Original Article

Effectiveness of Pandan Leaf Extract as An Irrigant Against *Enterococcus Faecalis:* A Laboratory Study

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Abstract

This study aimed to evaluate the pH, antimicrobial efficacy against *Enterococcus faecalis (E. faecalis)*, and the effects on dentin mineral density of *Pandanus amaryllifolius* leaf extract solutions, compared with distilled water and 2.5% sodium hypochlorite as root canal irrigants. Pandan leaf extracts at concentrations of 32, 64, and 128 mg/mL were prepared and tested against distilled water and 2.5% sodium hypochlorite. The sample size for each experimental group consisted of 10 root canals. The pH of each irrigant was measured with a calibrated pH meter. Antimicrobial activity was determined by measuring colony-forming units per milliliter (CFU/mL) of *E. faecalis* after irrigation. Dentin mineral density was assessed using micro-computed tomography (micro-CT). All experiments were performed in triplicated. Statistical analysis was performed using Kruskal-Wallis test, followed by multiple comparisons at a significance level of $p < 0.05$. Distilled water showed a slightly acidic pH (6.8 ± 0.1), while sodium hypochlorite was strongly alkaline. Pandan leaf extracts exhibited alkaline pH values that increased with increasing concentration. Antimicrobial testing demonstrated a dose-dependent reduction of *E. faecalis*, with 128 mg/mL producing the most pronounced effect, comparable to sodium hypochlorite. Micro-CT analysis showed no significant differences in dentin mineral density among all groups. Higher concentrations of pandan leaf extract exhibited notable antimicrobial activity against *E. faecalis*, with no adverse effect on dentin mineral density. Its alkaline nature may enhance anti-bacterial properties.

Keyword: Antimicrobial activity, Dentin mineral density, Micro-computed tomography, *Pandanus amaryllifolius*, Root canal irrigation

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Introduction

Sodium hypochlorite (NaOCl) has been widely recognized and used as an effective antimicrobial agent in various fields of medicine, including dentistry, due to its broad-spectrum bactericidal properties.[1] Chemically, sodium hypochlorite dissociates in aqueous solution to produce sodium ions (Na^+) and hypochlorite ions (OCl^-),

which exist in equilibrium with hypochlorous acid (HOCl)[2]. This equilibrium is strongly influenced by the pH of the solution: at alkaline pH values above 9.0, the predominant species is the OCl^-, whereas at acidic or neutral pH, HOCl prevails. While HOCl is known to exhibit stronger antimicrobial activity, OCl^- remains the dominant and more stable form in alkaline conditions. OCl^- acts as an effective oxidizing agent capable of disrupting microbial components and is widely used as a disinfectant and irrigant in dental and medical applications due to its stability and broad-spectrum antimicrobial efficacy.[2,3]

Historically, NaOCl was initially introduced in the 18th century as a bleaching agent. Later, in the 19th century, Labarraque pioneered its use in medicine for infection control, notably for preventing puerperal fever and various other infections. The landmark discoveries by Robert Koch and Louis Pasteur confirmed the strong antimicrobial properties of sodium hypochlorite, leading to its widespread adoption as a disinfectant and antiseptic in healthcare settings worldwide. Its ability to kill a wide range of microorganisms, including bacteria, viruses, fungi, and spores, makes it a versatile and indispensable agent in infection control.[4]

In the field of endodontics, NaOCl is the irrigant of choice for root canal therapy because of its excellent antimicrobial properties and its unique ability to dissolve organic tissue remnants within the root canal system. Effective root canal disinfection is essential for the success of endodontic treatment, as persistent microbial infection is a primary cause of treatment failure. The concentration of sodium hypochlorite used in clinical practice ranges from 0.5% to 6%, with higher concentrations providing faster and more effective antimicrobial action and tissue dissolution. However, the choice of concentration must balance efficacy and safety, as higher concentrations are associated with increased cytotoxicity and the potential for damage to periapical tissues if extruded beyond the apex.[5]

Despite its advantages, sodium hypochlorite also has notable limitations when used as a root canal irrigant. One major limitation is its inability to effectively remove the smear layer, a layer of organic and inorganic debris created on canal walls during instrumentation. While NaOCl can degrade the organic portion of the smear layer, it does not dissolve the inorganic components, necessitating the use of chelating agents such as ethylenediaminetetraacetic acid (EDTA) in subsequent irrigation steps. Furthermore, NaOCl is highly cytotoxic and can cause severe irritation or damage to periapical tissues, oral mucosa, and skin upon accidental extrusion or contact. Its unpleasant taste and strong odor can reduce patient compliance and comfort during treatment. Sodium hypochlorite is also corrosive to dental instruments and can degrade the organic matrix of dentin, which may adversely affect the mechanical properties of the tooth, including elasticity and flexural strength. Moreover, its antimicrobial efficacy is reduced in the presence of organic matter, which is abundant within the root canal system.[6,7]

Given these drawbacks, there has been increasing interest in exploring alternative root canal irrigants derived from natural products, particularly herbal extracts. Herbal medicines have been used for centuries across many cultures for treating a wide range of medical conditions. Compared to synthetic drugs, herbal extracts often have fewer side effects, are generally more biocompatible, and are more accessible and affordable, especially in resource-limited settings. Many plants contain bioactive compounds such as phenolics, flavonoids, alkaloids, and terpenoids, which exhibit antimicrobial, anti-inflammatory, and antioxidant properties. The incorporation of such herbal extracts into endodontic irrigation protocols could potentially improve antimicrobial effectiveness, while minimizing toxicity and other adverse effects.

Among various medicinal plants, pandan leaf (*Pandanus amaryllifolius*), commonly known as pandan or "toey hom" in Thai, has gained attention for its potential oral health benefits. Traditionally used in Southeast Asian cuisine and folk medicine, pandan leaf contains several bioactive compounds, including phenolic compounds, which have demonstrated antimicrobial activity against oral pathogens. Phenolic compounds can interfere with bacterial cell walls, disrupt membrane permeability, and inhibit enzyme activity, thus suppressing microbial growth. Previous studies have shown that ethanolic and aqueous extracts of pandan leaf can inhibit the

growth of oral bacteria such as *Streptococcus sanguinis*, *Streptococcus salivarius*, *Streptococcus mutans*, and *Porphyromonas gingivalis* at relatively low minimal inhibitory concentrations (MIC). These bacteria are known contributors to dental caries and periodontal diseases.[8] However, the effectiveness of pandan leaf extract against *E. faecalis*, a resilient and common pathogen associated with persistent root canal infections, appears to require higher concentrations. A prior in vitro study reported that ethanolic pandan leaf extract inhibited *E. faecalis* at an MIC of 32.5 mg/mL, indicating antimicrobial potential, albeit at relatively high concentration.

Despite promising antimicrobial activity, to date there has been no research investigating the use of pandan leaf extract as an irrigant in root canal treatment, particularly focusing on its effects against *E. faecalis.* Considering the limitations of NaOCl and the growing demand for safer, biocompatible alternatives, exploring pandan leaf extract as a component in root canal irrigation solutions is timely and warranted. The development of such herbal-based irrigants could offer benefits including reduced cytotoxicity, better patient tolerance, and potential cost-effectiveness, while harnessing the natural antimicrobial properties of the plant.

Therefore, this study aimed to formulate a root canal irrigant incorporating pandan leaf extract and to evaluate its physical properties and antimicrobial efficacy against *E. faecalis*. Understanding these characteristics would contribute to the development of novel endodontic irrigants that combine herbal medicine with modern dental practice, potentially improving clinical outcomes and patient safety.

Materials and methods

Preparation of Pandan Leaf Extract

The pandan leaf extract was prepared in liquid form using a method currently under petty patent application with the department of intellectual property of Thailand (application number 1-2403003729), filed by Nattapon Rotpenpian. Fresh pandan leaves were dried and ground into powder before extraction with 95% ethanol. The extract was filtered through muslin cloth, centrifuged to remove precipitates, and subsequently filtered through a 0.45 µm membrane filter. The filtrate was concentrated by rotary evaporation. Ethanol, a semi-polar solvent capable of dissolving both polar and non-polar compounds, was used as an extraction solvent. The yield of the extract was approximately 6–7%.[8]

Preparation of Root Canal Irrigants

Five experimental groups of irrigants were prepared: Group 1 – distilled water (negative control); Group 2 – pandan leaf extract at 32 mg/mL; Group 3 – pandan leaf extract at 64 mg/mL; Group 4 – pandan leaf extract at 128 mg/mL; and Group 5 – 2.5% NaOCl solution (positive control). Sample size was calculated based on previous studies with α = 0.05, power = 0.8, and five groups, resulting in a total of 50 samples (10 specimens per group).[9]

Measurement of Physical Properties

The pH of each irrigant solution was measured and compared among the groups to assess their physical properties.[10]

Tooth Preparation

Fifty extracted human single-rooted anterior and premolar teeth were selected based on the following criteria: single straight root canal with one apical foramen, closed apex, and absence of cracks at the root tip. Teeth were stored in 1% formalin solution and cleaned. The crowns were sectioned at the cementoenamel junction (CEJ) to achieve a standardized root length of 17 mm. Working length was established by inserting a size 10 K-file until it was visible at the apical foramen, then subtracting 1 mm (working length = 16 mm). Canal preparation involved coronal enlargement with Gates-Glidden drills sizes 2, 3, 4, and 5, followed by apical preparation with K-files to working length. Teeth were dried overnight at room temperature. The apical foramina were sealed with two layers of clear nail varnish to prevent bacterial leakage. All specimens were sterilized by autoclaving at 121°C and 15 PSI for 20 minutes.[9]

Bacterial Culture and Inoculation

E. faecalis (ATCC 29212) was obtained from Department of Oral biology and Occlusion, Faculty of Dentistry, Prince of Songkla University and cultured microaerobically on blood agar at 37 °C for 48 h. Pure

colonies were then grown in Brain Heart Infusion (BHI) broth at 37°C until the logarithmic growth phase was reached. The bacterial suspension was adjusted to an optical density of 0.5 at 600 nm corresponding to approximately 1.5×10^8 colony forming units per milliliter (CFU/mL). Thirty microliters of this suspension were injected into each root canal. The inoculated teeth were incubated in a biosafety cabinet at 37°C for 48 h to allow bacterial colonization.[11]

Root Canal Irrigation and Microbial Sampling

The infected teeth were initially sterilized by autoclaving to eliminate any pre-existing microorganisms and to standardize the experimental conditions. Subsequently, the root canals were artificially contaminated with a standardized bacterial suspension (1.5×10^8 CFU/mL) and incubated for 48 hours to allow bacterial colonization prior to irrigation procedures. The specimens were then randomly assigned to five irrigant groups. Root canals were irrigated with 5 mL of the assigned solution for 3 minutes. Following irrigation, 10 µL of fresh culture medium was transferred into the canals and incubated for an additional 48 hours to evaluate possible bacterial regrowth after irrigation, simulating the post-irrigation condition within the canal system. After incubation, samples were collected, serially diluted, and plated on agar to determine the bacterial count. The antimicrobial activity of each irrigant was expressed as the number of colony-forming units per milliliter (CFU/mL)

Bacterial count

E. faecalis bacterial counts were assessed using sterile microbrushes, followed by placement of sterile paper points into each canal for 5 minutes. Both the microbrushes and paper points were immediately transferred into 500 µL of sterile BHI broth and vortexed for 30 seconds to ensure bacterial release prior to plating. The cultures were incubated at 37 °C for 48 h. Colony growth was evaluated based on morphology on blood agar and confirmed by Gram staining. Colony-forming units (CFUs) were enumerated by two independent examiners. Each examiner visually inspected the agar plates, and CFUs were manually counted using a standard grid reference to ensure consistency. Independent counts were recorded separately to minimize bias and confirm reproducibility of results.[11]

Micro-CT scan Analysis

The specimens were analyzed using a high-resolution micro-computed tomography system (MicroCT35; SCANCO Medical, Bassersdorf, Switzerland). Scanning was conducted at 70 kVp, 114 µA, and 8 W power, with an integration time of 800 ms. Images were acquired at an isotropic voxel size of 15 µm to provide high-resolution visualization of internal microstructures. A total of 1000 projections were collected over a 360° rotation using a cone-beam geometry. The raw projection data were reconstructed with a filtered back-projection algorithm to generate axial cross-sectional images. The reconstructed datasets were exported as 16-bit grayscale images with a resolution of 2048 × 2048 pixels. Subsequent image processing and quantitative analyses were performed using Scanco Evaluation Software, with segmentation carried out by global thresholding to differentiate voids from solid phases. Calibration was performed using a hydroxyapatite phantom to ensure quantitative accuracy of the mineral density measurements.[12]

Statistical Analysis

Data were analyzed using SPSS software. Results were presented as mean ± standard deviation. Normality was tested with the Kolmogorov-Smirnov test. For non-normal data, the Kruskal-Wallis test followed by multiple comparisons was applied. A significance level of 0.05 was adopted.

Results

pH of chemical irrigations

The pH values of the root canal irrigants are presented in Table 1. Distilled water (Group 1) showed a slightly acidic pH of 6.8 ± 0.1. The pandan leaf extract solutions (Groups 2, 3, and 4) exhibited alkaline pH values ranging from 9.2 ± 0.2 to 9.8 ± 0.1, with pH increasing slightly as the concentration of the extract increased. The highest concentration group (128 mg/mL) showed the most alkaline pH among the pandan leaf extract groups. The NaOCl solution (Group 5) had the highest pH at 11.5 ± 0.3, consistent with its known strongly alkaline nature. Statistical analysis indicated significant differences in pH values among all groups ($p < 0.05$) as shown in table 1.

Table 1 *pH value of chemical irrigants*

Group	Solution	pH (mean ± SD)
1	Distilled water	6.8 ± 0.1
2	32 mg/mL Pandan leaf extract	9.2 ± 0.2*
3	64 mg/mL Pandan leaf extract	9.5 ± 0.15*
4	128 mg/mL Pandan leaf extract	9.8 ± 0.1*
5	2.5% NaOCl	11.5 ± 0.3*

*Note: * significantly different when compared to distilled water ($p < 0.05$).*

Colony formation after Pandan leaf extract irrigation

The antimicrobial efficacy of the tested irrigants against *E. faecalis* (ATCC 29212) was evaluated by measuring the average of CFU/mL after treatment, as shown in Table 2. As expected, the distilled water group (Group 1), which served as the negative control, exhibited no antimicrobial activity, with the highest mean bacterial count at 2.00 ± 0.23 CFU/mL. The pandan leaf extract groups demonstrated a concentration-dependent reduction in bacterial counts. Group 2 (32 mg/mL) showed a mean CFU of 1.98 ± 1.24, Group 3 (64 mg/mL) showed a further reduction with a mean CFU of 1.46 ± 3.33, and Group 4 (128 mg/mL) exhibited the most significant bacterial reduction among the pandan leaf extract groups, with a mean CFU of 0.42 ± 0.40. The positive control, 2.5% sodium hypochlorite (Group 5), showed a comparable antimicrobial effect with a mean CFU of 0.38 ± 0.17. However, there are no differences among all groups. These findings indicate that pandan leaf extract at higher concentrations can effectively reduce *E. faecalis* populations in root canals, with efficacy approaching that of sodium hypochlorite as shown in Table 2.

Table 2 *E.faecalis colony countt across different experimental group*

Group	Solution	Mean CFU/mL (± SD)
1	Distilled water	2.00 ± 0.23
2	32 mg/mL Pandan leaf extract	1.98 ± 1.24
3	64 mg/mL Pandan leaf extract	1.46 ± 3.33
4	128 mg/mL Pandan leaf extract	0.42 ± 0.40
5	2.5% NaOCl	0.380 ±0.17

no significant differences were found ($p > 0.05$).

Micro-CT scan in root canal

Micro-computed tomography (micro-CT) analysis demonstrated that dentin specimens irrigated with distilled water, pandan leaf extract at concentrations of 32 mg/mL, 64 mg/mL, and 128 mg/mL, as well as 2.5% NaOCl, exhibited no statistically significant differences in dentin mineral density ($p > 0.05$). The mean dentin density values across all experimental groups remained comparable and not significant difference as shown in table 3 and figure 1.

Table 3 *Dentin mineral density (mean ± SD) following immersion in distilled water, Pandan leaf extract at varying concentrations and sodium hypochlorite*

Group	Solution	Dentin mineral density (mgHA/cm^3)
1	Distilled water	0.92 ± 0.46
2	32 mg/mL Pandan leaf extract	0.91 ± 0.57
3	64 mg/mL Pandan leaf extract	0.91 ± 0.58
4	128 mg/mL Pandan leaf extract	0.89 ± 0.84
5	2.5% NaOCl	0.91 ±0.47

no significant differences were found ($p > 0.05$).

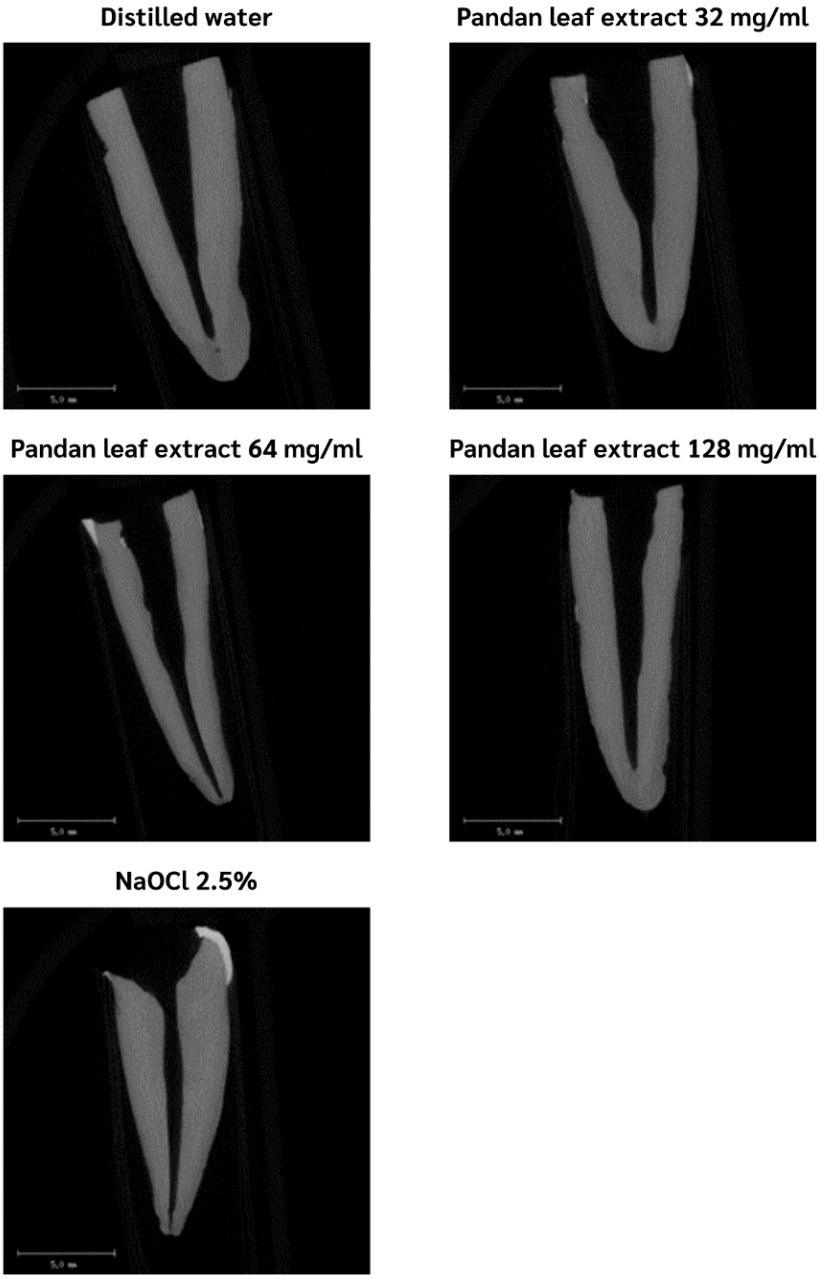

Figure 1 *The radicular dentin density by micro-CT scan with 5.0 mm magnification*

Discussion

The present study investigated the physicochemical properties, antimicrobial efficacy, and effects on dentin mineral density of Pandanus amaryllifolius (pandan leaf) extract solutions compared with distilled water and 2.5% NaOCl, when used as root canal irrigants. These findings provide important insights into the potential role of pandan leaf extract as a natural alternative or adjunct to conventional root canal irrigants.[13]

pH is a critical parameter influencing both antimicrobial activity and compatibility effects on dentin structure. In the present study, distilled water was slightly acidic (pH 6.8), consistent with prior reports of neutral-to-slightly-acidic pH values for deionized water. In contrast, pandan leaf extract solutions were alkaline, with pH ranging from 9.2 to 9.8. This alkalinity may be attributed to the presence of bioactive compounds, including flavonoids, alkaloids, and phenolic compounds, some of which can form basic salts or release hydroxyl ions in aqueous solution and might be compatible in root canal homeostasis. The pH increased modestly with extract

concentration, suggesting a concentration-dependent release of these constituents.[13]

NaOCl displayed the highest pH value (11.5), in agreement with its well-established strong alkalinity. The alkaline nature of NaOCl contributes to its antimicrobial action and ability to dissolve organic tissue. Pandan leaf extract, while less alkaline, still falls within a range that may inhibit bacterial growth. An alkaline pH has been reported to destabilize bacterial cytoplasmic membranes and interfere with enzymatic activity, potentially explaining part of the observed antimicrobial effect of pandan leaf extract.[14]

The antimicrobial assay revealed that pandan leaf extract exhibited concentration-dependent efficacy against *E. faecalis,* with the 128 mg/mL concentration achieving bacterial counts comparable to those of 2.5% NaOCl. *E. faecalis* is a well-documented pathogen associated with persistent endodontic infections and root canal treatment failures due to its ability to form biofilms, penetrate dentinal tubules, and survive in nutrient-poor environments.[15]

The marked reduction in CFU observed in the 128 mg/mL pandan leaf extract group may be linked to the phytochemical profile of *P. amaryllifolius.* Previous studies have reported the presence of antimicrobial agents such as 2-acetyl-1-pyrroline, phenolic acids, and essential oils. Phenolic compounds, in particular, can disrupt bacterial cell walls, cause protein denaturation, and interfere with nucleic acid synthesis. In addition, pandan leaf extract's alkaline pH may enhance cell wall disruption, similar to the mechanism proposed for calcium hydroxide.[16]

While NaOCl remains the gold standard irrigant due to its potent antibacterial and tissue-dissolving properties, its use is associated with drawbacks, including cytotoxicity, risk of extrusion injuries, and unpleasant taste or odor. The comparable antimicrobial performance of high-concentration pandan leaf extract observed in this study suggests that it may serve as a less cytotoxic, plant-based alternative, although further cytotoxicity and biocompatibility testings are essential before clinical application.

The search for herbal and plant-derived irrigants has been driven by the desire to find effective but biocompatible alternatives to NaOCl. Numerous plant extracts, such as *Azadirachta indica* (neem), *Camellia sinensis* (green tea), *Allium sativum* (garlic), and *Curcuma longa* (turmeric), have shown varying degrees of antimicrobial activity against endodontic pathogens. Pandan leaf extract shares common phytochemical traits with some of these plants, particularly the presence of phenolics and flavonoids, which are known to possess antibacterial, antioxidant, and anti-inflammatory properties.[17]

Compared with neem or green tea extracts, which tend to have a more neutral pH, pandan's alkaline nature could offer a distinct advantage in bacterial inhibition. However, unlike NaOCl, plant extracts generally lack proteolytic tissue-dissolving capability, which is essential for effective debridement. Therefore, pandan extract may be best positioned as an adjunctive irrigant or as part of a sequential irrigation protocol, rather than as a standalone replacement for NaOCl.[18]

From a clinical perspective, the findings of this study suggest that pandan leaf extract, particularly at higher concentrations, holds promise as a root canal irrigant. Its significant antimicrobial activity against *E. faecalis,* coupled with its lack of adverse effects on dentin mineral density, is encouraging.[9] Nevertheless, several limitations must be acknowledged. First, the study used a planktonic bacterial model rather than a mature biofilm model, which is more resistant to antimicrobial agents. Second, no cytotoxicity testing was conducted, and the safety of pandan leaf extract on periapical tissues remains unknown. Third, the absence of proteolytic activity limits its debridement potential compared with NaOCl.

Therefore, while pandan leaf extract may not replace NaOCl entirely, it could serve as a supplementary irrigant to reduce bacterial load while minimizing tissue damage. A potential application could be alternating NaOCl and pandan leaf extract during irrigation to balance efficacy and safety.[9]

Further researches are needed to confirm the antimicrobial effects of pandan leaf extract in more clinically relevant models, including multispecies biofilms and

dentinal tubule penetration assays. Cytotoxicity and genotoxicity studies will be critical to establish safety profiles. Investigating the optimal concentration, contact time, and combination protocols with other irrigants could help define its role in endodontic practice. Additionally, chemical analysis to identify and quantify the active compounds responsible for its antimicrobial action would aid in standardizing extract preparation and potency.

However, there are several limitations of this study including the proteolytic activity of pandan leaf extract, an important property of ideal endodontic irrigants, was not evaluated, and thus its role should be considered supplementary to conventional irrigants such as NaOCl. Antimicrobial testing was performed using planktonic bacteria rather than mature or multispecies biofilms, and further studies are warranted to assess its efficacy against biofilms and its penetration into dentinal tubules. In addition, cytotoxicity and biocompatibility were not investigated, highlighting the need for *in vitro* safety evaluation prior to clinical application. While dentin mineral density was unaffected, other mechanical properties, including microhardness, bonding strength as well as ultrastructural characteristics assessed by SEM, were not evaluated. The ability of the extract to remove smear layers was not examined, and its potential combination with chelating agents such as EDTA may be explored. Finally, although bioactive components were described, they were not quantified, and analytical techniques such as GC–MS or HPLC should be applied to ensure consistency and reproducibility. Further *in vivo* and cytotoxicity studies are necessary before clinical use.

Conclusion

This study demonstrates that *P. amaryllifolius* leaf extract possesses alkaline pH and significant antimicrobial activity against *E. faecalis*, with its highest concentration performing comparably to 2.5% NaOCl, while causing no measurable change in dentin mineral density. These findings support further investigation into pandan leaf extract as a potential natural irrigant or adjunct in root canal therapy. However, clinical validation, safety assessment, and optimization of its use remain essential steps before translation into practice.

Abbreviations NaOCl: Sodium hypochlorite
Micro-CT: Micro-computed tomography

Ethical Statements: All experiments were approved by Human research ethics committee of Faculty of Dentistry, Prince of Songkla university EC 6711-050

Conflicts of Interest: The authors declare no conflicts of interest

Author Contributions: PT: data curation, formal analysis, investigation, methodology, writing original draft; KK: formal analysis, investigation, validation, writing – review and editing; NR: data curation, investigation, writing – writing – review and editing; All authors reviewed and approved the final version of the manuscript.

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