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Antibacterial activity of 1 % Roselle flower nano-emulsion extract (Hibiscus sabdariffa) against peri-implantitis-related bacteria on orthodontic mini-implants: An in vitro study

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ABSTRACT

Objective: The orthodontic mini-implant (OMI) failure often occurs due to the accumulation of peri-implantitis bacteria surrounding it, which results in a stable, resistant form of absolute skeletal anchorage during orthodontic treatment. Administering doxycycline may be the solution, but long-term side effects result in antibiotic resistance. Roselle flowers (Hibiscus sabdariffa) possess beneficial active phytochemical substances, which may have potential as an OMI peri-implantitis alternative therapy. This study investigates the antibacterial activity of 1 % Roselle flower (H. sabdarifa) nanoemulsion (NE) extract (1 % RNE) toward peri-implantitis bacteria in OMIs.
Methods: A phytochemical analysis of 1 % RNE was carried out to examine the active substances possessed in it, such as flavonoids, quinone, saponin, alkaloids, tannins, terpenoids, and steroids. Nanoemulsion characterization was carried out using a particle size analyzer (PSA). The antibacterial activity of 1 % RNE toward Prevotella intermedia (Pi), Porphyromonas gingivalis (Pg), Aggregatibacter actinomycetemcomitans (Aa), and Fusobacterium nucleatum (Fn) was carried out to determine the minimum inhibitory concentration, minimum bactericidal concentration, and inhibitory zone compared with doxycycline as a positive control.
Results: Roselle flower NE extract (1 %) possessed flavonoids, quinone, saponin, alkaloids, tannins, terpenoids, and steroids positively. The PSA showed that the 1 % RNE had a size of 98.13 d nm. The antibacterial activity of 1 % RNE against Aa, Pg, Pi, and Fn bacteria at 3.125 % showed significant differences ($p < 0.05$). The antibacterial activity of 1 % RNE toward peri-implantitis bacteria is lower than that of doxycycline.
Conclusion: Roselle flower NE extract (1 %) has antibacterial activity against peri-implantitis bacteria at a concentration of 3.125 %.

1. Introduction

An orthodontic mini-implant (OMI) is a device used in fixed orthodontic treatment to provide absolute intraoral anchorage for moving teeth to the desired location. It is considered more optimal and efficient compared to other methods. These devices are widely used for treating skeletal malocclusion, correcting midline shifts, and modifying excessive overjet.[1] Several studies showed the overall success rate of

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mini-implants ranged from 79 % to 98.2 %, considering 5332 screws.[2] The success rate of OMIs is 86.5 % based on a study of 2281 patients.[3] A study showed that their success rate was >90 % in 573 patients.[4] An OMI can be inserted into the free marginal gingiva mucosa and anchored to the alveolar bone. It is widely used as an additional source of absolute anchorage due to its relatively low cost and effective results. Orthodontic mini-implants also offer better patient comfort because of their small size (1.3–2 mm in diameter), eliminating the need for surgical procedures. This minimizes pain, swelling, and the risk of damage to nerves, blood vessels, maxillary sinuses, and tooth roots.[1] Nevertheless, the clinical use of OMIs has some side effects and complications that may occur during insertion, use, or removal. One of the most commonly reported complications is contact between the OMI and the root of the tooth adjacent to the insertion site. This complication occurs more frequently at interradicular insertion sites, particularly in the posterior regions of both the mandibular and maxillary arches. If left untreated, this issue can lead to the loss of the OMI or compromise tooth vitality.[5] There are risks and errors in the installation of the OMI due to mobility and detachment caused by the progression of peri-implantitis.[6,7]

Peri-implantitis is a chronic inflammation that attacks the surrounding tissue of the dental implant, or an OMI, which is attached to the area around the gingival mucosa and alveolar bone.[7] It makes the OMI unstable and causes pain in the afflicted periodontal tissue, with a reported prevalence of 16.7%–35.5 % in 2024. Alveolar bones found around the implant undergo progressive pathological resorption and inflame the surrounding soft tissue. Clinical manifestations can be characterized by the presence of plaque and calculus around the OMI area, as well as bad oral hygiene, mobility of the OMI, mucosal hyperplasia, and pain in the area around the implant.[8] Peri-implantitis influences gene expression, protein production, and cytokine secretion, with the majority focusing on the roles of inflammatory cytokines such as interleukin-1 (IL-1), especially IL-1 beta. It can also be caused by the presence of gram-negative bacteria around the OMI.[9]

The prevalence of gram-negative bacteria related to OMI peri-implantitis, such as Porphyromonas gingivalis (Pg), Actinomycetemcomitans (Aa), Fusobacterium nucleatum (Fn), and Prevotella intermedia (Pi), is about 28%–56 %.[10] Peri-implantitis involves many cytokines and chemokines, especially pro-inflammatory cytokines. Peri-implant crevicular fluid mostly contains the Receptor Activator of Nuclear Factor-κβ Ligand and Tumor Necrosis Factor Alpha.[11] These cytokines and chemokines worsen the condition of peri-implantitis due to the high inflammatory response around the tissue, disrupting the osseointegration system and wound healing.[5] The treatment of peri-implantitis can be done in various ways, including non-surgical treatment. This can be done by administering antibiotics, host modulation, local debridement, laser treatment, and the application of chlorhexidine (CH) gel 0.12 %.[12] The long-term use of antibiotics has side effects such as antibiotic drug resistance.[13] Pg, Aa, Pi, and Fn displayed high resistance to tetracycline, doxycycline, metronidazole, clindamycin, and erythromycin.[14] Several systematic reviews have indicated that most surgical peri-implantitis procedures recommend the adjunctive implementation of systemic antimicrobials to target putative microorganisms.[15–17] In addition, a recent systematic review found that to avoid implant failure, antimicrobial prophylaxis was prescribed.[18] It has also been reported that most clinicians empirically use antimicrobials against peri-implantitis without a bacteriological examination.[19]

The drawbacks mainly include enhancing antimicrobial resistance and the risk of altering the normal microflora, as well as hypersensitivity reactions, among others.[15] It has been reported that non-steroidal inflammatory drugs exert an influence on bone healing by inhibiting the release of prostaglandin E2, which modulates bone metabolism through the suppression of the cyclooxygenase-2 pathway. Neither osseointegration nor bone healing was shown to be impaired in rat tibias following the two-week administration of a non-specific Cyclooxygenase (COX) inhibitor using Diclofenac sodium.[20] The development of herbal medicine in modern times now utilizes many safe and natural materials with minimal side effects and are adequate as alternative therapies for peri-implantitis in OMIs. Roselle flowers (Hibiscus sabdarifa) are a type of plant often found in Asia, especially in Indonesia, and have many benefits and are easy to cultivate. H. sabdarifa has high levels of antioxidants such as gossypetin, anthocyanins, flavonoids, phenolics, saponins, alkaloids, and tannins. Antioxidant mechanisms: Roselle flower petals contain quercetin and catechin. Quercetin is a flavonoid derivative, specifically a flavanol, that has antibacterial effects. It can disrupt bacterial DNA gyrase activity by binding to a bacterial DNA gyrase called GyrB and inhibiting the enzyme activity of adenosine triphosphate in the bacterial cell membrane.[21] Antibacterial mechanisms: Roselle flowers contain flavonoids, saponins, and tannins.[22] Flavonoids can interact with cell membranes, affect cell membrane bioactivity, and reduce the fluidity of bacteria through autolysis and, consequently, osmotic lysis. The mechanism of saponins as an antibacterial agent causes leakage of proteins and enzymes from the bacterial cell by increasing membrane permeability. The mechanism of action of tannins as an antibacterial agent targets the bacterial cell wall polypeptides, disrupting the formation of the cell wall and leading to a less-than-perfect structure, which causes the bacterial cell to lyse.[23] Nano-emulsion is a drug preparation technique that consists of various mixtures of ingredients, such as oil, water, and surfactants, with a diameter of less than 300 nm (nm). Nano-emulsion has several advantages, including being non-toxic to tissues, not altering the phytochemical properties of the extract, and being easy to produce. It also offers better surface stability than conventional topicals, such as emulsions and ointments, which enhances penetration into tissue.[24] As Roselle flowers (H. sabdariffa) possess a potential active compound, they can be used as an antibacterial strategy against peri-implantitis-related bacteria in the OMI. This study hypothesizes that the nanoemulsion (NE) of Roselle flowers (H. sabdariffa) has antibacterial activity related to peri-implantitis. Furthermore, this study aims to investigate the antibacterial activity of 1 % Roselle flower NE extract (1 % RNE) against peri-implantitis bacteria in OMIs using an in vitro approach.

2. Materials and methods

2.1. Ethical clearance

All methods in this research were carried out in accordance with the relevant guidelines and regulations by the Ethics Committee of the Faculty of Dentistry, Airlangga University, Surabaya, Indonesia, with appointment number 0652/HRECC.FODM/VII/2024.

2.2. Roselle flower (H. Sabdariffa) extract preparation

Roselle flowers *(H. sabdariffa)* were collected with fresh flowers, ensuring no signs of damage (physical or disease), by picking and then storing them in dark plastic containers in a clean box. The samples were then prepared at the Research Center of the Faculty of Dentistry, Airlangga University, Surabaya. The flowers were cleaned by washing them with running water to remove debris, then dried using tissue paper and cut into small pieces (simplicia). The dried Roselle flowers (H. *sabdariffa*) were placed in a clean container. They were then weighed at 1 kg. Next, the flowers were crushed into powder using a blender. The fine powder was then sieved to a 100-mesh size. Extraction was carried out using a single-solvent maceration method with 2.5 L of 96 % ethanol. Maceration with 96 % ethanol was performed for 3 days. The filtrate and residue were separated by filtering the macerated mixture through a sterile cloth. The resulting filtrate was measured in volume units and evaporated using a vacuum evaporator at 40 °C. The Roselle flower extract was then placed in an oven to remove any remaining ethanol solvent from the evaporation process. The extract, after oven treatment, was weighed.[25]

2.3. Roselle flowers (H. Sabdariffa) (1 %) nanoemulsion preparation

Roselle flower extract was placed into a beaker with a volume of 100 mL for each concentration. Then, 1 mL of polysorbate 80 was added to the solution and stirred using a homogenizer (model 300 131 V/T, USA) at a speed of 1000 rpm for 10 min. After that, 20 mL of 0.1 % sodium tripolyphosphate solution was added to the mixture and then homogenized at a speed of 4000 rpm for 60 min. During the mixing process, the stirring speed was gradually increased to reduce the particle size. This is because the faster the stirring process is, the smaller the particle size obtained, resulting in a nanoparticle formulation. All formulas were then stored at room temperature for further use.[24]

2.4. Particle sizer analysis of roselle flowers (H. Sabdariffa) nanoemulsion extract

The determination of particle size was carried out using a particle size analyzer (PSA) (Horiba SZ-100). The sample solution was placed into a cuvette and analyzed with the PSA, and particle size observations were made using it. The smaller the particle size was, the greater the amount of biomaterial that would come into contact with the target. The research involved preparing a sample of 0.05 g, then hydrating it with 5.0 mL of pH 6.0 phosphate buffer, followed by sonication for 20 min. The sample was then placed into a cuvette, and the particle size was determined.[24]

2.5. Phytochemical analysis of roselle flowers (H. Sabdariffa) nanoemulsion extract

2.5.1. Flavonoid test

Roselle flower NE extract was then combined with two drops of concentrated hydrochloric acid (HCl). After adding the HCl, the RNE was agitated homogeneously and then mixed with magnesium powder. The resulting mixture was agitated until it was homogeneous, at which point foam formed, and the solution turned orange if flavonoid antioxidants were present in the tube. After shaking the mixture until it was homogeneous, the RNE was mixed with two drops of 2-normal (2N) sulfuric acid (H_2SO_4). If the color of the solution in the tube changed to yellow, red, or brownish, this indicated the presence of flavonoids in the sample.[24]

2.5.2. Alkaloid test

Roselle flower NE extract was mixed with ammonia chloroform. After combining with ammonia chloroform, the mixture was filtered through cotton and separated into tubes A and B. Dragendorff's reagent was then added to the RNE in tube A, and Wagner's reagent was applied to the extract in tube B. If sediment formed in tube A and the color changed to red, alkaloids were present in the RNE; if sediment formed in tube B and the color changed to brown, alkaloids were present in the RNE.[24]

2.5.3. Saponin test

Roselle flower NE extract was rinsed with an ammonia chloroform solution. It was then filtered through cotton wool and placed in a fresh, sterile tube. The RNE solution was shaken for two to 3 min until it was homogeneous, then two drops of 2N HCl were added, and the mixture was agitated again until it was homogeneous. The homogenized tube was then left for 10 min and observed to determine if foam formed, indicating the presence of saponin in the RNE. The more foam that develops within 10 min, the higher the concentration of saponin in the tube.[24]

2.5.4. Triterpenoid and steroid test

The RNE residue was dried by heating and then extracted again using a 1:1 mixture of air and chloroform. Once it was determined that the two components were homogeneous after mixing, two drops of chloroform were added to the RNE on the spot plate, and it was allowed to dry. Next, changes were observed after adding one drop of anhydrous acetic acid. If the sample turned red or brown, it indicated the presence of positive triterpenoids; if it turned blue, purple, or green, it indicated the presence of steroids in the RNE.[24]

2.6. Antibacterial activity of 1 % RNE toward peri-implantitis bacteria Aa, Pg, Pi, and Fn

2.6.1. Bacterial culture and preparation porphyromonas gingivalis

On a tryptic soy broth (TSB) agar medium, Pg (ATCC33277, UK) was cultivated and then incubated for 18–24 h at 37 °C in an anaerobic environment. Using a loop needle that had been warmed in a Bunsen burner, bacterial colonies were extracted after incubation for a predefined amount of time. Next, a tube containing 3 mL of a brain heart infusion (BHI) solution was filled using the tube needle for fermentation, and the tube was incubated for 18 h at 37 °C. To ensure uniform growth on the agar media, the bacterial solution was equalized using the standard McFarland dilution of 0.5 (1.5×10^9 CFU/mL) and then transferred using a micropipette.[26]

2.6.2. Bacterial culture and preparation agregratibacter actinomycetemcomitan

To cultivate Aa (ATCC43718, United Kingdom), it was kept in an anaerobic environment for 24 h at 37 °C. Using a sterile tube needle, the bacteria were extracted from the stock solution and subsequently cultivated on a BHI medium. The stock solution was then diluted to 1.5×10^8 CFU/mL, or McFarland standard 0.5. Observations were made using a double-blind method, assessing the turbidity of the bacterial suspension by contrasting the tube containing the solution with paper on a black-and-white backdrop.[26]

2.6.3. Bacterial culture and preparation fusobacterium nucleatum

A TSB medium was used to cultivate Fn (ATCC22586, United Kingdom). The cultures were then kept in an incubator at 37 °C for 18–24 h in an anaerobic environment. Once the incubation period had passed, the bacterial colonies were removed from the medium by heating the tube needle over a Bunsen burner until it was sterile, waiting for it to stop glowing red. After that, the bacteria were cultured for 18 h at 37 °C in 3 mL of a BHI medium. The suspension was spread uniformly over the surface of the agar medium after adjusting it to the McFarland standard of 0.5 (1.5×10^9 CFU/mL).[26]

2.6.4. Bacterial culture and preparation prevotella intermedia

Five milliliters of thioglycolate (TG) broth (BBL™ Fluid, Becton Dickinson and Company) were used to cultivate Pi (ATCC25611, United Kingdom). The medium was cultivated in an anaerobic environment at 35 °C (Anaerogen, Oxoid) in an incubator for 8 days. Following that, the bacteria were cultured again on the Wilkins–Chalgren agar and blood agar. The medium was subsequently placed in an anaerobic environment (Anaerogen, Oxoid) for 8 days at 35 °C. Gram staining was then used to inspect bacterial colonies to ensure the bacteria were pure and had the correct form. Next, 3–5 bacterial colonies were removed and added to 4 mL of the BHI broth containing 1 g/mL menadione and 5 μg/mL hemin. The colonies were then cultured for 72 h at 35 °C in anaerobic conditions (Anaerogen, Oxoid). The McFarland standard (1.5×10^8 CFU/mL) was used to dilute the bacterial colonies.[26]

2.7. Minimum inhibitory concentration of 1 % RNE extract against peri-implantitis bacteria analysis

The sample size for this study was determined using Lameshow's minimum sample size calculation, resulting in four samples for each group. The samples were selected using simple random sampling. To create a stock solution of antibacterial compounds, RNE was weighed and then added to 1 mL of the TG medium at a ratio of 100 g/mL. Nine

tubes were then prepared for the minimum inhibitory concentration (MIC) technique by diluting the stock solution with a standard medium using the microdilution method. To achieve dilution, 20 μL of the stock solution was added to 380 μL of the TG solution in the first tube. This was followed by the addition of 200 μL of the TG solution to each of the next 9 tubes. The 200 μL solution from the first tube was then taken out and transferred to a second tube containing 200 μL of TG—this is known as 101 % dilution. Then, 200 μL was transferred from the first tube (101 % dilution) to the second tube (102 dilution). Up to 109 serial dilutions were carried out to accommodate the number of tubes. Next, 2 mL of the TG medium was combined with 10 mL of the organism culture containing 4 bacterial strains. After serially diluting each tube, 200 μL of the culture suspension was transferred to each tube using a micropipette. The MIC dilutions of the 1 % RNE were 100 %, 50 %, 25 %, 12.5 %, 6.25 %, 3.125 %, 1.56 %, and 0.78 % against Aa, Pg, Fn, and Pi. The tubes were then securely sealed to ensure airtightness. Afterward, the tubes were incubated under anaerobic conditions for 48 h. The minimum concentration of RNE in a tube that does not cause turbidity is known as the MIC.[26]

2.8. Minimum bactericidal concentration of 1 % RNE extract against peri-implantitis bacteria analysis

To monitor the development of microorganisms, four tubes with antibacterial sensitivity at lower concentrations were obtained and placed in the appropriate culture media, similar to the bacterial MIC approach. Next, the Petri dishes were anaerobically cultured for 48 h in a jar or airtight storage container before the bacterial colonies were removed.[26]

2.9. Peri-implantitis bacteria inhibitory zones analysis

The antibacterial inhibitory zone was examined using Pg, Aa, Fn, and Pi bacterial cultures. The treatment group of bacteria was treated with a 1 % RNE extract solution at concentrations of 3.125 % and 1.56 %, while the positive control was doxycycline on a paper disk. Afterward, each group's zone of inhibition was reproduced and measured in millimeters using digital calipers (Mitutoyo, Japan).[26]

2.10. Statistical analysis

Analysis of variance, the honest significant difference post-hoc test with varying significance values ($p < 0.05$), normality and homogeneity tests ($p > 0.05$), and the Statistical Program for Social Science version 20.0 for Windows (IBM Corporation, Illinois, Chicago, United States) were used to analyze the data.

3. Result

RNE contains tannin, terpenoids, quinone, alkaloids, flavonoids, and saponins (Fig. 1A). The 1 % RNE extract's PSA findings revealed a size of 96.13 nm, indicating that it had a good particle size and satisfied the <300 nm nanoparticle requirement. The phytochemical test findings for the 1 % RNE extract are shown in Fig. 1B. Significant differences ($p < 0.05$) were observed in the antibacterial activity of 1 % RNE against Aa, Pg, Pi, and Fn bacteria at 3.125 %. However, compared to doxycycline, 1 % RNE exhibited less antibacterial activity against the microorganisms that cause peri-implantitis (Figs. 2–5).

4. Discussion

In modern times, many discoveries have been made regarding materials derived from nature for therapy in the medical field, thereby minimizing the side effects of drug use, especially antibiotics. One alternative ingredient that can be used in peri-implantitis therapy is RNE. Phytochemical tests have shown that RNE contains several types of active antioxidant compounds, such as polyphenolic compounds, including flavonoids, tannins, saponins, alkaloids, steroids, and triterpenoids, which have been identified and could serve as an alternative therapy for peri-implantitis. Flavonoid compounds can eliminate pathogenic bacteria through various mechanisms, including inhibiting nucleic acid synthesis, DNA gyrase-linked enzymes, and disrupting bacterial membrane function, making them more susceptible to lysis. These compounds can also kill bacteria by denaturing bacterial proteins, which disrupt the permeability of the bacterial cell membrane. As a result, the cell membrane wall layer becomes unstable, causing nutrients needed by the bacteria to leak from the intracellular tissue, leading to gradual bacterial death. Saponin compounds also help reduce the surface tension of bacteria, making the bacterial cell membrane more permeable. The permeable cell wall creates gaps for intracellular compounds to escape, and enzyme proteins within the bacteria are destroyed. This results in the bacterial protein structure changing shape, becoming damaged, and undergoing lysis. Roselle flower NE extract also contains high levels of antioxidants such as anthocyanins and quercetin

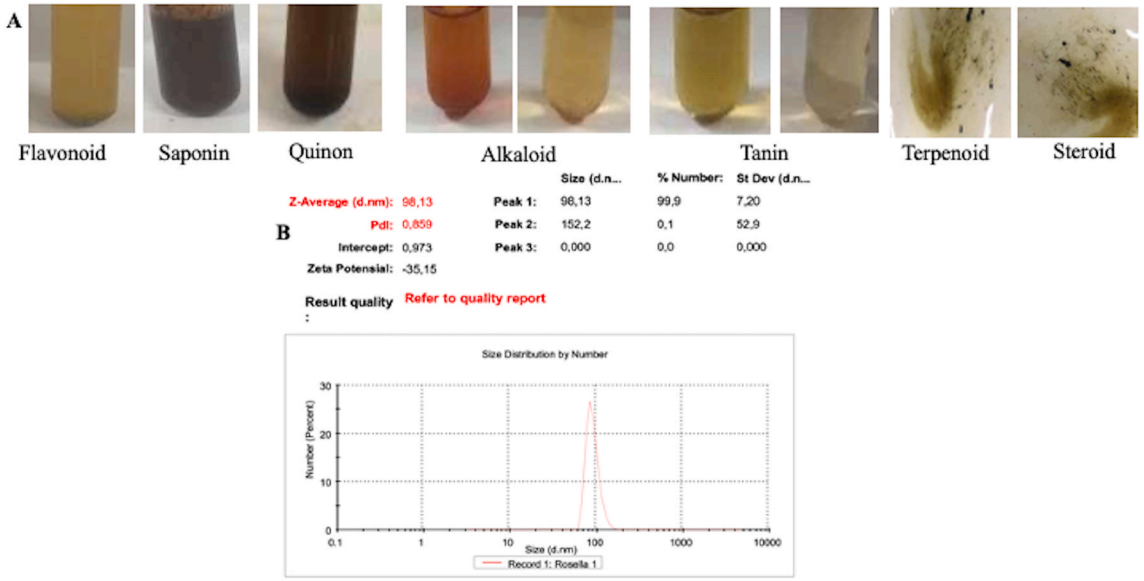

Fig. 1. (A) Phytochemical analysis of 1 % RNE. (B) Particle size analyzer result of 1 % RNE.

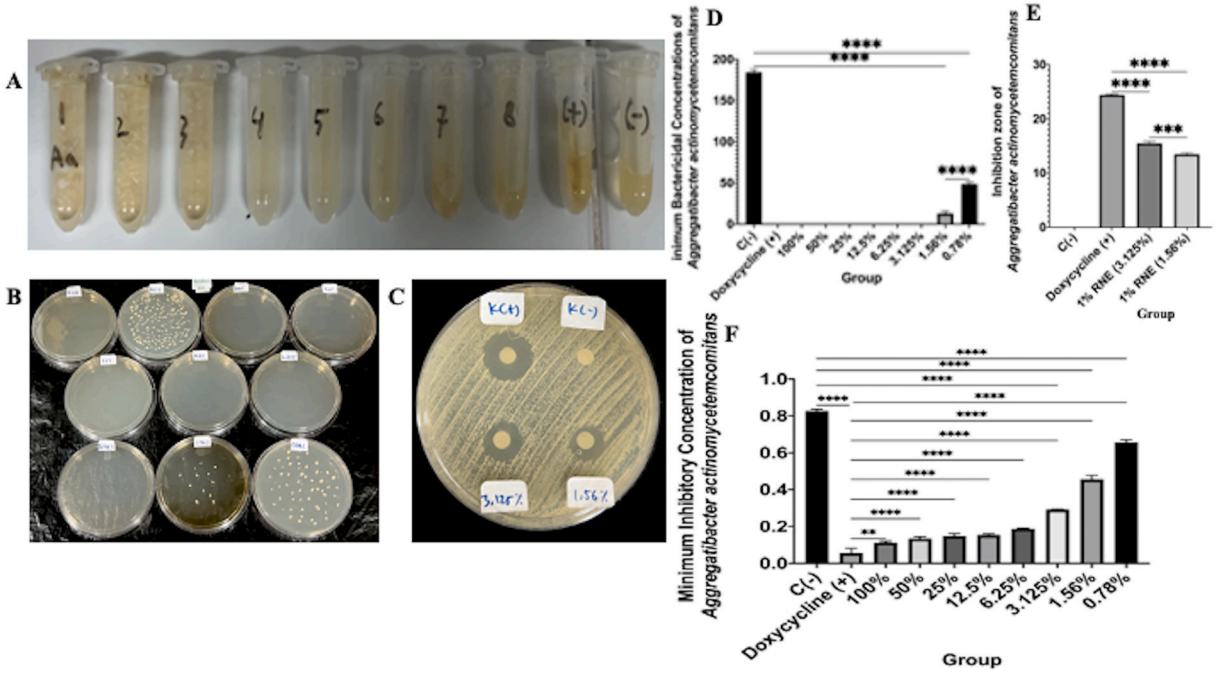

Fig. 2. 1 % RNE antibacterial activity against *Aggregatibacter actinomycetemcomitans* (Aa). (A) Minimum inhibitory concentration (MIC), (B) Minimum bactericidal concentration (MBC), (C) Inhibitory zone of Roselle nanoemulsion at a concentration of 3.125 % shows better antibacterial activity than 1.56 % against Aa bacteria, (D) MBC graph of Aa bacteria, (E) Inhibition zone graph of Aa, (F) MIC graph of Aa. Note: Significant differences between groups at $p < 0.05$***, $p < 0.01$****.

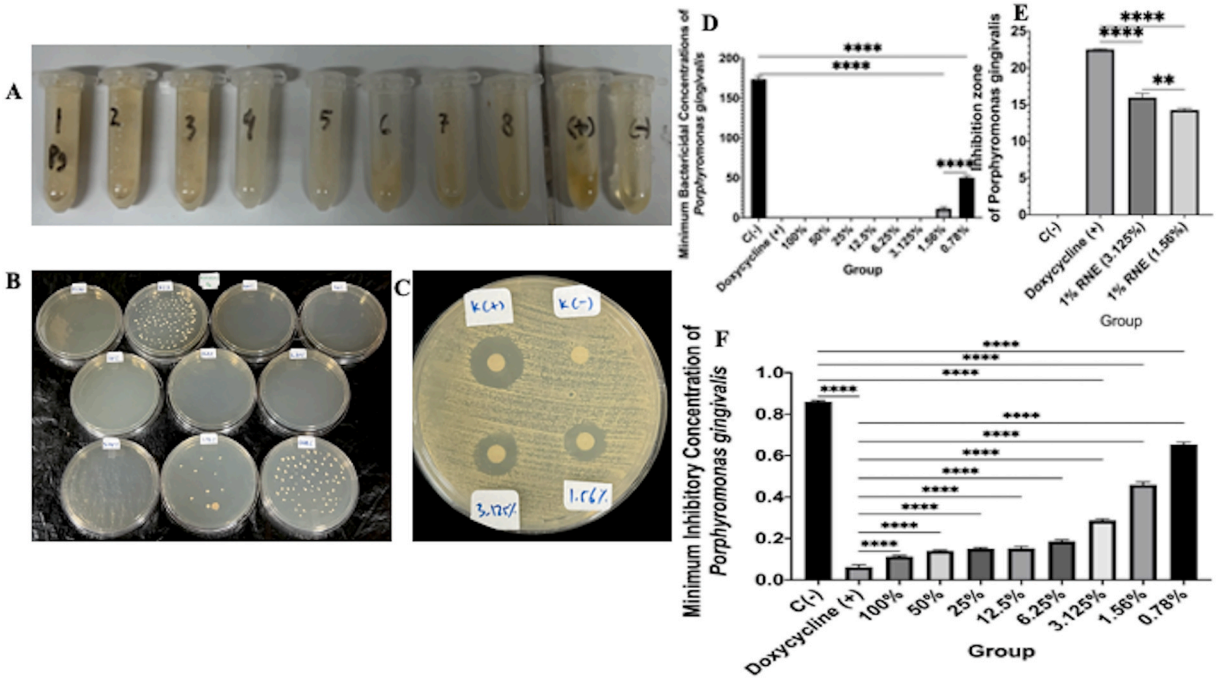

Fig. 3. 1 % RNE antibacterial activity against *Porphyromonas gingivalis* (Pg). (A) Minimum inhibitory concentration (MIC), (B) Minimum bactericidal concentration (MBC), (C) Inhibitory zone of Roselle nanoemulsion at a concentration of 3.125 % shows better antibacterial activity than 1.56 % against Pg bacteria, (D) MBC graph of Pg bacteria, (E) Inhibition zone graph of Pg, (F) MIC graph of Pg. Note: Significant differences between groups at $p < 0.05$***, $p < 0.01$****.

from Roselle flowers, which function in cancer cell apoptosis.[27,28]

In this research, the Roselle flower was developed into an NE preparation. Nanoemulsion consists of oil and air dispersed together, making drug preparations in this form relatively stable kinetically and thermodynamically on the tissue surface. Roselle flower NE extract has a particle size of less than 300 nm, specifically 96.13 nm, which indicates that the smaller the particle size is, the better. This smaller particle size facilitates good penetration into the tissue. When the NE preparation is applied to the tissue surface, it induces better penetration, leading to the destruction of the cell wall. This results in a change from semi-permeable or selectively permeable to permeable, alters extracellular metabolism, disrupts DNA synthesis in the cell nucleus, induces bacterial cell death, and causes the loss of nutrients from bacterial cells, which leads to the release of intracellular material and ultimately bacterial

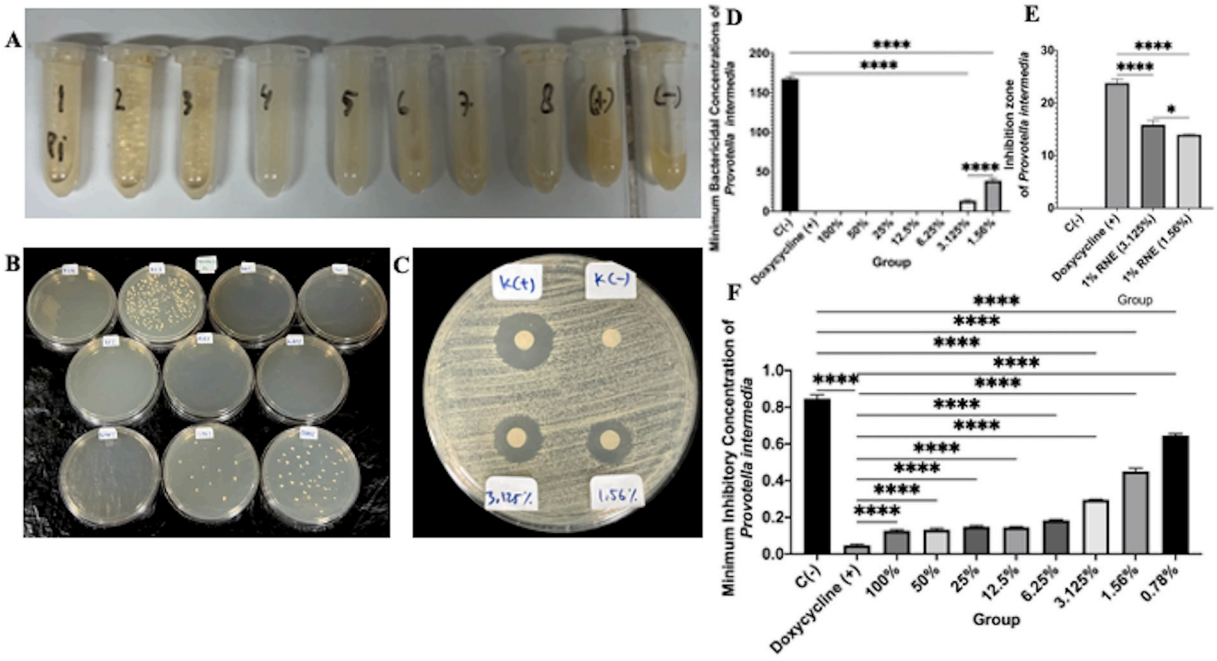

Fig. 4. 1 % RNE antibacterial activity against *Prevotella intermedia* (Pi). (A) Minimum inhibitory concentration (MIC), (B) Minimum bactericidal concentration (MBC), (C) Inhibitory zone of Roselle nanoemulsion at a concentration of 3.125 % shows better antibacterial activity than 1.56 % against Pi bacteria, (D) MBC graph of Pi bacteria, (E) Inhibition zone graph of Pi, (F) MIC graph of Pi. Note: Significant differences between groups at $p < 0.05$***, $p < 0.01$****.

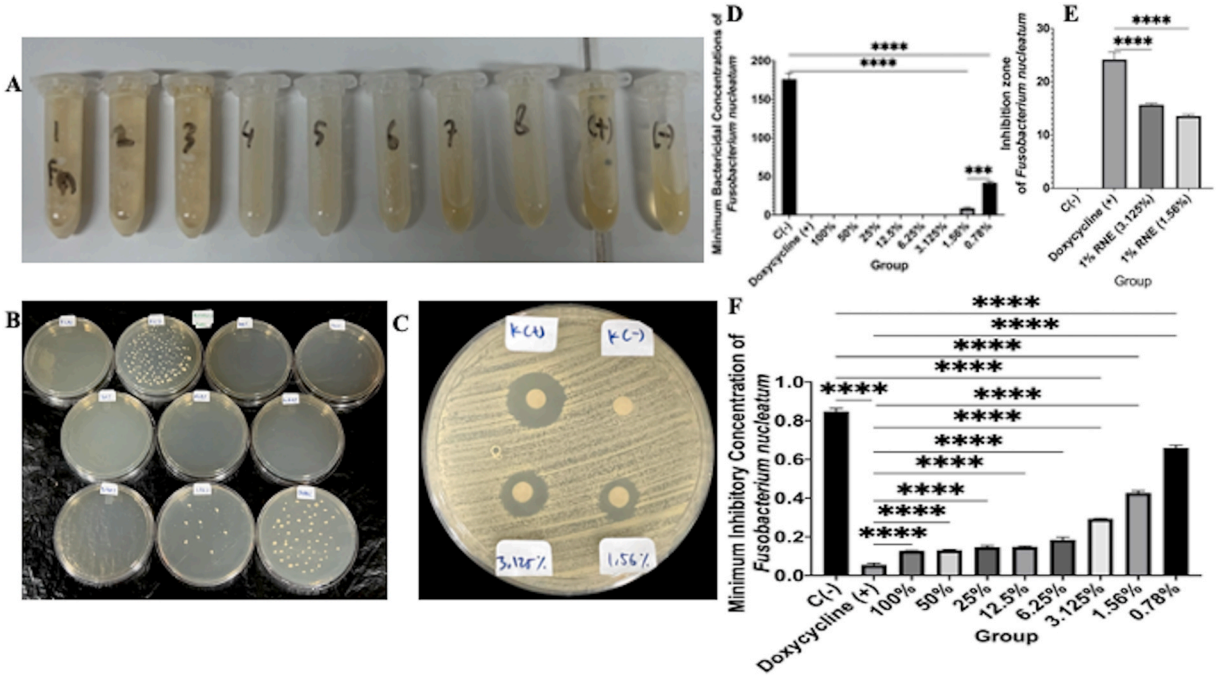

Fig. 5. 1 % RNE antibacterial activity against *Fusobacterium nucleatum* (Fn). (A) Minimum inhibitory concentration (MIC), (B) Minimum bactericidal concentration (MBC), (C) Inhibitory zone of Roselle nanoemulsion at a concentration of 3.125 % shows better antibacterial activity than 1.56 % against Fn bacteria, (D) MBC graph of Fn bacteria, (E) Inhibition zone graph of Fn, (F) MIC graph of Fn. Note: Significant differences between groups at $p < 0.05$***, $p < 0.01$****.

lysis. However, NE has several disadvantages. In the future, 1 % RNE extract could be developed into a microemulsion, as it has a particle size of <100 nm and achieves a very low interfacial tension of up to 10^{-4}–10^{-3} mN/m. Microemulsions (ME) can also bind oil better than NE, making the solution more stable on the wound surface and less prone to contamination, such as from saliva. However, ME have relatively higher manufacturing costs compared to NE, so these costs must be considered despite their significant advantages.[24,29]

The results of the MIC, MBC, and diffusion zone tests for the bacteria that cause peri-implantitis (Aa, Pg, Fn, and Pi) were observed at concentrations of 3.125 % and 1.56 %, with significant differences ($p < 0.05$). This is further supported by several studies showing that Roselle flower extract can act as an antibacterial agent against both gram-positive and gram-negative bacteria. In this study, RNE functions by

preventing bacterial adhesion, damaging the bacterial plasma membrane, and disrupting bacterial metabolism. The antioxidants in RNE can inhibit nucleic acid synthesis in cells by forming bonds with DNA and ATP gyrase. When DNA replication is hindered, bacteria are unable to perform DNA transcription and replication, leading to a failure in the formation of enzymes required for bacterial growth. Bacteria rely on an enzyme called gyrase for lysis, which helps prevent further DNA replication. The gyrase enzyme induces changes in DNA, leading to changes in intracellular bacteria that result in lysis. Other studies have shown that quercetin can inhibit the replication of antibiotic-resistant bacteria by breaking the DNA chain through the formation of DNA gyrase in the cell nucleus, causing the bacteria to lyse.[30] In other research, it has been stated that Roselle extract (RE) with concentrations of 50 mg/mL and 100 mg/mL shows results comparable to CH at concentrations of 0.2 % and 2 %, which are used for endodontic treatment irrigation. In addition, RE concentrations of 75 mg/mL and 100 mg/mL produced better MIC and MBC values compared to the use of metronidazole and tetracycline antibiotics against the bacteria *Streptococcus mutans*, *Staphylococcus aureus*, and *Enterococcus faecalis*.[31] This present study found that doxycycline had better results compared to 1 % RNE. The antibacterial activity of the extract was compared with the positive control (doxycycline), which indicated that RNE has potent antibacterial properties. Doxycycline works by reversibly inhibiting bacterial protein synthesis in the 30S ribosomal subunit and preventing the association of aminoacyl-tRNA with bacterial ribosomes. Oral use of doxycycline has been reported to cause the emergence of systemic diseases such as systemic lupus erythematosus, hepatitis, and changes in intracranial pressure. Additionally, the use of antibiotics can cause discoloration of the teeth, although only a few people experience this.[32] One of the therapies for peri-implantitis is the use of antibiotics, but long-term use can often lead to drug resistance in patients. Prolonged antibiotic use can also cause the formation of reactive oxygen species in tissues, which may induce the development of cancer in the human body. Therefore, antibiotics must be used at the right time and efficiently.[33]

This research has limitations regarding the specific types of bacteria used, as the study was conducted randomly in vitro with limited research methods. These limitations provide opportunities for future development, such as altering the route of drug administration, using an effective dose, testing toxicity, changing drug preparations, and examining the side effects of RNE use in alternative therapy for peri-implantitis in OMIs. Future research could be expanded by conducting *in vivo* studies using experimental animals. This treatment could be tested by modifying animals to induce peri-implantitis conditions, inserting implants, and isolating gram-negative bacteria from peri-implantitis patients, as this study only used American Type Culture Collection (ATCC) bacteria.

5. Conclusion

From the study results above, it can be concluded that 1 % RNE has potential antibacterial activity against peri-implantitis bacteria (Aa, Pg, Pi, and Fn) at a concentration of 3.125 %. Further studies are needed to elucidate the efficacy and effectiveness of 1 % RNE as an antibacterial agent and its anti-inflammatory activity in a peri-implantitis animal model using various investigational research methods.

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Declaration of competing interest

The authors declare that there is no conflict of interest in this study.

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