



ORIGINAL ARTICLE

Antimicrobial activity of red dragon fruit peel extract against *Staphylococcus aureus* for root canal irrigation

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ABSTRACT

Staphylococcus aureus is a type of bacteria that can colonise root canals, leading to infection and pain. Root canal irrigants are substances used during root canal treatment, and sodium hypochlorite is the gold standard, despite its toxicity. Red dragon fruit peel has several active compounds with antibacterial properties. This study aimed to determine the effectiveness of red dragon fruit peel extract (*Hylocereus polyrhizus*) against *S. aureus* as a root canal irrigant. This was an experimental study with a post-test-only group design. The study population was pure *S. aureus*. The study consisted of five treatment groups: red dragon fruit peel extract at concentrations of 80%, 40%, and 20%, 0.2% chlorhexidine, and DMSO, with five repetitions. Antibacterial effectiveness was tested using the disc diffusion method. Data were analysed using the Kruskal-Wallis statistical test and Post Hoc LSD. The study results showed average inhibition zone diameters of red dragon fruit peel extract against *S. aureus* of 12.18 ± 0.228 mm, 11.16 ± 0.305 mm, and 9.54 ± 0.603 mm for concentrations of 80%, 40%, and 20%, respectively. The highest average inhibition zone diameter was found in the positive control (0.2% chlorhexidine), which was 13.20 ± 0.255 mm, while the negative control (DMSO) showed no inhibition against *S. aureus*. The Kruskal-Wallis test results indicated that there was a significant effect of red dragon fruit peel extract against *S. aureus* ($p=0.0001$; $p<0.05$). The Post Hoc LSD test results showed that there were differences in the effectiveness of red dragon fruit peel extract between the two different groups ($p=0.0001$; $p<0.05$). Red dragon fruit peel extract can be used as a root canal irrigant, with a concentration of 80% being the most effective concentration.

Keywords: irrigating solutions, sodium hypochlorite, Dragon fruit peel, *Staphylococcus aureus*, root canals

Introduction

Dental and oral diseases represent a significant health concern within the general population, with dental caries being a prevalent condition that can progress to pulpal and periapical diseases. Endodontic treatment, or root canal therapy, is the standard approach for managing these pulpal and periapical infections.¹ The primary objective of root canal treatment is to restore the natural tooth's form and function, enabling its continued use in efficient mastication.² Following disinfection, the root canal system is obturated to prevent microbial re-entry and proliferation.³

A diverse range of bacteria can be found within root canals, including *Staphylococcus aureus*.⁴ This bacterium is frequently identified as a normal commensal flora and is a Gram-positive coccus with a diameter of 0.7-1.2 μ m. It typically forms irregular clusters resembling grapes and exhibits optimal growth at 37°C, although pigment production is more pronounced at room temperature (20-25°C).^{5,6,7} *S. aureus* is among the

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most frequently isolated microorganisms from dentoalveolar abscesses and is pathogenic in various oral diseases, such as oral mucositis, periodontitis, peri-implantitis, endodontic infections, and dental caries. Its capacity to penetrate dentinal tubules renders biomechanical preparation alone insufficient for bacterial eradication.^{7,8,9}

The persistent survival of bacteria within root canals can lead to endodontic treatment failure. Consequently, irrigating solutions are essential for removing organic debris, microorganisms, residual pulp tissue, smear layers, and endotoxins from the root canal system.^{10,11} These irrigants are medicated liquids designed to eliminate microorganisms within the tooth's cavity and root canals.¹¹ Sodium hypochlorite, at concentrations of 0.5% to 5.25%, is a commonly used root canal irrigant and considered a gold standard due to its effectiveness in tissue dissolution and its antiseptic properties.¹² However, sodium hypochlorite exhibits certain limitations, including toxicity to periapical tissues and the potential to compromise the micromechanical properties of dentin within the root canal walls. Therefore, exploring alternative root canal irrigants is warranted.¹¹

Indonesia possesses a rich biodiversity, including the dragon fruit (*Hylocereus polyrhizus*), a cactus species that has recently garnered public attention.¹² Red dragon fruit is a vitamin-rich herb with reported benefits in aiding digestion due to its fibre content, preventing colon cancer and diabetes, neutralizing toxins such as heavy metals, and assisting in the reduction of cholesterol levels and hypertension.¹¹ While the utilization of dragon fruit has primarily focused on its pulp, its peel, which constitutes 30-35% of the total fruit, also holds significant potential. Despite this, the peel is often discarded as waste. Several studies have indicated that red dragon fruit peel possesses antibacterial and antifungal properties due to the presence of various active compounds, including alkaloids, terpenoids, and flavonoids.¹³ Furthermore, the peel exhibits higher antioxidant activity than the pulp, suggesting its potential as a natural antioxidant source.^{14,15}

Previous research by Astridwiyanti et al.¹³ demonstrated the inhibitory effect of red dragon fruit peel ethanol extract against *S. aureus* ATCC 25923, with a minimum inhibitory concentration observed at 25%. Subsequent research by Sukmawati et al.¹⁶ reported antibacterial activity of red dragon fruit peel extract against *S. aureus* at a 50% concentration, resulting in an inhibition zone diameter of 28.3 mm. Similarly, Sari et al.¹⁰ found that red dragon fruit peel ethanol extracts at 20%, 40%, and 80% concentrations exhibited weak antibacterial inhibitory activity against *Propionibacterium acnes*. This study aims to investigate the effectiveness of red dragon fruit peel extract against *S. aureus* as a potential root canal irrigant.

Method

This experimental study employed a post-test only group design to evaluate the antibacterial properties of red dragon fruit peel extract. The extract was prepared at the Pharmaceutical Phytochemistry Laboratory of Universitas Sumatera Utara (USU), and the antibacterial testing was conducted at the Pharmaceutical Microbiology Laboratory of USU between October and November 2024. The study utilized a pure culture of *Staphylococcus aureus* as the bacterial sample. The sample size was determined using Federer's formula, resulting in a total of 25 samples distributed across 5 groups, with 5 repetitions per group.

The following equipment was used: masks, gloves, micropipettes, Bunsen burners, sterile inoculation loops, digital balances, test tubes, test tube racks, laminar air flow (LAF) hoods, Petri dishes, spreaders, incubators, digital calipers, forceps, cotton swabs, stirrers, graduated cylinders, blenders, knives, Erlenmeyer flasks, beakers, autoclaves, drying ovens, Buchner funnels, filter paper discs, and rotary vacuum evaporators. The materials included: *S. aureus*, 70% ethanol, red dragon fruit peel, cotton buds, tissue paper, Mueller Hinton Agar (MHA) media, barium chloride, sulfuric acid, and sterile distilled water.

The red dragon fruit peel was separated from the fruit, washed under running water, cut into small pieces, and sorted. Subsequently, the peel was dried in an oven and ground into powder using a blender. Extraction of the red dragon fruit peel's colored compounds was performed using maceration with 70% ethanol (6-7 liters) for 3 x 24 hours, followed by filtration. The filtrate was then concentrated using a rotary vacuum evaporator at 60-70°C until the extract volume was reduced. The evaporated product was collected to obtain a thick extract of red dragon fruit peel.¹³

S. aureus bacteria were cultured from a pure culture using a sterile loop and then streaked onto Mueller Hinton Agar (MHA) media, followed by incubation for 24 hours at 37°C.⁷ The diffusion agar test, using the Kirby-Bauer method with filter paper discs, was employed to assess antibacterial activity. The revitalized pure culture of *S. aureus* was taken and cultured in sterile distilled water, then homogenized using a vortex mixer. The MHA media in Petri dishes was inoculated with the *S. aureus* culture by spreading it across the

agar surface using sterile cotton swabs. Filter paper discs (6 mm diameter) impregnated with the test solution were placed onto the inoculated media.¹⁷

Observations were made after 24 hours of incubation. The diameter of the inhibition zone, characterized by a clear area surrounding the filter paper disc, indicated the extent of bacterial growth inhibition by the antibacterial substance. The inhibition zone formed around the disc was measured using digital calipers, recording both vertical and horizontal diameters in millimeters.¹⁷

Results

Phytochemical screening was conducted to identify secondary compounds within the stem bark extract of the red dragon fruit, employing established methods. Table 1 presents the results of this screening, demonstrating the presence of several active compounds, including flavonoids, alkaloids, tannins, saponins, triterpenoids/steroids, and glycosides.

Secondary Metabolite	Reagent	Result
Flavonoid	MgHCl + H ₂ SO ₄	+
Alkaloid	Bouchardat's, Mayer's, Dragendorff's	+
Tannin	FeCl ₃	+
Saponin	Aquades	+
Steroid	Liebermann-Burchard	+
Glycoside	Molisch + H ₂ SO ₄	+

The diameter of the inhibition zones resulting from the application of red dragon fruit stem bark extract at concentrations of 80%, 40%, and 20% against *S. aureus* was measured. As shown in Table 2, the inhibition zone diameters were 12.18 ± 0.228 mm, 11.16 ± 0.305 mm, and 9.54 ± 0.603 mm, respectively. The highest inhibition zone diameter was observed in the positive control (0.2% chlorhexidine), measuring 13.20 ± 0.255 mm. Conversely, no inhibition zone was observed in the negative control (DMSO).

Group	Diameter of the zone of inhibition (mm)					
	Replicates					
	1	2	3	4	5	
Red dragon fruit peel ethanol extracts 80%	12,1	12,5	11,9	12,3	12,1	12,18±0,228
Red dragon fruit peel ethanol extracts 40%	11,3	11,6	11,1	11	10,8	11,16±0,305
Red dragon fruit peel ethanol extracts 20%	10,5	9,4	9,7	9,1	9	9,54±0,603
K+ (Chlorhexidine 0,2%)	13,0	13,3	13,6	13,1	13,0	13,20±0,255
K- (DMSO)	0	0	0	0	0	0

As shown in Table 3, the results of the normality and homogeneity tests indicated that the data were not normally distributed ($p < 0.05$) but were homogeneous ($p > 0.05$). Therefore, the data analysis for this study employed the Kruskal-Wallis statistical test and Post Hoc LSD.

Group	Normality	Homogeneity
	p value	p value
Red dragon fruit peel ethanol extracts 80%	0,003	0,175
Red dragon fruit peel ethanol extracts 40%		
Red dragon fruit peel ethanol extracts 20%		
K+ (Chlorhexidine 0,2%)		
K- (DMSO)		

According to Table 4, the Kruskal-Wallis statistical test revealed a significant difference in the mean diameter of the inhibition zone against *S. aureus* among the groups ($p = 0.0001$; $p < 0.05$). Thus, red dragon fruit peel extract at concentrations of 80%, 40%, and 20% was effective in artificial saliva irrigation.

Table 4. Effectiveness of red dragon fruit peel extract against *S. aureus* in artificial saliva irrigation

Group	Diameter of the zone of inhibition	p
K+ (Chlorhexidine 0,2%)	13,20±0,255	0,0001
K- (DMSO)	0	
Red dragon fruit peel ethanol extracts 80%	12,18±0,228	
Red dragon fruit peel ethanol extracts 40%	11,16±0,305	
Red dragon fruit peel ethanol extracts 20%	9,54±0,603	

As presented in Table 5, the Post Hoc LSD test indicated significant differences between 80% red dragon fruit peel extract and 40% and 20% red dragon fruit peel extract ($p = 0.0001$), between 40% and 20% red dragon fruit peel extract ($p = 0.0001$), between the positive control and 80%, 40%, and 20% red dragon fruit peel extract ($p = 0.0001$), and between the negative control and 80%, 40%, and 20% red dragon fruit peel extract ($p = 0.0001$).

Table 5. Comparison of effectiveness of red dragon fruit peel extract against *S. aureus* in artificial saliva irrigation

Group	p value
K-	0,0001
K+ (Klorheksidin 0,2%)	Red dragon fruit peel ethanol extracts 80%
	Red dragon fruit peel ethanol extracts 40%
	Red dragon fruit peel ethanol extracts 20%
K- (DMSO)	Red dragon fruit peel ethanol extracts 80%
	Red dragon fruit peel ethanol extracts 40%
	Red dragon fruit peel ethanol extracts 20%
Red dragon fruit peel ethanol extracts 80%	Red dragon fruit peel ethanol extracts 40%
	Red dragon fruit peel ethanol extracts 20%
Red dragon fruit peel ethanol extracts 40%	Red dragon fruit peel ethanol extracts 20%

Discussion

Certain antibacterial compounds function by inhibiting bacterial growth through various mechanisms, including disrupting bacterial cell metabolism.¹⁹ Phytochemical screening of red dragon fruit peel extract (Table 1) revealed the presence of several active compounds, including flavonoids, alkaloids, tannins, saponins, steroids, and glycosides, which contribute to its inhibitory effect against *S. aureus*. This aligns with the findings of Siregar and Daniel¹⁹, who reported that ethanolic extracts of red dragon fruit peel tested positive for alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids/steroids.

Flavonoids are a group of phenolic compounds known for their antiviral, antibacterial, and antifungal properties.¹⁷ The mechanism of action of flavonoids involves disrupting the bacterial cell membrane, leading to uncontrolled water influx and ultimately cell lysis.¹⁸ Alkaloids are polar compounds with bioactive properties. They can interfere with peptidoglycan synthesis in bacterial cell walls, inhibiting cell wall formation and causing cell death.¹⁹ Other compounds, such as tannins, exert antibacterial effects by coagulating bacterial protoplasm and inhibiting cell wall synthesis.¹⁸ Saponins exhibit antibacterial activity through a detergent-like mechanism, disrupting bacterial cell wall integrity and increasing membrane permeability.¹⁹ Steroids interact with phospholipid cell membranes, altering membrane permeability due to their lipophilic nature, leading to membrane integrity disruption, morphological changes, and ultimately cell rupture and lysis.²⁰

The 80% red dragon fruit peel extract demonstrated the highest inhibition zone diameter against *S. aureus*, measuring 12.18 ± 0.228 mm, while the 20% concentration showed a diameter of 9.54 ± 0.603 mm. This indicates a direct correlation between extract concentration and inhibition zone diameter. Sartika et al.²¹ categorized inhibition zone diameters from agar diffusion tests: <5 mm as weak, 5-10 mm as moderate, 10-19 mm as strong, and ≥ 20 mm as very strong. Based on these criteria, the 80% and 40% red dragon fruit peel extracts exhibited strong inhibition, while the 20% concentration showed moderate inhibition.²¹

Normality and homogeneity tests (Table 3) revealed non-normal data distribution ($p < 0.05$) but homogeneity ($p > 0.05$), necessitating Kruskal-Wallis and Post Hoc LSD statistical analyses. The Kruskal-Wallis test indicated a significant inhibitory effect of red dragon fruit peel extract on *S. aureus* growth ($p = 0.0001$; $p < 0.05$), suggesting its potential as an oral irrigation solution. This finding is consistent with Siregar and Daniel¹³, who reported antibacterial activity of ethanolic red dragon fruit peel extract against *S.*

aureus. Furthermore, other studies have shown the potential of ethanolic red dragon fruit peel extract against *Enterococcus faecalis* as an oral irrigation solution in endodontic procedures.¹⁵

However, the antibacterial effectiveness of red dragon fruit peel extract at all concentrations remained lower than that of the positive control (0.2% chlorhexidine), which exhibited the highest inhibition zone diameter against *S. aureus* (13.20 ± 0.255 mm) (Table 5). This is supported by Widiaastuti et al.⁵, who demonstrated the antibacterial effect of NaOCl against *S. aureus* cultures at concentrations as low as 50 ppm.⁵

Conclusion

The research conducted yielded the following key findings. Extracts of red dragon fruit peel demonstrated effectiveness against *S. aureus*. A concentration of 80% was found to be the most effective concentration compared to other concentrations tested. Further research should be conducted to investigate the antibacterial effects of various types of dragon fruit peel against different strains of *S. aureus*. Additional studies employing dilution methods are warranted to explore the efficacy of red dragon fruit peel extracts at concentrations of 80%, 40%, and 20% in inhibiting the growth of *S. aureus*. More extensive research on red dragon fruit peel extracts is recommended, including the study of smear layer effects and the combination of extracts with other irrigation solutions. This would provide a wider range of alternative irrigation options for saline water.

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