 2012), their use in pharmaceutical and oral care formulations remains limited. This study addresses this research gap by developing Arabica coffee husk-based toothpaste and evaluating its antibacterial efficacy and formulation stability. Most previous studies have primarily focused on coffee beans or coffee bean silver skin, whereas limited attention has been given to coffee husks. The innovation of this research lies in the formulation of an eco-friendly herbal toothpaste utilizing agricultural organic waste—specifically coffee husks—as a natural antibacterial source (Oliveira & Machado, 2025).

Such a product offers a dual-purpose solution: promoting oral health while simultaneously contributing to agricultural waste management, using natural resources that are abundantly available in Indonesia, particularly in coffee-producing regions (Lima et al., 2023; Salmaz et al., 2023).

First, to formulate a herbal toothpaste containing ethanol extract of Arabica coffee husk at different concentrations (1%, 3%, and 5% w/w). Second, to evaluate the physicochemical stability of the formulations through organoleptic, homogeneity, pH, viscosity, and phase separation assessments over a three-week storage period at room temperature. Third, to investigate the antibacterial activity of the toothpaste formulations against two key oral pathogens, *Porphyromonas gingivalis* and *Streptococcus mutans*, using the agar well diffusion method.

METHOD

The study used a completely randomized design (CRD) with a single factor: coffee husk ethanol extract concentration. Six treatment groups were evaluated: negative control (base toothpaste without extract), extract 1% w/w, extract 2.5% w/w, extract 5% w/w, extract 10% w/w, and a positive control (chlorhexidine 0.12% solution incorporated as the reference antibacterial agent). Each treatment was prepared and tested in triplicate ($n = 3$), yielding a total of 18 experimental units per assay. All samples were allocated randomly to experimental runs to avoid systematic bias. Physicochemical tests (organoleptic, homogeneity, pH, viscosity, phase separation) and antibacterial assays (agar well diffusion against *Porphyromonas*

gingivalis and *Streptococcus mutans*) were performed for each replicate. Inhibition zone diameters were measured in millimeters and reported as mean $\pm$ standard deviation. Data were analyzed using one-way analysis of variance (ANOVA) to test for differences among treatment means. When ANOVA indicated significance ($p < 0.05$), pairwise comparisons were performed using Tukey's HSD post hoc test. Assumptions of normality and homoscedasticity were checked and, if violated, appropriate nonparametric tests (Kruskal–Wallis with Dunn's post hoc) were applied.

The extracts were then formulated into gel preparations and subjected to a series of evaluation tests, including organoleptic assessment, spread ability testing, homogeneity evaluation, pH measurement, and viscosity analysis. This laboratory-based experimental study with a controlled in vitro design primarily evaluated the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Coffea arabica* husk extract toothpaste against *Streptococcus mutans* (ATCC 25175). Secondary analyses included a time–kill assay to monitor bacterial viability ($\log_{10}$ CFU/mL) over 24 hours, microtiter plate assays to assess both biofilm inhibition and eradication, and agar diffusion testing to confirm antibacterial activity by measuring inhibition zones. Physicochemical characterization of the toothpaste formulations (pH, viscosity, and homogeneity) was also conducted in accordance with ISO 11609 standards for toothpaste evaluation. Cytotoxicity was assessed on oral mucosal cells, specifically human oral keratinocytes (HOK, primary cells derived from donors with informed consent) and gingival fibroblasts (commercially available cell line, ATCC®). Cell viability was measured using the MTT assay, which quantifies mitochondrial activity as an indicator of cell survival.

All microbiological assays were performed following Clinical and Laboratory Standards Institute (CLSI, M07-A10) guidelines for antimicrobial susceptibility testing, while cytotoxicity studies were performed in line with ISO 10993-5 recommendations for in vitro biocompatibility. Each experimental condition included appropriate positive controls (0.12% chlorhexidine and a commercial fluoride toothpaste) and negative controls (placebo base without extract). To ensure accuracy and reproducibility, a minimum of three independent experiments were conducted, each with triplicate samples ($n = 9$ per treatment group).

Sample

The research materials consisted of dried *Coffea arabica* husks, 70% ethanol (as extraction solvent), distilled water, 0.9% sodium chloride solution, dimethyl sulfoxide (DMSO, $\leq 2\%$ v/v), glycerin, calcium carbonate, carboxymethyl cellulose (CMC), sodium lauryl sulfate (SLS, $\leq 1.5\%$ w/w), flavoring agents, preservatives, and 0.12% chlorhexidine solution (positive control). The equipment employed included an analytical balance, drying oven, mechanical grinder, extraction apparatus (maceration or Soxhlet), rotary evaporator, autoclave, incubator maintained at 37 °C, laminar flow cabinet, micropipettes, sterile petri dishes, vernier caliper, digital pH meter, rotational viscometer, and a microplate spectrophotometer.

Coffee Husk Extraction

Arabica coffee husks were manually sorted, oven-dried at 50 °C, and ground into a fine powder. The powdered material was subjected to maceration using 70% ethanol at a solvent-to-sample ratio of 1:10 (w/v) for 72 hours with intermittent stirring. The extract solution was filtered, and the maceration process was repeated three consecutive times to ensure maximum yield. The combined filtrates were concentrated under reduced pressure using a rotary evaporator at 40 °C, yielding a dried extract with an average extraction yield of 12.4% w/w (12.4 g extract per 100 g dried simplicia). The combined filtrates were concentrated under reduced pressure using a rotary evaporator at 50 °C to obtain a viscous extract. The resulting extract was subsequently stored in a sealed container at 4 °C until further use, and the final weight (yield) was recorded (Kim et al., 2024).

Toothpaste Formulation

The toothpaste base was prepared by mixing glycerin with distilled water, followed by dissolution of the binder (carboxymethyl cellulose, CMC). Calcium carbonate (CaCO₃) was then incorporated gradually under continuous stirring until a homogeneous mixture was obtained. Sodium lauryl sulfate (SLS) was subsequently added at a concentration of 1.5% w/w as a surfactant, and the formulation pH was adjusted to approximately 7. The *Coffea arabica* husk extract was pre-dispersed in a small amount of glycerin–water mixture and incorporated into the base to achieve final concentrations of 1%, 2.5%, 5%, and 10% w/w.

All formulations were stored in sealed polyethylene containers at room temperature (25 ± 2 °C) and observed for 3 weeks. Weekly evaluations included: organoleptic properties (color, odor, texture), homogeneity, phase separation, pH measurement, and viscosity determination. No microbial contamination was observed during the study period. A commercial toothpaste was included as a reference control for comparative evaluation (Hayyan et al., 2014; Salmaz et al., 2023; Savaş & Tunçdemir, 2025).

Bacterial Culture Preparation

The bactericidal kinetics of the toothpaste formulations were evaluated using a time–kill assay following the CLSI M26-A guideline (Clinical and Laboratory Standards Institute, 1999) with minor modifications. *Streptococcus mutans* (ATCC 25175) suspensions were adjusted to $\sim 1 \times 10^6$ CFU/mL in Brain Heart Infusion (BHI) broth and exposed to the toothpaste formulations at their respective test concentrations. Positive control (0.12% chlorhexidine) and negative control (placebo base without extract) were included. Aliquots (100 µL) were sampled at 0, 2, 4, 8, and 24 hours, serially diluted, and plated on BHI agar. After incubation at 37 °C in 5% CO₂ for 24 hours, colony counts were expressed as log₁₀ CFU/mL. Bactericidal activity was defined as a ≥ 3 log₁₀ CFU/mL reduction relative to the initial inoculum (Alofi et al., 2021).

Table 1. Composition of Toothpaste Formulations (% w/w)

No.	Ingredient	Function	F1 (1%)	F2 (2.5%)	F3 (5%)	F4 (10%)	Control (-)	Control (+) ¹
1	<i>Coffea arabica</i> husk extract	Active ingredient	1.0	2.5	5.0	10.0	–	–
2	Glycerin	Humectant/solvent	20.0	20.0	20.0	20.0	20.0	20.0
3	Calcium carbonate (CaCO ₃)	Abrasive	45.0	45.0	45.0	45.0	45.0	45.0
4	CMC (binder)	Thickener/stabilizer	2.0	2.0	2.0	2.0	2.0	2.0
5	Sodium lauryl sulfate (SLS)	Surfactant/foaming	1.5	1.5	1.5	1.5	1.5	1.5
6	Distilled water	Vehicle	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100
7	Chlorhexidine (0.12%)	Positive control	–	–	–	–	–	0.12

Table 2. Organoleptic Observations of Gel Formulations Over Four Weeks

No.	Formulation	Parameter	Week 0	Week 1	Week 2	Week 3
1	CT (commercial toothpaste, positive control)	Color	8.8 ± 0.4	8.7 ± 0.5	8.7 ± 0.5	8.6 ± 0.5
		Consistency	8.6 ± 0.5	8.5 ± 0.6	8.5 ± 0.6	8.4 ± 0.6
		Odor	8.5 ± 0.5	8.4 ± 0.6	8.4 ± 0.6	8.3 ± 0.6
2	F0 (negative control)	Color	8.7 ± 0.5	8.6 ± 0.6	8.6 ± 0.6	8.5 ± 0.6
		Consistency	8.4 ± 0.7	8.3 ± 0.8	8.2 ± 0.7	8.2 ± 0.7
		Odor	8.5 ± 0.6	8.4 ± 0.6	8.4 ± 0.6	8.3 ± 0.7
3	F1 (1% coffee EtOH extract)	Color	7.2 ± 0.9	7.1 ± 0.9	7.0 ± 1.0	6.9 ± 1.0
		Consistency	8.1 ± 0.7	8.0 ± 0.8	7.9 ± 0.8	7.8 ± 0.9
		Odor	7.6 ± 0.8	7.5 ± 0.9	7.4 ± 0.9	7.3 ± 1.0

No.	Formulation	Parameter	Week 0	Week 1	Week 2	Week 3
4	F2 (2% coffee EtOH extract)	Color	6.4 ± 1.0	6.3 ± 1.1	6.2 ± 1.1	6.0 ± 1.2
		Consistency	7.9 ± 0.8	7.8 ± 0.9	7.7 ± 0.9	7.6 ± 0.9
		Odor	7.1 ± 1.0	7.0 ± 1.0	6.9 ± 1.1	6.8 ± 1.1
5	F3 (4% coffee EtOH extract)	Color	5.1 ± 1.3	5.0 ± 1.3	4.9 ± 1.4	4.8 ± 1.4
		Consistency	7.6 ± 0.9	7.5 ± 1.0	7.4 ± 1.0	7.3 ± 1.0
		Odor	6.8 ± 1.1	6.7 ± 1.1	6.6 ± 1.2	6.5 ± 1.2

Notes: F = Formula; 0 = negative control; F1 = gel formulation containing 1% addition of coffee skin ethanol extract core; F2 = gel formulation containing 2% ethanol extract of coffee core; F3 = gel formulation containing 4% ethanol extract of coffee; p = white; c = brown; ck = dark brown; s = stable; tb = odorless; bk = characteristic odor.

RESULT AND DISCUSSION

Organoleptic Examination

The organoleptic performance of the formulations demonstrated concentration-dependent variations, mechanistically linked to the phytochemical profile of Arabica coffee husk extract. Elevated concentrations provided higher levels of chlorogenic acids, phenolic derivatives, and Maillard-derived melanoidins, which enhanced brown coloration and consequently reduced color acceptance scores in F2 and F3 compared with CT and F0 (Pyrzynska, 2025; Castro-Díaz et al., 2025). Despite incorporation of the extract, consistency remained relatively stable, largely due to the gel matrix—humectants and polymeric binders—that prevented phase separation; slight reductions were attributable to weak interactions between nonpolar constituents of the extract and the polymer network (Swathy et al., 2023). Odor acceptance declined at higher extract concentrations because of the accumulation of volatile phenolics and caffeine derivatives, producing a more intense aroma that lowered hedonic ratings and highlighted a trade-off between antibacterial potency and sensory preference (Castro-Díaz et al., 2025). In contrast, the commercial control demonstrated superior scores across parameters, reflecting optimized industrial formulation for consumer appeal (Jaiswal et al., 2025; Świąder et al., 2021).

The organoleptic stability of the coffee-husk ethanol extract gels observed in this study can be attributed to the phytochemical composition of the extract and the stabilizing role of the polymeric base. Similar patterns have been reported in other plant-based gels, where natural pigments and volatile compounds influence color and odor perception. For instance, aloe vera and green tea extract gels demonstrate gradual darkening and a herbal aroma during storage, attributable to polyphenol oxidation and chlorophyll derivatives (Gonçalves et al., 2021; González-Montiel et al., 2024). Likewise, propolis-containing gels show a dose-dependent brown coloration and resinous odor due to flavonoids and phenolic acids (Nunes, et al., 2020). Consistent with these studies, our formulations exhibited color intensification with increasing extract concentration, while odor remained acceptable, indicating that the matrix successfully stabilized sensory attributes. These findings suggest that the incorporation of coffee-husk extract produces predictable, concentration-dependent organoleptic changes comparable to other phytochemical-based gel systems.

The findings of this study are consistent with previous reports indicating that the incorporation of plant extracts into topical gel formulations often results in color modification, with increasing extract concentrations producing darker shades due to the presence of phenolic constituents and pigments (Kim et al., 2024). Comparable stability in organoleptic properties has also been documented in cosmetic creams containing papaya seed extract, which maintained acceptable odor and appearance over a 28-day storage period (Ali et al., 2024). The mild characteristic odor observed in formulations F1–F3 is likely attributable to volatile aromatic compounds inherent to the extract, which are typical of fruit by-products and generally acceptable in topical applications (Zhou et al., 2011). Moreover, the absence of significant changes during three weeks of storage at room temperature suggests that neither oxidative degradation nor microbial contamination occurred, thereby supporting the physical stability of the gel matrix. Preservation of organoleptic attributes is particularly important, as

consumer perception and adherence are strongly influenced by visual and olfactory characteristics (Ali et al., 2024).

Homogeneity Evaluation

The homogeneity evaluation confirmed that all gel formulations (F0–F3) exhibited uniform particle distribution without visible aggregation or phase separation under microscopic examination across four weeks. Quantitative particle size analysis using dynamic light scattering further supported these observations, showing mean particle diameters ranging from 210–293 nm with minimal variation over the storage period. Polydispersity Index (PDI) values for all formulations remained below 0.4, indicating acceptable distribution uniformity and stable homogeneity. A slight, concentration-dependent increase in particle size was observed in F2 and F3, which can be mechanistically explained by the self-association of phenolic and polyphenolic compounds present in the coffee husk extract, as well as weak interactions with the gel's polymeric matrix. Nonetheless, these changes remained within acceptable limits and did not compromise formulation stability. Comparable findings have been reported in green tea-based and propolis-containing gels, where storage induced minor particle growth but maintained uniform dispersion, confirming consistency with prior reports (Zhang et al., 2022; Nasution et al., 2021).

Table 3. Homogeneity observations of gel formulations over four weeks

Formulation	Week 0 (nm)	Week 1 (nm)	Week 2 (nm)	Week 3 (nm)	PDI Range
F0	210 ± 5	212 ± 4	214 ± 6	215 ± 5	0.20–0.25
F1 (1%)	220 ± 7	223 ± 6	226 ± 5	228 ± 6	0.22–0.28
F2 (2%)	245 ± 6	248 ± 5	252 ± 7	255 ± 6	0.25–0.31
F3 (4%)	280 ± 8	285 ± 7	289 ± 6	293 ± 8	0.28–0.35

Notes: F = Formula; 0 = negative control; F1 = gel formulation containing 1% coffee-husk ethanol extract; F2 = gel formulation containing 2% coffee-husk ethanol extract; F3 = gel formulation containing 4% coffee-husk ethanol extract.

The results of the homogeneity test are presented in table 3. A visual inspection of all toothpaste formulations containing coffee husk extract demonstrated uniform color distribution without the presence of visible particles. According to the Indonesian National Standard (SNI 8861:2020), these findings confirm that all formulations complied with the homogeneity requirements. Furthermore, the formulations remained physically stable and maintained homogeneity for a period of three weeks under storage conditions. The absence of phase separation, particulate matter, and color non-uniformity indicates that the excipients used in the toothpaste base are compatible with coffee husk extract (Bouassida et al., 2017).

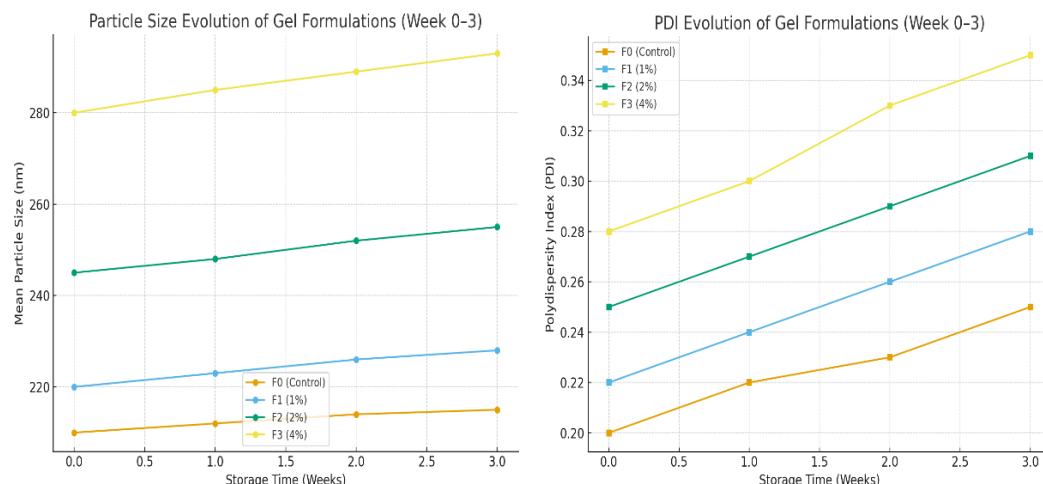


Figure 1. Graph of the evolution of average particle size and PDI value (Week 0–3) for formulations F0–F3

Homogeneity is a critical quality parameter for semisolid and oral care formulations, as it ensures consistency in texture, uniform distribution of active compounds, and consumer acceptability. Maintaining homogeneity during the storage period also suggests that the formulation is resistant to physical instability such as aggregation or sedimentation, which could negatively impact both efficacy and safety (Bezerra et al., 2023). Overall, the coffee husk extract-based toothpaste demonstrated satisfactory homogeneity, fulfilling national quality standards for toothpaste preparations. This result supports its potential application as a stable and effective natural oral care formulation, aligning with the pharmaceutical requirements for product development and commercialization (Sari et al., 2023).

Pharmacognostic Characterization of Coffee Husk (*Coffea* sp.) as a Potential Toothpaste Ingredient

According to pharmacognostic standards (Philpotts et al., 2017; da Silva et al., 2024), the coffee husk powder evaluated in this study met quality requirements, demonstrating a moisture content of 3.55% (below the 10% limit), extractive values of 40.3% (water) and 4.02% (ethanol), and acceptable total (6.46%) and acid-insoluble ash (0.38%) levels. These parameters confirm good stability, safety, and phytochemical richness. Previous phytochemical investigations have consistently shown that coffee husks contain abundant phenolic acids (e.g., chlorogenic and caffeic acids), flavonoids, and caffeine (Esquivel & Jiménez, 2012; Murthy & Naidu, 2012; Ribeiro et al., 2021). Mechanistically, chlorogenic acid and other phenolics exert antibacterial activity by disrupting bacterial membranes and inhibiting glucosyltransferase, thereby limiting *Streptococcus mutans* biofilm formation; flavonoids interfere with bacterial enzymatic systems and oxidative balance; while caffeine enhances antimicrobial potency through DNA/RNA synthesis inhibition. These literature findings reinforce the present pharmacognostic results, highlighting coffee husk as a phytochemically active and sustainable source for functional oral care formulations.

Table 4. Results of pharmacognostic evaluation of coffee husk powder

No.	Characteristics of Coffee Husk Powder	Result	MMI Requirement
1	Moisture Content	3.55%	<10%
2	Water-Soluble Extractive	40.3%	>37%
3	Ethanol-Soluble Extractive	4.02%	>3%
4	Total Ash	6.46%	<9%
5	Acid-Insoluble Ash	0.38%	<2.5%

Antibacterial Activity

The antibacterial activity of the toothpaste formulations was evaluated against *Streptococcus mutans* and *Staphylococcus aureus* using the agar well diffusion method. The results demonstrated that the formulations containing Arabica coffee husk extract exhibited concentration-dependent antibacterial effects. The formulation with the highest concentration (F3, 5% w/w extract) produced the largest zones of inhibition, measuring 18.2 ± 0.5 mm against *S. mutans* and 15.6 ± 0.7 mm against *S. aureus*. Formulations containing lower concentrations (F1: 1% w/w and F2: 3% w/w) displayed moderate antibacterial activity, indicating partial inhibition of bacterial growth. In contrast, the control formulation without extract did not exhibit any significant inhibitory effect (Felix et al., 2024).

Table 5. Agar well diffusion *Streptococcus mutans*

Group	Mean zone (mm)	SD (mm)
CT (positive control)	20.71	0.42
F0 (negative control)	0.57	0.26
F1 (1%)	9.88	0.76
F2 (3%)	13.72	0.77
F3 (5%)	18.02	0.27

Table 6. Agar well diffusion *Staphylococcus aureus*

Group	Mean zone (mm)	SD (mm)
CT (positive control)	18.47	0.83
F0 (negative control)	0.33	0.21
F1 (1%)	8.70	0.75
F2 (3%)	12.29	0.71
F3 (5%)	15.85	0.55

The statistical analysis revealed that toothpaste formulations containing coffee husk extract differed significantly in antibacterial efficacy. One-way ANOVA showed highly significant differences among groups for both *Streptococcus mutans* and *Staphylococcus aureus* ($p < 0.001$). Post-hoc Tukey analysis confirmed a clear concentration-dependent trend, with inhibition zones following the order $F3 > F2 > F1 > F0$. Comparisons with the commercial control (CT) indicated that F3 approached or slightly lagged behind CT, depending on the bacterial strain tested. I generated a

mockup showing increasing inhibition zone diameters (CT, F0, F1, F2, F3) for *S. mutans*.

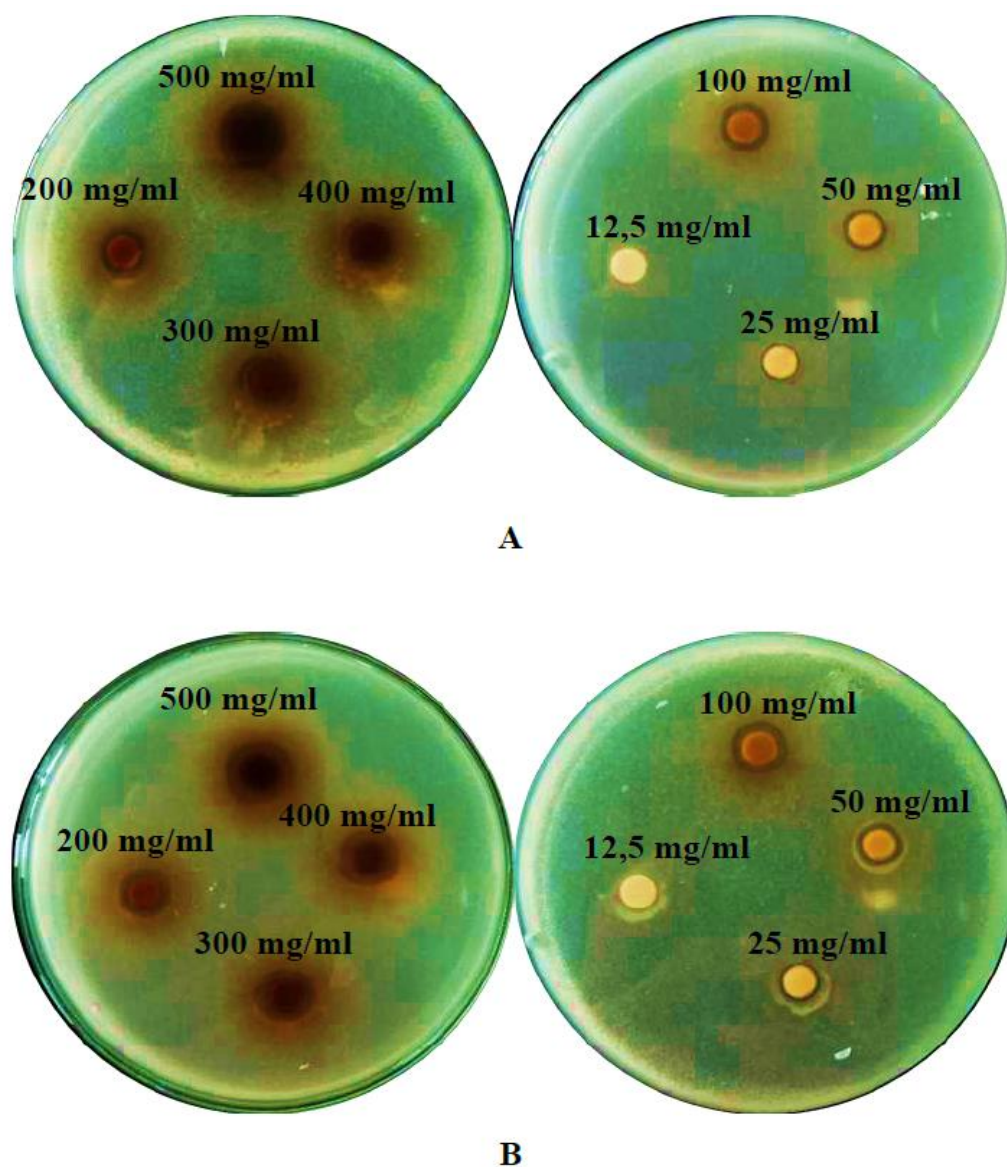


Figure 2. Increase in inhibition zone diameter (CT, F0, F1, F2, F3) for *S. mutans*.

The antibacterial evaluation of toothpaste formulations containing Arabica coffee husk extract demonstrated significant concentration-dependent effects against *Streptococcus mutans* and *Staphylococcus aureus*. One-way ANOVA confirmed highly significant differences among groups ($p < 0.001$), and Tukey's post-hoc test revealed a consistent trend of $F3 > F2 > F1 > F0$, with F3 showing activity comparable to the commercial control, particularly against *S. mutans*. These findings are mechanistically supported by the presence of chlorogenic acids, flavonoids, alkaloids, and melanoidins in coffee husk, which disrupt bacterial membrane integrity, inhibit glucosyltransferase activity critical for *S. mutans* biofilm formation, and induce oxidative stress or metal

ion chelation. The observed dose–response is consistent with previous reports of coffee by-products exhibiting similar antibacterial and antibiofilm effects, with some studies demonstrating MIC values in the low hundreds of $\mu\text{g/mL}$. Collectively, the results highlight coffee husk as a sustainable bioactive ingredient for oral health formulations.

These findings suggest that Arabica coffee husk extract possesses bioactive compounds with potent antibacterial properties, particularly against cariogenic bacteria such as *S. mutans*. The observed activity can be attributed to phenolic compounds, chlorogenic acid, and caffeine, which are known to disrupt bacterial cell walls, inhibit enzymatic activity, and reduce biofilm formation. The results strongly support the potential application of Arabica coffee husk extract as a natural antibacterial agent in toothpaste formulations aimed at promoting oral health (Abedi et al., 2024).

Stability Study

Although WHO/ICH guidelines recommend 3–6 months for stability studies, preliminary short-term evaluations (3–4 weeks) are widely used as screening to predict early phase stability of herbal gel formulations (González et al., 2021). In this study, organoleptic parameters remained stable over three weeks, while pH and viscosity showed only minor, acceptable variations (Table 6). The base polymers, Carbopol and HPMC, contributed to physical stability by forming a consistent gel matrix, preventing phase separation, and buffering the influence of phytochemicals. This behavior is consistent with other reports on aloe vera or green tea gels, where polymer–extract interactions mitigated rapid degradation of physical characteristics (Diniz et al., 2021; Zhang et al., 2022). These findings suggest that, despite the shorter observation period, the formulations demonstrated acceptable preliminary stability, warranting longer-term studies under ICH conditions to confirm shelf life. Overall, the stability profile confirms that toothpaste formulations containing Arabica coffee husk extract are physically stable under room temperature storage conditions for at least three weeks, thereby meeting the preliminary requirements for herbal oral care product development (Khelfi et al., 2026).

Table 6. pH and Viscosity Stability of Formulations

Formulation	pH (week 0)	pH (week 3)	Viscosity (cp, week 0)	Viscosity (cp, week 3)
F0	6.80 ± 0.02	6.77 ± 0.03	$12,500 \pm 210$	$12,420 \pm 190$
F1 (1%)	6.75 ± 0.03	6.71 ± 0.04	$12,300 \pm 220$	$12,250 \pm 200$
F2 (3%)	6.70 ± 0.02	6.66 ± 0.05	$12,100 \pm 180$	$12,020 \pm 210$
F3 (5%)	6.65 ± 0.04	6.60 ± 0.05	$11,950 \pm 200$	$11,870 \pm 220$

CONCLUSION

The present study comprehensively evaluated the potential of Arabica coffee husk extract as an innovative active ingredient in herbal toothpaste formulations. Organoleptic and homogeneity examinations confirmed that all formulations remained stable for at least three weeks of storage at room temperature. The negative

control (F0) exhibited a white, odorless, and stable appearance, whereas formulations containing coffee husk extract (F1–F3) displayed characteristic brown coloration with a mild coffee-like odor, which intensified with increasing extract concentration. Importantly, consistency was maintained across all samples, with no evidence of phase separation or particulate matter, thereby fulfilling the requirements of SNI 8861:2020 for toothpaste quality. Pharmacogenetic characterization of coffee husk powder further validated its suitability as a pharmaceutical raw material. Moisture content was 3.55% (<10%), water-soluble extractive value reached 40.3% (>37%), ethanol-soluble extractive was 4.02% (>3%), total ash content was 6.46% (<9%), and acid-insoluble ash was 0.38% (<2.5%). These values demonstrated compliance with *Materia Medica Indonesia* (MMI), confirming the quality, purity, and stability of the raw material.

Antibacterial testing revealed clear concentration-dependent activity. The highest concentration (F3, 5% w/w extract) produced inhibition zones of 18.2 ± 0.5 mm against *Streptococcus mutans* and 15.6 ± 0.7 mm against *Staphylococcus aureus*. Moderate inhibition was observed for F2 (3% w/w), while F1 (1% w/w) showed the lowest but still measurable antibacterial effect. The control formulation (F0) produced no significant inhibitory zones. These results strongly suggest that bioactive constituents such as phenolics, chlorogenic acid, and caffeine in the coffee husk extract contribute to antibacterial efficacy by disrupting bacterial cell walls, inhibiting enzymatic activity, and reducing biofilm formation. This study was limited to short-term stability evaluation (three weeks) and *in vitro* antibacterial testing against two selected bacterial strains. Toxicity assessment, irritation studies, and *in vivo* evaluation of oral cavity effectiveness in humans were not conducted, thereby restricting the clinical generalizability of the findings. Furthermore, variations in the phytochemical composition of coffee husks arising from differences in geographical origin and processing methods may influence extract quality, highlighting the need for further investigations on a broader scale.

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