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Toothpaste Formulation Containing 24-Methylenecycloartanol Isolated from Pometia pinnata Fruit Peel as an Antibiofilm Agent Against Streptococcus mutans and Candida albicans

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ABSTRACT:

Background: Early Childhood Caries (ECC) remains a significant public health concern in Indonesia, with Streptococcus mutans and Candida albicans identified as the principal cariogenic microorganisms responsible for biofilm formation and caries progression. Natural phytochemicals isolated from tropical plants represent a promising strategy for addressing the antimicrobial resistance limitations associated with conventional agents.

Objective: This study aimed to optimise toothpaste formulations incorporating 24-methylenecycloartanol (24-MCA) at varying concentrations and to evaluate their antibiofilm efficacy against S. mutans (ATCC 25175) and C. albicans (ATCC 10231).

Materials and Methods: Six toothpaste formulations (F0–F5) were prepared with 24-MCA concentrations ranging from 0% to 0.05% (w/w). Physicochemical characterisation was performed in compliance with the Indonesian National Standard (SNI) 8861:2020. Antibiofilm activity was assessed using a microdilution crystal violet assay, with absorbance read at 540 nm and chlorhexidine gluconate (CHX) as the positive control.

Results: All six formulations complied with the Indonesian national toothpaste standard with respect to pH, viscosity, spreadability and foam height, and remained stable after accelerated storage cycling. Formulation F3 (0.03% w/w 24-MCA) exhibited the most potent antibiofilm activity, achieving 92.28% inhibition at 24 h and 89.30% inhibition at 48 h against the dual-species biofilm of S. mutans and C. albicans.

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Conclusion: The 0.03% (w/w) 24-MCA toothpaste formulation demonstrated optimal physicochemical properties and superior dual antibiofilm efficacy against the primary cariogenic pathogens.

1. INTRODUCTION

Dental caries constitutes one of the most prevalent chronic diseases affecting children globally. According to the 2023 Indonesian Health Survey, dental caries in children represents the leading oral disease recorded nationwide.[1] Early Childhood Caries (ECC), defined as the presence of one or more decayed, missing or filled tooth surfaces in children under six years of age, is characterised by rapid and aggressive destruction of the primary dentition.[2] The aetiology of ECC is multifactorial, involving a complex interplay among cariogenic microorganisms, fermentable dietary carbohydrates, host susceptibility and time.[3,4]

The microbial community central to ECC pathogenesis is organised into a structured biofilm — the dental plaque — that adheres to the tooth surface. Biofilm constitutes a dynamic three-dimensional microbial community embedded within a self-produced extracellular polymeric matrix consisting of exopolysaccharides (EPS), proteins and extracellular DNA.[5,6] The two principal pathogens driving biofilm-mediated cariogenesis are *Streptococcus mutans* and *Candida albicans*. *S. mutans* synthesises insoluble glucan via glucosyltransferases (GtfB and GtfC), promoting adhesion and biofilm maturation, while *C. albicans* synergistically enhances biofilm stability through adhesin-mediated co-aggregation and EPS production.[7,8,9] The resulting polymicrobial biofilm exhibits markedly increased resistance to antimicrobial agents compared with planktonic cells.

Toothpaste constitutes the most widely used vehicle for delivering therapeutic antibacterial agents to the oral cavity. Effective toothpaste formulations must balance physicochemical stability, patient acceptability and pharmacological efficacy. Conventional antibacterial agents — including chlorhexidine, triclosan and fluoride — remain standard therapeutic options; however, concerns regarding antimicrobial resistance, disruption of the oral microbiome and adverse effects such as tooth staining have been raised with prolonged use.[10,11] Consequently, growing scientific interest has focused on plant-derived compounds as alternative therapeutic agents.

Pometia pinnata J.R. Forst. & G. Forst. (Sapindaceae), commonly known as Matoa, is a tropical fruit tree indigenous to Papua, Indonesia, and widely distributed across Southeast Asia and the Pacific.[12] Phytochemical investigations of *P. pinnata* have revealed a diverse secondary metabolite profile encompassing alkaloids, saponins, flavonoids, tannins, polyphenols, monoterpenes, sesquiterpenes and triterpenoids.[13] Among these, the cycloartane-type triterpenoid 24-methylenecycloartanol (24-MCA) has been identified in the fruit peel and has demonstrated significant *in vitro* antibiofilm activity against both *S. mutans* and *C. albicans*.[14]

Despite these promising preliminary findings, the systematic integration of 24-MCA into a clinically applicable toothpaste delivery system has not been previously investigated. Formulation optimisation is essential to ensure stabilisation of the active compound within the excipient matrix and to confirm that the resulting preparation meets established quality standards for safety, stability and rheological performance. Furthermore, concentration–response data for 24-MCA in a toothpaste matrix against polymicrobial biofilm targets are currently lacking in the literature.

The present study therefore aimed to: (1) isolate and structurally confirm 24-MCA from the n-hexane fraction of *P. pinnata* fruit peel; (2) develop and optimise six toothpaste formulations incorporating 24-MCA at concentrations of 0–0.05% (w/w); (3) characterise their physicochemical properties in accordance with SNI 8861:2020; and (4) determine the biofilm inhibitory activity of each formulation against *S. mutans* ATCC 25175 and *C. albicans* ATCC 10231. This work provides novel data toward the development of a natural antibiofilm toothpaste for ECC prevention, in alignment with global efforts to address antimicrobial resistance.

2. MATERIALS AND METHODS

2.1 Ethical Approval

This study did not involve human participants, human-derived clinical specimens or animal subjects. The protocol was reviewed and approved by the Health Research Ethics Committee, Faculty of Dentistry, Universitas Muhammadiyah Semarang (Approval No. 021/EC/KEPK/FKM-UNIMUS/II/2025). Plant material was collected in accordance with institutional and national

regulations governing access to plant genetic resources, and a voucher specimen was deposited at the Herbarium of the Faculty of Pharmacy, Universitas Muhammadiyah Semarang (voucher no. UNIMUS-PP-2024/07).

2.2 Plant Material and Isolation of 24-Methylenecycloartanol

Fresh Matoa (*P. pinnata*) fruit was procured from Pati Regency, Central Java, Indonesia. A total of 30 kg of fresh fruit was collected, peeled, washed, sliced and oven-dried at 40 °C, yielding 3 kg of dried crude drug. The crude drug was macerated with methanol (1:10 w/v) in six sequential batches of 2 × 24 h each. The combined methanol filtrates were concentrated under reduced pressure using a rotary evaporator to yield a viscous methanol extract.

The methanol extract underwent liquid–liquid partitioning with n-hexane, ethyl acetate and distilled water, yielding three polarity-separated fractions. Based on the anticipated triterpenoid character of the target compound, the n-hexane fraction was selected for further fractionation by vacuum liquid chromatography (VLC) using silica gel as the stationary phase and a stepwise gradient of hexane–ethyl acetate–methanol (incrementing by 10% v/v) as the mobile phase. Fractions exhibiting similar thin layer chromatography (TLC) profiles (hexane:ethyl acetate 8:2, UV 254/366 nm) were combined, yielding six pooled fractions.

Pooled Fraction 3, which displayed a spot consistent with the target compound, was subjected to gravity column chromatography (GCC) under isocratic conditions (hexane:ethyl acetate, 9:1 v/v). Resulting sub-fractions were monitored by TLC, pooled and recrystallised to yield pure white crystalline 24-MCA. Compound identity and purity were confirmed by: (i) single-spot TLC (R*f* = 0.65, hexane:ethyl acetate 8:2); (ii) melting point determination using a Fisher–Johns apparatus; (iii) ultraviolet (UV), infrared (IR), electron impact mass spectrometry (EIMS), ^{1}H nuclear magnetic resonance (NMR), ^{13}C-NMR and two-dimensional NMR (COSY, HMQC, HMBC) spectral analysis, with data matched against literature values for 24-methylenecycloartanol; and (iv) specific optical rotation measurement.

2.3 Preparation of Toothpaste Formulations

Six toothpaste formulations (F0–F5) were prepared incorporating 24-MCA at concentrations of 0%, 0.01%, 0.02%, 0.03%, 0.04% and 0.05% (w/w), respectively. The base excipient composition remained constant across all formulations, as detailed in Table 1.

The preparation procedure was as follows: 24-MCA was dispersed in glycerin with a small amount of distilled water under continuous stirring (Step A). Separately, sodium lauryl sulfate (SLS) was dissolved in a small volume of glycerin (Step B), and menthol was dissolved in peppermint oil (Step C). Mixtures from Steps A and C were combined and stirred until homogeneous (Step D). Xylitol and a portion of potassium carbonate were incorporated into Step D (Step E). The remaining potassium carbonate was blended into the SLS mixture from Step B (Step F). Mixtures from Steps E and F were combined with stirring until a homogeneous mass was obtained (Step G). Peppermint oil was then incorporated to form the final paste (Step H). Prepared formulations were stored in sealed aluminium tubes and subjected to an accelerated stability protocol (25 °C/ambient humidity; 4 °C; 40 °C) in accordance with International Council for Harmonisation (ICH) Q1A(R2) guidelines.

2.4 Physicochemical Characterisation

All formulations were evaluated in triplicate before and after accelerated storage, in accordance with the requirements of SNI 8861:2020 for toothpaste quality. Organoleptic evaluation assessed colour, odour and physical form by visual and olfactory inspection. Homogeneity was assessed by spreading samples collected from three tube positions (top, middle and bottom) onto glass slides and examining them visually for uniformity. pH was measured using a calibrated digital pH meter and reported as the mean of three replicate readings. Viscosity was determined using a Brookfield viscometer. Spreadability was assessed by the parallel plate method using incremental loads (50 g and 100 g), with the mean diameter recorded at three positions. Foam height was measured in a graduated cylinder following vigorous manual agitation for 30 s.

2.5 Preparation of Microbial Suspensions

S. mutans ATCC 25175 and *C. albicans* ATCC 10231 were obtained from the Central Laboratory, Universitas Padjadjaran, Indonesia. A single colony of each organism was cultured in brain heart infusion (BHI) broth at 37 °C overnight. The resulting suspension was adjusted to a 0.5 McFarland turbidity standard (approximately 1.5 × 10^{8} CFU/mL) prior to use.

2.6 Antibiofilm Assay

Antibiofilm activity was evaluated against a dual-species biofilm of *S. mutans* ATCC 25175 and *C. albicans* ATCC 10231 using a microdilution crystal violet assay. Briefly, 50 µL of BHI broth supplemented with 2% sucrose, 5 µL of *S. mutans* suspension, 5 µL of *C. albicans* suspension and 40 µL of serial dilutions of each toothpaste formulation (F0–F5) were transferred into 96-well polystyrene microtitre plates (Iwaki, Japan). Phosphate-buffered saline (PBS) and 0.2% chlorhexidine gluconate (CHX) served as negative and positive controls, respectively. Plates were incubated at 37 °C under anaerobic conditions for 24 h and 48 h.

Following incubation, planktonic cells were removed by rinsing twice with PBS. Adherent biofilms were stained with 125 µL of 0.1% crystal violet solution for 15 min, then rinsed with PBS to remove unbound dye. Bound crystal violet was solubilised with 200 µL of 96% ethanol, and absorbance was recorded at an optical density (OD) of 540 nm using a spectrophotometer (Thermo Scientific, USA). Experiments were performed in quadruplicate, and the percentage of biofilm inhibition was calculated according to the following formula:

$$\text{Biofilm inhibition (\%)} = [(OD_{\text{growth control}} - OD_{\text{sample}}) / OD_{\text{growth control}}] \times 100$$

The minimum biofilm inhibitory concentration (MBIC) was defined as the lowest concentration of the test preparation producing at least a 50% reduction in biofilm biomass relative to the untreated growth control, and was determined from the same microdilution series.

2.7 Statistical Analysis

Physicochemical data are expressed as the mean ± standard deviation (SD) of triplicate measurements. Biofilm inhibition percentages are reported as geometric means across three independent experiments. Data were analysed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test; a significance threshold of $p < 0.05$ was applied. All statistical analyses were performed using SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

3. RESULTS

3.1 Isolation and Structural Identification of 24-Methylenecycloartanol

Maceration of 3 kg of dried *P. pinnata* fruit peel with methanol yielded a viscous brown extract. Subsequent liquid–liquid partitioning produced three fractions with distinct polarities. VLC of the n-hexane fraction yielded six pooled sub-fractions. TLC screening identified Pooled Fraction 3 as containing the target triterpenoid, evidenced by a characteristic spot at R_f 0.65 under UV 254 nm and a grey zone after anisaldehyde–sulfuric acid spray with heating.

Gravity column chromatography of Pooled Fraction 3 (hexane:ethyl acetate, 9:1), followed by recrystallisation, yielded 30 g of white needle-like crystals with a sharp melting point consistent with the literature value for 24-MCA. Purity was confirmed by single-spot TLC under three different solvent systems. IR spectroscopy revealed characteristic absorption bands for the cyclopropane ring, an exocyclic methylene group (=CH_2 stretching) and hydroxyl functionality. The ^{1}H-NMR spectrum displayed signals for the cyclopropyl and exocyclic methylene protons, while ^{13}C-NMR data corroborated the cycloartanol carbon framework. Full connectivity was established by two-dimensional NMR (COSY, HMQC, HMBC), and all data were consistent with previously reported values for 24-methylenecycloartanol.

Table 1. Composition of the six toothpaste formulations (F0–F5) containing 24-methylenecycloartanol.

Material	F0 (0%)	F1 (0.01%)	F2 (0.02%)	F3 (0.03%)	F4 (0.04%)	F5 (0.05%)	Function
24-Methylenecycloartanol*	0	0.01	0.02	0.03	0.04	0.05	Active ingredient
Sodium carboxymethyl cellulose (NaCMC)	1	1	1	1	1	1	Binder
Potassium carbonate	20	20	20	20	20	20	Abrasive
Sodium lauryl sulfate (SLS)	0.81	0.81	0.81	0.81	0.81	0.81	Surfactant

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Material	F0 (0%)	F1 (0.01%)	F2 (0.02%)	F3 (0.03%)	F4 (0.04%)	F5 (0.05%)	Function
Glycerin	20	20	20	20	20	20	Humectant/sweetener
Menthol	0.5	0.5	0.5	0.5	0.5	0.5	Flavouring agent
Peppermint oil	0.3	0.3	0.3	0.3	0.3	0.3	Flavouring agent
Ethanol 95%	3	3	3	3	3	3	Solvent
Distilled water	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	Solvent

Isolated from Pometia pinnata* fruit peel. All values are expressed as % w/w. NaCMC: sodium carboxymethyl cellulose; SLS: sodium lauryl sulfate.

3.2 Physicochemical Characterisation of Toothpaste Formulations

All six toothpaste formulations exhibited consistent physicochemical profiles before and after accelerated storage; the per-formulation data are presented in Table 2. Organoleptic evaluation revealed a uniform, light-green, semi-solid paste with a characteristic peppermint aroma across all formulations, which remained unchanged after thermal cycling. All formulations demonstrated homogeneity, with no phase separation detected across top, middle and bottom tube samples. Mean pH values ranged from 8.00 to 8.04 before storage and from 8.08 to 8.12 after storage, remaining within the acceptable range of 6.0–10.0 specified by SNI 8861:2020. Viscosity values of 20,280–21,024 cps conformed to the standard range (2,000–50,000 cps). Spreadability (6.1–6.3 cm) and foam height (5.7–6.0 cm) similarly satisfied the respective standard criteria. No statistically significant differences were observed between pre- and post-storage measurements for any formulation ($p > 0.05$), confirming formulation stability under the accelerated storage protocol.

Table 2. Physicochemical characteristics of each toothpaste formulation (F0–F5) before and after accelerated stability testing.

Parameter	F0	F1	F2	F3	F4	F5	SNI 8861:2020
pH (before)	8.02 ± 0.03	8.04 ± 0.02	8.01 ± 0.04	8.03 ± 0.03	8.00 ± 0.02	8.02 ± 0.03	6.0–10.0
pH (after)	8.11 ± 0.04	8.12 ± 0.03	8.09 ± 0.03	8.10 ± 0.04	8.08 ± 0.03	8.11 ± 0.02	6.0–10.0
Viscosity, cps (before)	21,024 ± 96	20,980 ± 88	20,912 ± 104	20,876 ± 92	20,840 ± 86	20,804 ± 98	2,000–50,000
Viscosity, cps (after)	20,412 ± 84	20,380 ± 90	20,344 ± 78	20,312 ± 86	20,296 ± 80	20,280 ± 92	2,000–50,000
Spreadability, cm (before)	6.3 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	5–7
Spreadability, cm (after)	6.2 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	5–7
Foam height, cm (before)	6.0 ± 0.2	6.0 ± 0.1	5.9 ± 0.2	5.9 ± 0.1	5.8 ± 0.2	5.8 ± 0.1	0–15
Foam height, cm (after)	5.9 ± 0.1	5.8 ± 0.2	5.8 ± 0.1	5.7 ± 0.2	5.7 ± 0.1	5.7 ± 0.2	0–15
Colour	Light green	Light green	Light green	Light green	Light green	Light green	Uniform

Parameter	F0	F1	F2	F3	F4	F5	SNI 8861:2020
Form	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid paste
Odour	Peppermint	Peppermint	Peppermint	Peppermint	Peppermint	Peppermint	Characteristic
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous

Quantitative values are expressed as mean ± SD of three independent replicates. "Before" denotes measurement prior to accelerated storage (25 °C); "after" denotes measurement following accelerated thermal cycling (4 °C and 40 °C). Organoleptic and homogeneity findings were identical before and after storage. SD: standard deviation; SNI: Standar Nasional Indonesia (Indonesian National Standard). No significant difference was detected between pre- and post-storage values for any parameter ($p > 0.05$, one-way ANOVA).

3.3 Antibiofilm Activity of Toothpaste Formulations

The antibiofilm activity of toothpaste formulations F0–F5 against the dual-species biofilm of *S. mutans* and *C. albicans* is presented in Table 3. At 24 h, biofilm inhibition percentages were 5.20 ± 0.02% (F0, vehicle control), 83.20 ± 0.15% (F1), 87.60 ± 0.16% (F2), 92.28 ± 0.60% (F3), 86.45 ± 0.12% (F4) and 80.36 ± 0.18% (F5). The positive control (0.2% CHX) yielded 93.20 ± 0.20% inhibition. At 48 h, inhibition values were 2.96 ± 0.02% (F0), 80.24 ± 0.12% (F1), 84.20 ± 0.26% (F2), 89.30 ± 0.28% (F3), 82.40 ± 0.26% (F4) and 78.20 ± 0.22% (F5), with the positive control yielding 88.40 ± 0.22%.

Biofilm inhibition increased with ascending 24-MCA concentration up to 0.03% (w/w) (F3) but declined at higher concentrations (F4 and F5). One-way ANOVA with Tukey's post hoc analysis revealed no statistically significant difference ($p > 0.05$) between F3 and the 0.2% CHX positive control at either 24 h or 48 h, indicating comparable antibiofilm efficacy. The MBIC of F3 was 62.5 µg/mL against the dual-species biofilm, representing a twofold to fourfold improvement relative to the remaining active formulations (125–250 µg/mL).

Table 3. Biofilm inhibition (%) and minimum biofilm inhibitory concentration (MBIC) of toothpaste formulations F0–F5 against the dual-species biofilm of *S. mutans* and *C. albicans* at 24 h and 48 h.

Formulation	24-MCA concentration (% w/w)	Biofilm inhibition at 24 h (%)	Biofilm inhibition at 48 h (%)
F0	0 (vehicle control)	5.20 ± 0.02	2.96 ± 0.02
F1	0.01	83.20 ± 0.15	80.24 ± 0.12
F2	0.02	87.60 ± 0.16	84.20 ± 0.26
F3	0.03	92.28 ± 0.60	89.30 ± 0.28
F4	0.04	86.45 ± 0.12	82.40 ± 0.26
F5	0.05	80.36 ± 0.18	78.20 ± 0.22
CHX 0.2%	Positive control	93.20 ± 0.20	88.40 ± 0.22

Values are expressed as mean ± SD of four replicate wells across three independent experiments. 24-MCA: 24-methylenecycloartanol; CHX: chlorhexidine gluconate; MBIC: minimum biofilm inhibitory concentration, defined as the lowest concentration producing ≥ 50% reduction in biofilm biomass relative to the untreated growth control; SD: standard deviation. No significant difference was observed between F3 and 0.2% CHX at either time point ($p > 0.05$, one-way ANOVA with Tukey's post hoc test).

4. DISCUSSION

This study represents the first systematic formulation and antibiofilm evaluation of a toothpaste incorporating 24-methylenecycloartanol isolated from *Pometia pinnata* fruit peel. The successful isolation of 30 g of pure 24-MCA from 3 kg of dried plant material demonstrates a commercially viable yield and supports the feasibility of scale-up production. Spectroscopic confirmation consistent with published reference data validates the structural integrity of the isolated compound.[14]

The physicochemical characterisation results demonstrated that all six formulations satisfied the quality criteria stipulated by SNI 8861:2020, Indonesia's national standard for toothpaste. The pH range of 8.00–8.12 falls within the acceptable 6.0–10.0 interval and reflects the alkaline contribution of potassium carbonate as the abrasive excipient. Alkaline toothpaste pH values are well tolerated by the oral mucosa and may confer an additional carioprotective benefit through pH buffering in the oral environment, thereby mitigating cariogenic demineralisation.[15] The viscosity range (20,280–21,024 cps) ensured paste cohesiveness and adequate spreadability without excessive extrusion force. Spreadability values of 6.1–6.3 cm are within the clinically appropriate range to ensure adequate surface coverage during toothbrushing. Crucially, all parameters remained stable following accelerated temperature cycling, confirming adequate shelf-life resilience of the formulations.

The most compelling finding of this study is the superior antibiofilm activity of formulation F3 (0.03% w/w 24-MCA), which produced the highest biofilm inhibition against the dual-species biofilm of *S. mutans* and *C. albicans*. The dual-target efficacy is of particular clinical significance given the synergistic pathogenicity of these organisms in ECC. *S. mutans* produces insoluble glucan via Gtf enzymes that scaffold biofilm architecture, while *C. albicans* hyphae physically reinforce biofilm structure and elaborate quorum-sensing molecules that modulate bacterial behaviour.[7,8] An agent capable of simultaneously inhibiting both organisms at sub-inhibitory concentrations represents a strategically advantageous approach to caries prevention.

The nonlinear dose–response relationship observed — whereby inhibition rose to a maximum at 0.03% (w/w) (F3) and declined at higher concentrations — warrants mechanistic investigation. One plausible explanation relates to the physicochemical behaviour of 24-MCA within the excipient matrix: at concentrations above 0.03% (w/w), the compound may form microscopic aggregates with sodium carboxymethyl cellulose (NaCMC) or potassium carbonate, reducing effective solubilisation and bioavailability at the biofilm interface. The apparent plateau and subsequent decline in efficacy in F4 and F5 may also be attributable to saturation of the antibiofilm mechanism or to increased hydrophobicity that limits penetration into the hydrated biofilm matrix.[16] These observations underscore the importance of systematic formulation optimisation rather than assuming a linear dose–activity relationship for poorly water-soluble active compounds.

The proposed mechanism of antibiofilm activity of 24-MCA is likely multifactorial. Cycloartane-type triterpenoids have been reported to intercalate into microbial cell membranes, disrupting membrane integrity and impeding quorum-sensing signalling pathways critical for biofilm initiation and EPS biosynthesis.[17] Furthermore, inhibition of glucosyltransferase (Gtf) enzymatic activity — as demonstrated for structurally related triterpenoids — may contribute to the observed activity against *S. mutans*.[18] For *C. albicans*, inhibition of hyphal morphogenesis and adhesin expression by triterpenoids has been documented, thereby limiting co-aggregation and biofilm reinforcement.[19,20]

Compared with the positive control (0.2% chlorhexidine gluconate), the biofilm inhibition percentage of F3 was comparable, with no statistically significant difference detected ($p > 0.05$). Chlorhexidine, however, is associated with well-documented adverse effects including dental and restoration staining, taste disturbance, oral mucosal desquamation and disruption of the oral microbiome equilibrium with prolonged use.[10] Systematic evidence indicates that herbal oral care preparations can achieve clinically meaningful plaque and gingivitis reduction with a favourable tolerability profile,[21] while the intrinsic resistance of *C. albicans* biofilms to conventional agents reinforces the value of alternative plant-derived chemistries.[22,23] A natural triterpenoid-based formulation with equivalent antibiofilm efficacy and a substantially improved safety profile therefore represents a clinically meaningful therapeutic option, particularly for chronic daily use in paediatric populations, in whom untreated ECC carries consequences extending well beyond the dentition.[24]

Several limitations of the present study should be acknowledged. Antibiofilm assays were conducted under static, dual-species *in vitro* conditions, which may not fully recapitulate the hydrodynamic complexity and polymicrobial diversity of the *in vivo* oral environment. The long-term chemical stability of 24-MCA within the toothpaste matrix under real-world storage conditions requires further investigation. Additionally, cytotoxicity and genotoxicity evaluations represent essential prerequisites for clinical

translation. Future studies should incorporate *in vivo* biofilm inhibition models, pharmacokinetic assessment of 24-MCA release from the paste matrix during simulated toothbrushing, and randomised controlled clinical trials in paediatric populations.

5. CONCLUSION

This study successfully isolated 24-methylenecycloartanol (24-MCA) from *P. pinnata* fruit peel and incorporated it into six toothpaste formulations at varying concentrations. All formulations complied with the Indonesian national toothpaste standard (SNI 8861:2020) and maintained physicochemical stability under accelerated storage conditions. Formulation F3 (0.03% w/w 24-MCA) was identified as the optimal preparation, achieving biofilm inhibition of 92.28% at 24 h and 89.30% at 48 h against the dual-species biofilm of *S. mutans* ATCC 25175 and *C. albicans* ATCC 10231, with efficacy comparable to that of 0.2% chlorhexidine gluconate. These findings position *P. pinnata*-derived 24-MCA as a promising natural active ingredient for the development of antibiofilm toothpaste formulations for ECC prevention.

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AUTHOR CONTRIBUTIONS

Risyandi Anwar: conceptualisation, methodology, isolation and structural elucidation of the active compound, supervision, funding acquisition, and writing — review and editing. **Ayu Kristin Rakhmawati:** investigation, formulation development, physicochemical characterisation, data curation, and writing — original draft. **Susi Sukmasari:** methodology, microbiological assay design, validation, and writing — review and editing. **M. Fadhil Zidni H:** investigation, execution of the antibiofilm assays, formal analysis, and visualisation. All authors have read, critically revised and approved the final version of the manuscript, and agree to be accountable for all aspects of the work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ETHICS STATEMENT

The protocol was reviewed and approved by the Health Research Ethics Committee, Faculty of Dentistry, Universitas Muhammadiyah Semarang (Approval No. 021/EC/KEPK/FKG-UNIMUS/II/2025). The study did not involve human participants, human-derived clinical specimens or animal subjects.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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