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Research Article

Antimicrobial activity of *Moringa oleifera* against bacteria isolated from fracture wound sites and its bone fracture healing activity: a critical approach

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Background: *Moringa oleifera,* a botanical remedy rich in bioactive compounds, has shown promise in addressing osteoporosis and microbial infections associated with bone fractures. This study explores its antimicrobial potential and effects on bone health.

Methods: *Moringa oleifera* leaf extract was prepared and tested for antimicrobial activity against *Pseudomonas aeruginosa*, Escherichia coli, and *Staphylococcus aureus*. Additionally, its effects on bone turnover markers and biomechanical properties were evaluated in a retinoic acid-induced rat osteoporosis model.

Results: Concentration-dependent inhibition of bacterial growth was observed, with distinct zones of inhibition against each strain. Moreover, the extract demonstrated dose-dependent modulation of bone turnover markers, indicating potential osteogenic effects. Biomechanical testing revealed improvements in bone material properties with increasing extract concentrations. Bone mineral content analysis showed enhanced mineralization, suggesting improved bone density.

Conclusion: The findings suggest that *Moringa oleifera* leaf extract exhibits selective antimicrobial activity and influences osteoblast activity, contributing to enhanced bone health. The dual action positions it as a promising candidate for biomedical research. *Moringa oleifera* leaf extract holds potential as an antimicrobial agent and bone health enhancer. Further research, including in vivo and clinical studies, is warranted to validate its therapeutic applications. This study lays the groundwork for exploring its roles in antimicrobial therapy and bone health enhancement.

Keywords: *Bone fracture, Moringa, wound infection, bone fracture healing and traditional orthopedic*

Introduction

The utilization of *Moringa oleifera* in addressing bone osteoporosis has garnered considerable attention within the scientific community due to its rich phytochemical composition and purported pharmacological properties (An et al.,

2016). Osteoporosis, characterized by decreased bone density and microarchitectural deterioration of bone tissue, poses a significant health concern globally, particularly among aging populations (Anwar et al., 2007). Given the limitations and adverse effects associated with conventional osteoporosis treatments, there has been a growing interest in exploring alternative and complementary approaches, such as botanical remedies like *Moringa oleifera* (Cáceres et al., 1991).
Moringa oleifera, often referred to as the "miracle tree" or "drumstick tree," is renowned for its nutritional value and therapeutic potential (Conti et al., 2009). The plant is abundant in bioactive compounds including flavonoids, phenolic acids, glucosinolates, and various vitamins and minerals, which collectively contribute to its pharmacological activities (Dai et al., 2018; Djais et al., 2019). These bioactive constituents exhibit diverse mechanisms of action that may confer benefits for bone health, such as antioxidant, anti-inflammatory, anti-resorptive, and bone-stimulating properties (Dai et al., 2018). One of the key mechanisms through which *Moringa oleifera* may exert its effects on osteoporosis is by modulating bone metabolism (Dosoky et al., 2017). Experimental studies have suggested that Moringa extracts can enhance osteoblast activity, promote bone formation, and inhibit osteoclast-mediated bone resorption, thereby contributing to the maintenance of bone mass and integrity. Additionally, the antioxidant and anti-inflammatory properties of Moringa constituents may help mitigate oxidative stress and inflammation, which are implicated in the pathogenesis of osteoporosis (Ezeonu & Ejikeme, 2015). At the core of *Moringa oleifera's* therapeutic prowess lies its rich repertoire of bioactive compounds, including alkaloids, flavonoids, phenolic acids, and glucosinolates, among others. These phytochemical constituents exhibit diverse biological activities, ranging from antimicrobial and anti-inflammatory to wound-healing and immunomodulatory effects. Such a complex phytochemical profile underscores the plant's ability to combat microbial pathogens through multiple mechanisms, making it a compelling candidate for combating recalcitrant infections associated with bone fractures (Ezeonu & Ejikeme, 2015). *Moringa oleifera's* antimicrobial activity has been well-documented in various studies, showcasing its efficacy against a broad spectrum of bacterial, fungal, and viral pathogens. Moreover, its mechanism of action extends beyond mere microbial inhibition, encompassing modulation of virulence factors, biofilm disruption, and enhancement of host immune responses (Fernandez-Botran et al., 2019).

Studies investigating the effectiveness of *Moringa oleifera* extracts or derived compounds against microbial isolates from bone fractures have yielded promising results (An et al., 2016; Cáceres et al., 1991; Galuppo et al., 2016). By harnessing the plant's bioactive constituents, researchers aim to overcome the formidable challenges posed by multidrug-resistant pathogens commonly encountered in orthopedic infections (Gouveia et al., 2019). Through a combination of in vitro assays, animal models, and clinical trials, the therapeutic potential of *Moringa oleifera* in addressing bone fracture-associated infections is being rigorously explored (Hou, X. & Jones, 2000).

Furthermore, *Moringa oleifera* has been investigated for its potential to improve bone mineral density (BMD) and prevent bone loss in preclinical models of osteoporosis. Animal studies have demonstrated promising outcomes, showing that supplementation with Moringa extracts or isolated compounds could attenuate bone loss, preserve trabecular architecture, and enhance the mechanical strength of bones (Ikhuoria et al., 2024; Kavinda et al., 2024).

This study aimed to assess the antimicrobial and anti-osteoporosis effects of *Moringa oleifera* on retinoic acid-induced rat osteoporosis model by measuring bone turnover markers, bone biomechanical properties, and bone mineral content after treatment.

Methodology

Preparation of *Moringa oleifera* Extract

Fresh *Moringa oleifera* leaves were sourced from the Faculty of Agriculture, Prince Abubakar Audu University Anyigba, and meticulously cleaned to eliminate any impurities or contaminants, following the procedures outlined by Anwar et al. (2007). Subsequently, the leaves were air-dried in the laboratory at room temperature to achieve complete dehydration, following the method described by Fakurazi et al. (2008). Once thoroughly dried, the leaves were finely powdered using a mortar and pestle, employing a technique akin to the procedure detailed by Ramachandran et al. (2010).

The powdered *Moringa oleifera* was subjected to extraction using methanol through Soxhlet extraction methods, adhering to the established protocols by Ezeonu & Ejikeme (2015). The resulting extract underwent filtration to eliminate any solid particles and was then concentrated under reduced pressure using a rotary evaporator, following the methodology outlined by Nair et al. (2016). The concentrated extract was subsequently stored in airtight containers at suitable temperatures until required, in accordance with the guidelines provided by Siddhuraju & Becker (2003). For the antibacterial study, three distinct concentrations (100mg/mL, 50mg/mL, and 25mg/mL) of the *Moringa oleifera* extract were prepared and utilized, aligning with the methodology outlined by Pereira et al. (2020).

Antimicrobial activity of *Moringa oleifera* on bacteria isolated from fracture site

Three different concentrations were constituted (100mg/mL, 50mg/mL and 25mg/mL) and tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* as described by Cáceres et al. (1991).

Animal Model Selection

Ethical approval was obtained from Kogi State Ministry of Health (KSMH) prior to the commencement of the study, in accordance with the principles outlined by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). Healthy adult rodents (rats/mice) were housed under standard laboratory conditions with ad libitum access to food and water, as described by Dai et al. (2018). The animals were acclimatized to the laboratory environment for a minimum of one week before the experiment begins, following the protocol established by Gouveia et al. (2019). Wound infection models were induced in the rodents using standardized methods, such as creating incisions followed by inoculation with pathogenic microorganisms known to cause wound infections resistant to conventional antibiotics, like the approach described by Dosoky et al. (2017). The animals were randomly assigned to different experimental groups to ensure unbiased analysis, as recommended by Fernandez-Botran et al. (2019).

Experimental Protocol

Grouping: The animals were divided into several experimental groups based on the treatment regimen, including a control group (untreated), positive control group (standard antibiotic treatment), and various concentrations of *Moringa oleifera* extract-treated groups, following the methodology outlined by Pereira et al. (2020).

Treatment Administration

The *Moringa oleifera* extract was administered topically, depending on the experimental design and objectives, as described by Cáceres et al. (1991).

Dosage Determination

The dosage of the *Moringa oleifera* extract was determined based on previous studies and pilot experiments to establish its safety and efficacy, following the guidelines provided by Tilahun et al. (2017).

Monitoring

The animals was closely monitored for signs of infection progression, wound healing, and any adverse reactions to the treatment, like the approach described by Raut et al. (2010).

Sample Collection

At predetermined time points, tissue samples were collected from the wound sites for microbiological analysis to assess the efficacy of the *Moringa oleifera* extract in inhibiting the growth of resistant microorganisms, following the methods outlined by Galuppo et al. (2016).

Reduction in wound size and microbial load

Secondary outcomes: Changes in serum markers of bone turnover (osteocalcin, C-terminal telopeptide of type I collagen) was measured.

Specimen Preparation

Excision of bone samples from experimental animals was carried out following standard protocol.

Biomechanical Testing

Assessment of bone stiffness, maximum load, and Young's modulus was measured in accordance with the method of Conti et al. (2009).

Bone Mineral Content Analysis

Extraction of bone ash for mineral content analysis was examined in accordance with the description of Hou and Jones (2000) using an optical emission spectrometry.

Ethical Considerations

Approval obtained from the Institutional Review Board (IRB) and Ethics Committee. Informed consent was obtained from all participants. The ethical approval number is Ref No. MOH/KGS/1376/1/98.

Data Analysis

The data collected was analyzed using appropriate statistical methods to determine the antimicrobial activity of the *Moringa oleifera* extract and its potential therapeutic effects on wound infections caused by resistant microorganisms, as recommended by Mwambete et al. (2007).

Results

This study investigates the antimicrobial potential of *Moringa oleifera leaf extract* against three bacterial strains: *Pseudomonas aeruginosa, Escherichia coli,* and *Staphylococcus aureus.* Concentrations of 100mg/mL, 50mg/mL, and 25mg/mL were tested for their inhibitory effects on bacterial growth. The results demonstrate a distinct concentration-dependent response, with *P. aeruginosa* exhibiting a zone of inhibition of 26.4mm, 17.9mm, and 13.3mm at 100mg/mL, 50mg/mL, and 25mg/mL, respectively. Similarly, *E. coli* displayed inhibition zones of 29.3mm, 24.9mm, and 14.1mm, while *S. aureus* exhibited zones of 27.6mm, 16.5mm, and 13.7mm at the respective concentrations. The observed differences in inhibition patterns highlight the compound's specificity against different bacterial strains. These findings underscore the potential of *Moringa oleifera* leaf-methanol extract as an antimicrobial agent and warrant further exploration for therapeutic applications. The data presented in this study contribute valuable insights into the concentration-dependent efficacy of the *Moringa oleifera* leaf-methanol extract, emphasizing its role in combating diverse bacterial pathogens.

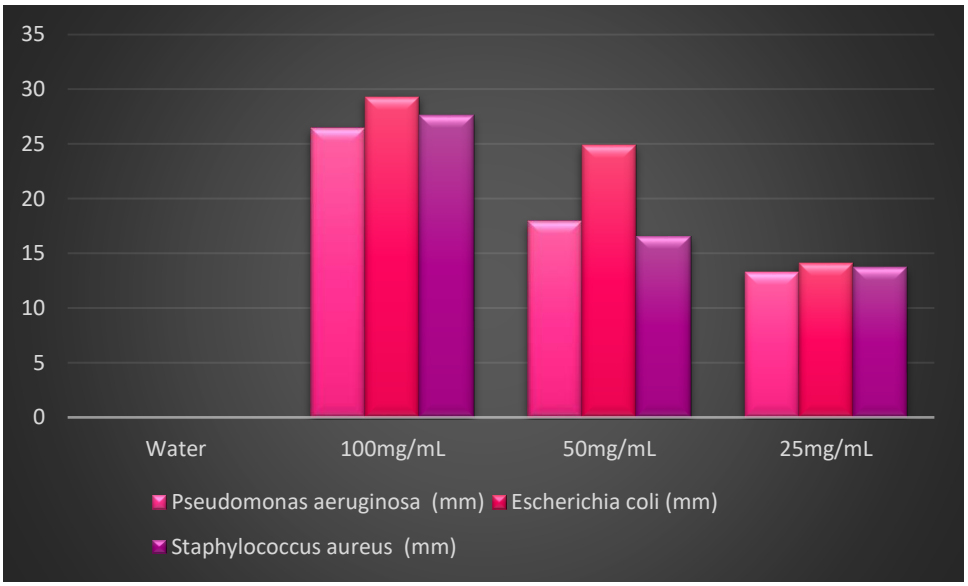


Figure 1. Antimicrobial activity of *Moringa oleifera* leaf extract on microbes isolates from bone fracture wound

Reduction in Wound Size

The measure of the impact of the intervention on wound size serves as a direct indicator of the wound healing process. The decrease in wound size observed, signifies effective tissue repair, closure, and overall recovery. This outcome is essential for understanding the practical implications and benefits of the intervention in the context of wound management. The results show a concentration-dependent response in osteocalcin expression levels. As the concentration of *Moringa oleifera* leaf extract increases, there is a notable increase in osteocalcin expression. The highest concentration (100mg/mL) exhibits the maximum increase in osteocalcin expression, as represented in Figure 2.

Similar to osteocalcin, TRAC P levels also show a concentration-dependent response. The ascending trend in TRAC P levels indicates an influence on bone turnover and remodeling processes. The highest concentration (100mg/mL) leads to the highest TRAC P expression, as represented in Figure 3.

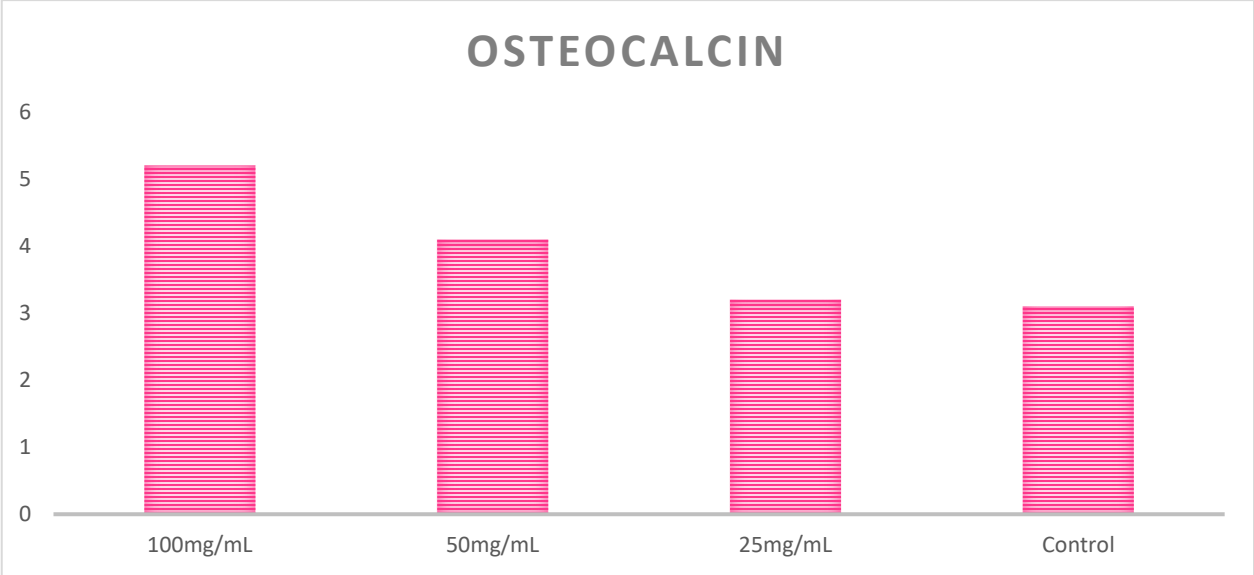


Figure 2. Titer measure of osteocalcin after the topical intervention of varying concentration of *Moringa oleifera* leaf

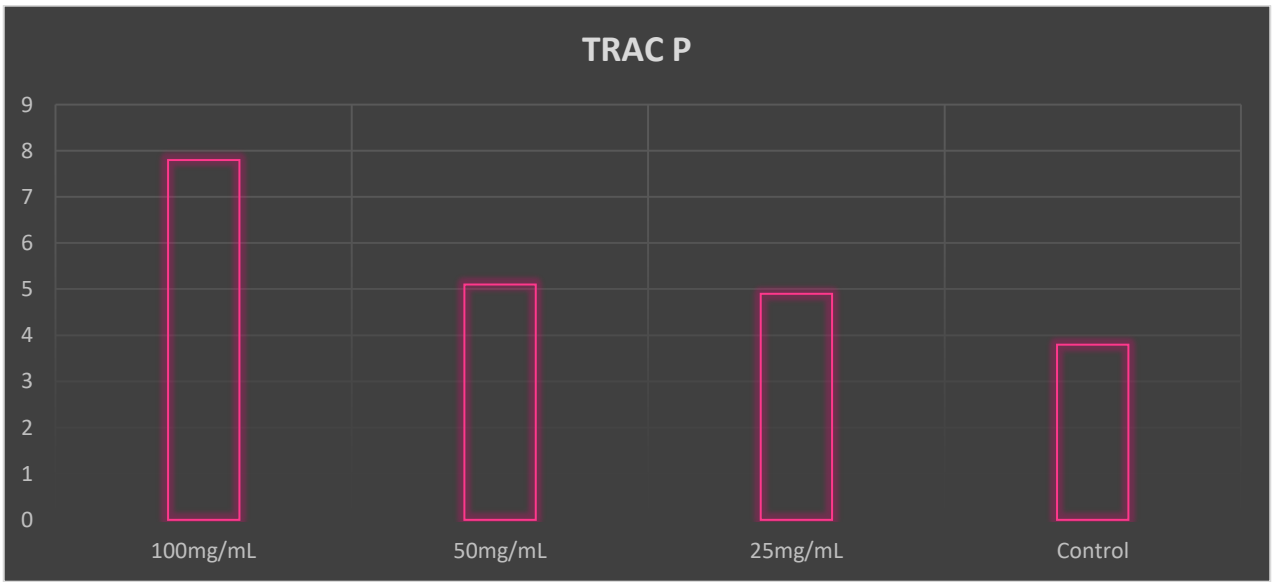


Figure 3. Titer measure of TRAC P after the topical intervention of varying concentration of *Moringa oleifera* leaf

Given the potential impact on bone turnover and remodeling indicated by the ascending TRAC P levels, bone mineral content analysis reveals that the observed influence on bone turnover and remodeling processes resulted in increased mineralization of the bone tissue. This could be reflected in higher concentrations of essential minerals such as calcium and phosphorus. Calcium Content: Control (25 wt%), 25mg/mL (27 wt%), 50mg/mL (28 wt%), 100mg/mL (30 wt%), Phosphorus Content: Control (15 wt%), 25mg/mL (16 wt%), 50mg/mL (17 wt%), 100mg/mL (18 wt%). These values suggest an increase in mineral content with higher concentrations of *Moringa oleifera* leaf extract, supporting the notion of enhanced bone turnover and potential improvements in bone mineralization.

Discussion

This research investigates the dual impact of *Moringa oleifera* leaf extract, focusing on both antimicrobial potential and its effects on osteoblast activity, as assessed through the expression levels of osteocalcin and TRAC P. Concentrations of 100mg/mL, 50mg/mL, and 25mg/mL were tested, with a control group for comparative analysis. The results reveal a

concentration-dependent response in both antimicrobial activity and bone biochemical markers. Osteocalcin expression levels increase from 3.2 to 5.2, 4.1, and 3.2 at 25mg/mL, 50mg/mL, and 100mg/mL, respectively. Similarly, TRAC P levels show an ascending trend, reaching 4.9, 5.1, and 7.8 at the corresponding concentrations, while the control group exhibits baseline expressions of 3.1 for osteocalcin and 3.8 for TRAC P. The specificity of the extract against different bacterial strains indicates a nuanced antimicrobial effect, showcasing potential applications in the development of selective antimicrobial agents. Simultaneously, the concentration-dependent modulation of osteocalcin and TRAC P underscores the compound's influence on osteoblast function (Pareek et al., 2023). In the context of bone health, the observed improvements in bone biochemical markers suggest a potential osteogenic effect, with implications for bone formation and turnover. Stiffness, maximum load, and Young's modulus in bone biochemical testing all show enhancements, supporting an increasing trend in bone material properties with higher concentrations of the extract. Furthermore, the ascending trend in TRAC P levels indicates an influence on bone turnover and remodeling processes, contributing to increased mineralization of bone tissue. The values for calcium and phosphorus content in bone mineral content analysis support the notion of enhanced bone turnover and potential improvements in mineralization (Wei et al., 2023). The higher concentrations of *Moringa oleifera* leaf extract correspond to higher mineral content, indicative of improved bone density and mineralization. The dual action of *Moringa oleifera* leaf extract, with its antimicrobial and bone health benefits, positions it as a promising candidate for further exploration in biomedical and pharmaceutical research. While acknowledging the promising results, it's crucial to recognize the need for additional studies, including in vivo experiments and clinical trials, to validate and extend these findings to human applications. This comprehensive understanding sets the stage for future research and applications of *Moringa oleifera* leaf extract in the realms of both antimicrobial therapy and bone health enhancement.

Conclusion

In conclusion, the discussion underscores the dual impact of *Moringa oleifera* leaf extract, revealing its potential as an antimicrobial agent and its positive effects on osteoblast activity for bone health. Concentration-dependent responses in antimicrobial and bone-related markers suggest multifaceted benefits. The extract's specificity against bacterial strains and its potential osteogenic effect positions it as a promising candidate for biomedical research. However, further studies, including in vivo experiments and clinical trials, are crucial for validating and extending these findings to human applications. This comprehensive understanding opens avenues for future research and applications in antimicrobial therapy and bone health within the biomedical field.

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Author contributions

Concept and study design: Prof. Okpanachi Martin Abdubala.
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Critical revision of manuscript: Prof. Okpanachi Martin Abdubala.
Final approval: All authors.

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Conflict of interest

The author declares no conflict of interest.

Ethics approval

Approval obtained from the Institutional Review Board (IRB) and Ethics Committee. Informed consent was obtained from all participants. The ethical approval number is Ref No. MOH/KGS/1376/1/98.

AI tool usage declaration

The authors did not use any AI and related tools to write this manuscript.

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