

## Flaxseed-Coated Titanium: A Natural Antibacterial Strategy for Dental Implants

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### Abstract

Flaxseed (*Linum usitatissimum*) has been used since ancient times for its nutritional and medicinal properties. It is increasingly recognized for its rich content of bioactive compounds, including antioxidants and antimicrobial agents. In the field of dentistry, titanium (Ti) is widely utilized for implants due to its biocompatibility; however, implant-associated infections remain a clinical concern. This has led to interest in natural bioactive coatings that could enhance antimicrobial protection.

This study aimed to evaluate the in-vitro antimicrobial activity of flaxseed-coated titanium plates against common oral pathogens.

Titanium specimens were coated with flaxseed extract and tested against *Staphylococcus aureus* and *Porphyromonas gingivalis* using agar diffusion methods. Zones of inhibition were measured and compared to positive (broth) and negative (ethanol) controls. Experiments were conducted in triplicate, and data were analyzed using SPSS version 25.

The flaxseed-coated titanium exhibited measurable antibacterial activity, with zones of inhibition comparable to those produced by ethanol and broth. Notably, the combination of flaxseed and ethanol showed enhanced antimicrobial effects against both bacterial strains.

The findings demonstrate that flaxseed extract has promising antimicrobial potential when used as a coating on titanium surfaces. This natural, plant-based approach may contribute to the development of antimicrobial dental implants and help reduce postoperative complications related to infection.

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### Introduction

Flaxseed (*Linum usitatissimum*) is well-known for its protective properties and nutritive, which are related to the existence of antioxidants like tocopherols, phenolics, lignin and carotenoids, high-quality omega-3 unsaturated fatty acids, and alpha-linolenic acid (ALA)<sup>1</sup>. Alpha linolenic acid (ALA) is abundant in flaxseed,

accounting for 45 to 52 percent of total flaxseed oil. Flaxseed is unusual among oilseed crops because of its high ALA content. ALA is a good anti-oxidative agent that can protect some cells against oxidative stress and apoptosis. An in vivo study showed that ALA promotes bone formation and inhibits bone resorption<sup>2</sup>. Flaxseed (*L. usitatissimum*) has been used as a conventional healing agent for decades. Many beneficial effects of flaxseed extract have been reported, including anti-inflammatory effects, antioxidant, and antimicrobial. Flaxseed has been indicated to have positive effects on many types of wounds, including burns and burn scar healing, bone healing, skin wound healing and the healing of oral ulceration, in addition to exhibiting

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antibacterial effects in the oral cavity<sup>3</sup>.

The application of flaxseed in biomaterials has gained attention due to its potential antimicrobial properties. Flaxseed has been shown to inhibit the growth of a broad range of bacteria, making it a promising candidate as a coating materials in biomedical devices. The previous study worked on the development of a novel organic polymer coating for the prevention of growth of medically significant bacteria on three-dimensional solid surfaces, this study examines the effect of surface coating with flaxseed on the adhesion and proliferation tendencies of bacteria such as *Staphylococcus aureus* and compares them to previous investigations on *Pseudomonas aeruginosa*<sup>4</sup>. The results of this study could provide valuable insights into the use of flaxseed-coated titanium as a potential antimicrobial material for various applications in the biomedical field.

Titanium is used in most dental implants. Because of its ability to osseointegrate with bone, titanium (Ti) is frequently regarded as an ideal material for dental implants<sup>5</sup>. Titanium with various bioactive coatings is thought to be a good material for subgingival implants<sup>6</sup>. According to not recent research, some novel silane coatings on Ti implants may reduce biofilm formation<sup>7</sup>. Titanium dental implants have increased in number over the years. Titanium is used for dental and orthopedic implants because of its excellent biocompatibility and biomechanical properties<sup>8</sup>. However, there are few clinical concerns such as delay in osseointegration and healing during early stage of implant placements. Another problem is implant failure caused by bacteria infection. A higher implant failure rate was found in patients with periodontal diseases and smoker patients<sup>9</sup>. Currently, the methods mainly used in clinical practice to enhance osseointegration of titanium implants are sandblasted and dualacid-etched treatment<sup>10</sup>. Surface modification strategies aimed at promoting osseointegration of titanium implants and peri-implant bone formation are needed to increase the clinical success rate of implant surgery<sup>11</sup>. Late treatment complications are expected to occur after achieving full osseointegration and functionality; these complications are classified as severe in dental and orthopaedic applications<sup>12</sup>. Nonetheless, issues with post-infection in cases of dental Ti implants warrant further study looking into the

possibility of coating the implant with potential natural-based extract with antimicrobial activity.

Therefore, in this study, flaxseed extract was applied as a titanium coating to improve bone healing and prevent bacterial infection.

## Materials and methods

### Study Design

This study is a quantitative descriptive study conducted in laboratory (in-vitro) condition.

### Study Setting

This study was conducted at Research Laboratory and Microbiology Laboratory, Kulliyah of Dentistry IIUM, Kuantan. Malaysia.

### Methodology

Flaxseed (*Linum usitatissimum*) seeds were mechanically ground into a fine powder. The powdered seeds were then extracted using 99% undenatured ethanol in a Soxhlet apparatus. The resulting extract was concentrated with a rotary vacuum evaporator under reduced pressure and controlled temperature, yielding a clear to yellowish flaxseed oil, as shown in Figure 1<sup>13,14</sup>. The supernatant was initially stored at -80 °C for 12 hours, followed by freeze-drying for seven days, and subsequently preserved at -20 °C for further use<sup>15</sup>.



Figure 1: flaxseed crude extract.

The titanium samples underwent surface pre-treatment by polishing with 220- and 320-grit silicon carbide papers under running water, followed by rinsing with deionized water and air drying at room temperature (Figure 2a). Surface activation was achieved through sodium hydroxide etching and thermal treatment, where the samples were immersed in a 5M sodium hydroxide solution and heated at 60 °C for 24 hours<sup>16</sup>. After the alkali treatment, the samples were again rinsed with deionized water and dried at room temperature. For the spin coating process, 10 µL of flaxseed extract was applied onto each titanium plate at a spinning rate of 3000 rpm. The coated titanium samples were then oven-dried at 100 °C for 2 hours (Figure 2b)<sup>17</sup>.



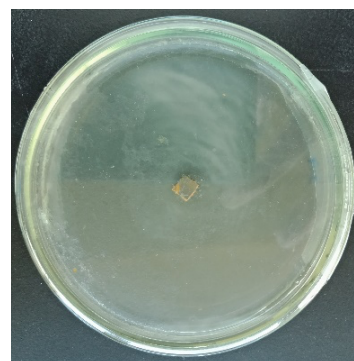
**Figure 2a:** Sandblasting.



**Figure 2b:** Ti coated with flaxseed.

For the antibacterial test, the titanium (Ti) samples were placed in Petri dishes, and 100 µL of bacterial suspensions—at a concentration of  $10^4$ – $10^5$  CFU/mL—were applied directly onto the titanium surfaces, as illustrated in Figure 3.

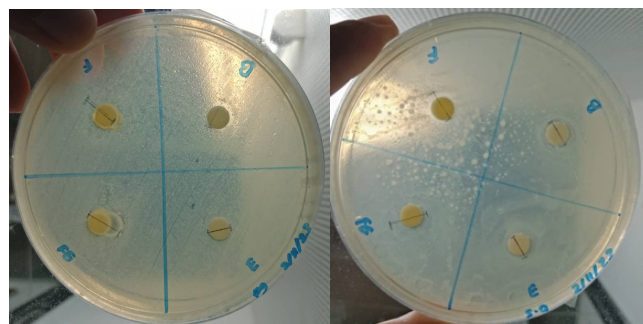
The samples were then incubated with *Staphylococcus aureus* and *Porphyromonas gingivalis* at 37 °C for 1 and 7 days. Following incubation, each sample surface was rinsed with 900 µL of sterile phosphate-buffered saline (PBS). From this, 100 µL of the resulting solution was collected and inoculated onto nutrient agar plates. After a further 24-hour incubation at 37 °C, bacterial colonies were counted<sup>18</sup>. All measurements were statistically analyzed using SPSS software, version 25.



**Figure 3:** Ti coated with flaxseed in bacterial suspension.

## Results

*Staphylococcus aureus* and *Porphyromonas gingivalis* were inoculated onto agar Petri dishes, as illustrated in Figure 4. Each dish was divided into four regions for testing. In two regions, discs impregnated with flaxseed extract and a combination of flaxseed extract with ethanol were placed. The remaining two regions contained the control groups: a positive control (blank disc) and a negative control disc containing ethanol. This setup allowed for comparative analysis of antimicrobial activity. The experiment was conducted in triplicate to obtain reliable and consistent data.



**Figure 4.** Left side (*P.gingivalis*) and right side (*S.aureus*).

	Positive control (blank) [vol:10µm]	Negative control (ethanol) [vol:10µm]	Flaxseed [vol:10µm, conc:100µm]	Flaxseed + ethanol [vol:10µm, conc:100µm]
<i>S.aureus</i>	6.33±0.27	7.00±0.00	8.33±0.27	9.00±0.00
<i>P.gingivalis</i>	6.67±0.54	6.67±0.27	9.00±0.82	10.00±1.41

**Table 1.0** As shown in Table 1.0, the mean zones of inhibition indicate that both the flaxseed extract and the combination of flaxseed with ethanol consistently demonstrated antimicrobial activity against the tested bacteria. These stable inhibition values suggest that the antimicrobial



effect of flaxseed may be attributed to its bioactive components, such as lignans, phenolic compounds, and long-chain unsaturated fatty acids. This supports the potential application of flaxseed extract as a coating material to help prevent bacterial infections.

## Discussion

The results of this in-vitro study provide compelling evidence for the antimicrobial potential of flaxseed-coated titanium, particularly against *Staphylococcus aureus* and *Porphyromonas gingivalis*, two significant pathogens implicated in peri-implant infections. The observed zones of inhibition for flaxseed and flaxseed-ethanol combinations indicate that flaxseed possesses intrinsic antimicrobial activity, likely due to its rich phytochemical profile—including lignans, phenolic compounds, and omega-3 fatty acids such as alpha-linolenic acid (ALA) <sup>1</sup>. These bioactive compounds have been previously associated with antimicrobial, anti-inflammatory, and antioxidative properties, which support the findings of this study.

Notably, the flaxseed-coated titanium demonstrated greater inhibition zones than both the positive (broth) and negative (ethanol) controls, especially in the combination group. This suggests a potential synergistic effect when flaxseed is combined with ethanol, possibly enhancing the bioavailability or diffusion of active compounds across the titanium surface. This synergy merits further investigation, as it could inform more effective formulation strategies for coating biomaterials.

The antimicrobial mechanism of flaxseed is presumed to involve disruption of bacterial cell membranes, inhibition of biofilm formation, and interference with bacterial metabolic processes. The phenolic compounds and lignans in flaxseed may bind to bacterial proteins and enzymes, impairing their function. Additionally, the fatty acids may integrate into bacterial membranes, altering their permeability and leading to cell lysis <sup>19,20</sup>.

In the context of dental implantology, titanium is widely recognized for its favorable mechanical and biocompatibility properties. However, microbial colonization and subsequent peri-implantitis remain major causes of implant failure. Incorporating a bioactive coating that provides antimicrobial protection during the early

healing phase could be a pivotal step toward improving clinical outcomes. The ability of flaxseed extract to inhibit early bacterial adhesion may thus reduce inflammation, support faster osseointegration, and lower the incidence of post-surgical infections.

The results align with prior findings that surface modifications of titanium can significantly affect bacterial adherence and biofilm formation. However, unlike synthetic antimicrobial coatings, flaxseed offers a biocompatible, natural alternative that may pose fewer risks of resistance development or cytotoxicity. Additionally, the antioxidant potential of flaxseed may play a supportive role in reducing oxidative stress at the implant site, which has been associated with delayed healing and inflammation in implant dentistry.

Another important advantage of plant-derived coatings like flaxseed is their potential contribution to tissue regeneration. Flaxseed's active compounds have been found to stimulate fibroblast proliferation and angiogenesis in wound healing models, which could be beneficial in promoting soft tissue integration around implants<sup>3</sup>. These regenerative effects may complement the antimicrobial action, leading to more predictable long-term implant stability. Nonetheless, this study has limitations. The experiments were conducted in vitro under controlled conditions, which may not fully reflect the complexity of the oral environment. Factors such as saliva enzymes, immune response, dietary variations, and dynamic mechanical stresses (e.g., mastication and brushing forces) can all influence the behavior of coatings in vivo. Additionally, the oral microbiome is composed of polymicrobial communities that interact in complex biofilms, and the efficacy of flaxseed extract in such environments remains to be tested.

Furthermore, the durability, wear resistance, and long-term stability of the flaxseed coating are unknown. In clinical settings, implant surfaces are exposed to fluctuations in pH, salivary flow, and microbial by-products, all of which can compromise the integrity of organic coatings. Future studies should focus on evaluating how well flaxseed coatings adhere under such conditions and whether they can maintain their antimicrobial properties over time.

It would also be beneficial to assess the cytocompatibility of flaxseed-coated titanium with

osteoblasts and epithelial cells to ensure the safety of these coatings in direct contact with host tissues. Longitudinal animal studies and human clinical trials would help confirm the translation of these findings from bench to bedside.

## Conclusions

The in-vitro evaluation of the antimicrobial activity of flaxseed-coated titanium indicates that it possesses significant antibacterial properties against pathogenic bacteria. These findings suggest its potential as an effective antimicrobial coating for medical devices. However, further research is necessary to validate these results and to evaluate the long-term efficacy of flaxseed-coated titanium in clinical applications. Future investigations may focus on optimizing coating techniques and exploring synergistic effects with other antimicrobial agents to enhance its performance.

Moreover, the insights gained from this study contribute to a deeper understanding of the biological effects of flaxseed, supporting its potential integration into dental implant technology. Flaxseed-coated titanium emerges as a promising, natural, plant-based antibacterial coating with potential applications in dental surgery and implantology.

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## Declaration of Interest

The authors report no conflict of interest.

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