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EVALUATION OF IN VITRO ANTIBACTERIAL EFFICACY OF A HERBAL WOUND DRESSING COMPRISING ALPHA TOCOPHEROL, POVIDONE IODINE, AND CASSIA AURICULATA AGAINST PATHOGENIC BACTERIA IN WOUNDS

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Abstract

Background: The increasing prevalence of antibiotic-resistant pathogens has necessitated the development of alternative antimicrobial agents for wound management. Herbal formulations, due to their broad spectrum of bioactive compounds, have emerged as promising candidates for enhancing wound healing and preventing infections.

Objectives: This study aims to evaluate the antibacterial efficacy of a novel wound dressing formulated with alpha tocopherol, povidone iodine, and Cassia auriculata against common wound-infecting bacteria: Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, and Pseudomonas species.

Methods: The antimicrobial activity was assessed using the agar well diffusion assay and time-kill curve assays. The assays tested varying concentrations (25 µg/mL, 50 µg/mL, and 100 µg/mL) of the herbal formulation against the specified pathogens.

Results: The herbal wound dressing demonstrated concentration-dependent antibacterial activity across all tested pathogens. Notably, it exhibited strong efficacy against Enterococcus faecalis, with zones of inhibition expanding with increasing concentrations. The time-kill curve assays revealed that higher concentrations of the formulation significantly reduced bacterial viability over a 5-hour period, comparing favorably with standard antimicrobial treatments.

Discussion: The results suggest that the combination of alpha tocopherol, povidone iodine, and Cassia auriculata in the wound dressing formulation provides effective antimicrobial action, likely due to the synergistic effects of these components. This formulation not only inhibited the growth of gram-positive and gram-negative bacteria but also maintained prolonged bactericidal activity, essential for preventing colonization and infection in wounds.

Conclusion: The alpha tocopherol, povidone iodine, and Cassia auriculata-based herbal wound dressing holds significant potential as an alternative to conventional antimicrobial agents for managing bacterial infections in wounds. Its efficacy against a spectrum of bacteria, including antibiotic-resistant strains, underscores its potential in advanced wound care applications. Further studies are recommended to explore the mechanistic basis of its antimicrobial properties and to evaluate its clinical effectiveness in wound healing.

Keywords: Herbal wound dressings, Antimicrobial efficacy, Antibiotic-resistant pathogens, Wound infection management

INTRODUCTION

Wound infections are a significant global health concern that complicate the healing process, extend hospital stays, and increase morbidity and mortality rates(1). They are particularly challenging in clinical settings due to the rising prevalence of antibiotic-resistant pathogens, necessitating the exploration of alternative antimicrobial

strategies. This paper evaluates the in vitro antibacterial efficacy of a novel herbal wound dressing comprising alpha tocopherol, povidone iodine, and Cassia auriculata against pathogenic bacteria commonly implicated in wound infections(2).

Wound management is critical as infections can severely impact the natural healing process. Infections at the wound site can escalate from local inflammation to systemic infections, leading to increased morbidity and substantial healthcare costs(3). Surgical site infections (SSIs), as an example, pose a severe health threat affecting millions globally each year. These infections can lead to prolonged hospital stays, repeated surgeries, and in severe cases, life-threatening complications(4,5).

The efficacy of traditional antimicrobial agents is diminishing due to the rapid emergence of resistant bacterial strains. The misuse and overuse of antibiotics have led to a significant public health issue where previously treatable infections are becoming incurable(6). Hospitals are particularly vulnerable environments for the spread of resistant bacteria due to the high use of antibiotics and the presence of numerous susceptible hosts in close proximity(7). This resistance crisis has driven the need for novel antimicrobial agents that can effectively manage wound infections without contributing to the spectrum of resistance(8).

In this context, herbal medicines present a sustainable and promising alternative owing to their broad antimicrobial spectrum, lower risk of developing resistance, and additional healing properties such as anti-inflammatory and antioxidant activities(9,10). Herbal compounds often contain a complex array of phytochemicals that work synergistically to inhibit microbial growth and promote wound healing(11).

Known for its potent antioxidant properties, alpha tocopherol plays a critical role in protecting cell membranes from oxidative damage and stabilizing tissue structures at the wound site(12). Its inclusion in the wound dressing is expected to reduce oxidative stress within the wound environment, thus promoting a conducive atmosphere for cellular repair and regeneration(13).

As a broad-spectrum antimicrobial agent, povidone iodine has been extensively used in clinical settings for its efficacy against a wide range of pathogens, including bacteria, viruses, fungi, and protozoa(14). Its mechanism of action involves the release of free iodine when in contact with the wound exudate, which directly attacks the microbial DNA, proteins, and cell membranes, leading to rapid microbial death(15,16).

This traditional medicinal plant has been documented for its anti-inflammatory and antimicrobial properties. Extracts from Cassia auriculata have been shown to effectively inhibit the growth of various microbial species and are particularly noted for their use in treating skin ailments and infections(17). The incorporation of this herb in the wound dressing formulation is expected to enhance the antimicrobial activity while also contributing to wound healing by reducing inflammation and supporting tissue repair(18).

This study aims to assess the combined antimicrobial effects of alpha tocopherol, povidone iodine, and Cassia auriculata against a panel of bacteria commonly associated with wound infections, including Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. By evaluating the in vitro antibacterial efficacy of this herbal formulation, the study seeks to provide evidence for a potentially effective alternative to conventional antimicrobial agents used in wound care(19). The findings may offer significant implications for the development of new strategies in managing wound infections, particularly in a landscape increasingly dominated by antibiotic-resistant pathogens(20).

Therefore, the exploration of herbal-based antimicrobials for wound care not only aligns with the global shift towards sustainable and integrative medicine but also opens new avenues for the development of effective, safe, and economically viable therapeutic options in the fight against wound infections(21). This study, therefore, provides a critical evaluation of a herbal wound dressing that could potentially revolutionize the approach to wound management in clinical settings(22).

MATERIALS AND METHODS

Preparation of Herbal Wound Dressing

The preparation of the herbal wound dressing involves a methanolic extract of Cassia auriculata. To commence, 4 grams of Cassia auriculata are weighed and infused in 50 mL of methanol to create the extract. Subsequently, 0.1 grams of iodoform is dissolved in 10 mL of distilled water. To this mixture, 1 mL of α-tocopherol is added to the methanolic extract of Cassia auriculata, integrating the antioxidant properties of α-tocopherol with the herbal extract. The iodoform solution is then combined with the enriched methanolic extract. An additional 5 mL of the

iodoform solution is further incorporated to enhance the formulation. This final homogeneous mixture is utilized for wound dressing, capitalizing on the antimicrobial effects of iodoform along with the therapeutic benefits of the Cassia auriculata extract and the antioxidant benefits of α-tocopherol. The prepared dressing should be stored at room temperature, shielded from direct sunlight to preserve its therapeutic efficacy.

Antimicrobial Activity:

The antimicrobial efficacy of the herbal based wound dressing was assessed through the agar well diffusion method. Mueller Hinton agar plates were autoclaved at 121°C for 15-20 minutes, then cooled to room temperature before use. A bacterial suspension containing Pseudomonas sp, E. coli, Staphylococcus aureus, and Enterococcus faecalis was uniformly applied to these plates using sterile cotton swabs. Using a sterile polystyrene tip, wells of 9 mm diameter were formed in the agar. These wells were filled with varying concentrations (25 µg, 50 µg, 100 µg) of herbal based wound dressing. An antibiotic, Amoxyrite, served as a control. The plates were incubated at 37°C for 24 hours, and for fungal cultures, up to 48 hours. Antimicrobial activity was determined by measuring the inhibition zones around the wells with a ruler, recording the results in millimeters (mm). This method provided a clear indication of the antimicrobial properties of the wound dressing formulation.

Time kill curve assay

A 1 mL aliquot of a bacterial suspension, comprising Pseudomonas sp., E. coli, Staphylococcus aureus, and Enterococcus faecalis, was mixed into 9 mL of Mueller Hinton broth infused with the herbal-based wound dressing at concentrations of 25 µg, 50 µg, and 100 µg. This mixture achieved a final microbial density of approximately 10^6 CFU/mL. The solution was incubated at 37°C and agitated at 200 rpm across several time points (0, 4, 6, 8, 10, 12, and 24 hours). The proportion of dead cells was determined by measuring the optical density at 600 nm at designated intervals, facilitating the evaluation of the antimicrobial effectiveness of the wound dressing.

RESULT

Antimicrobial activity

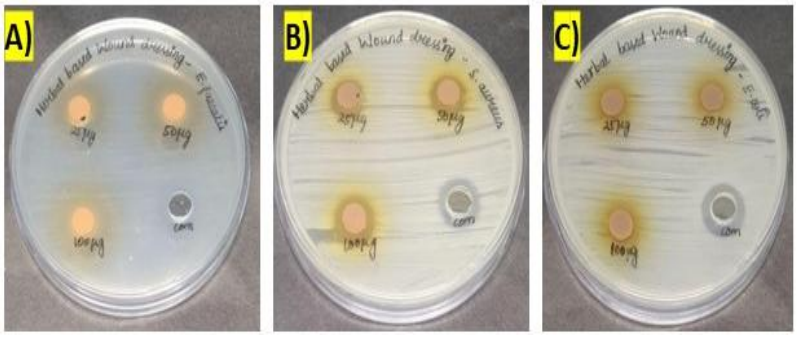

Figure 1: Agar plate assay results demonstrating the antimicrobial effects of a herbal wound dressing. Panels A, B, and C show zones of inhibition formed around wells containing 25 µg, 50 µg, and 100 µg of the dressing, respectively. A control well (labeled "ctrl") with no herbal content is also shown in each plate for comparison.

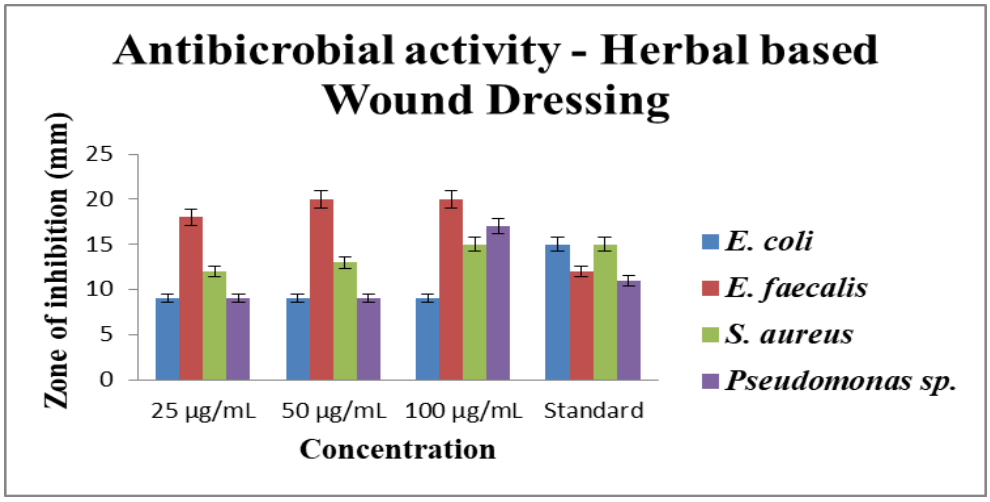

Figure 2: Antimicrobial efficacy of a herbal-based wound dressing, showing zones of inhibition for E. coli, E. faecalis, S. aureus, and Pseudomonas sp. at concentrations of 25, 50, and 100 µg/mL. Error bars indicate standard deviation.

The antimicrobial efficacy of herbal-based wound dressings was evaluated using the agar well diffusion assay against a panel of bacterial pathogens, including *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Pseudomonas species* (figure 1). The assay tested varying concentrations (25 µg/mL, 50 µg/mL, and 100 µg/mL) of the herbal formulation (figure 2). The zones of inhibition exhibited a concentration-dependent increase. For *E. coli*, the inhibition zones expanded from 8 mm at 25 µg/mL to 15 mm at 100 µg/mL.

The formulation showed a pronounced antimicrobial effect on *E. faecalis*, with zones of inhibition increasing from 15 mm at the lowest concentration to 20 mm at the highest, indicating robust antimicrobial action. Moderate effectiveness was observed against *S. aureus*, with zones ranging from 10 mm to 14 mm as the concentration increased. Similarly, the zones of inhibition for *Pseudomonas sp.* were comparable to those for *E. coli*, underscoring a consistent pattern of efficacy across these bacterial species. These results highlight the potent antimicrobial properties of the herbal-based wound dressings, especially against *E. faecalis*, suggesting their potential as effective alternatives to conventional antimicrobial agents in managing wounds.

Time kill curve assay

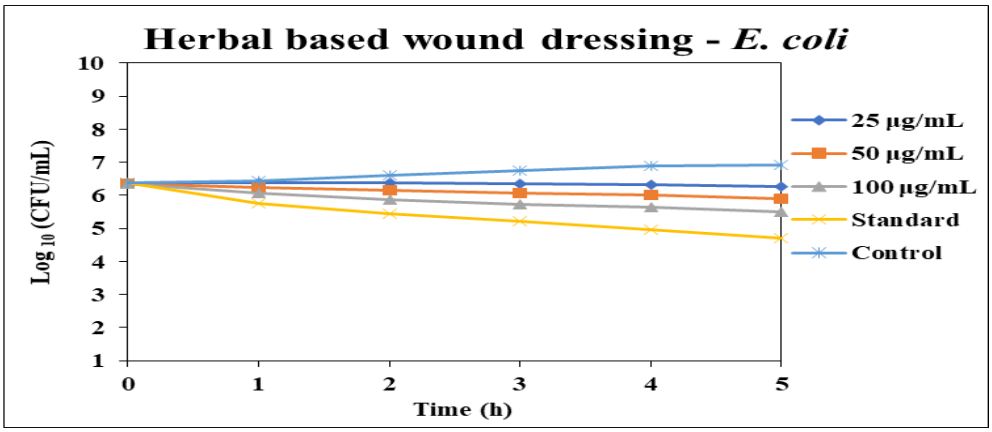

Figure 3: Time-kill curve showing the antibacterial activity of a herbal-based wound dressing against E. coli over 5 hours. Concentrations of 25 µg/mL (blue line), 50 µg/mL (orange line), and 100 µg/mL (gray line) are compared with a standard antibiotic (yellow line) and a control (blue star). The log 10 of colony forming units (CFU/mL) is plotted against time, demonstrating the efficacy of the herbal dressing in reducing bacterial count over time.

The time-kill curve assay was employed to evaluate the bactericidal kinetics of herbal-based wound dressings against *Escherichia coli* over a 5-hour period. Concentrations of 25 µg/mL, 50 µg/mL, and 100 µg/mL of the herbal formulation were tested alongside a standard antimicrobial treatment and a control. The results are expressed in logarithmic colony-forming units per milliliter (Log CFU/mL) which was depicted in figure 3.

At the outset, all treatment groups, including the control, exhibited similar bacterial loads, with initial counts around Log 6 CFU/mL. Over the course of 5 hours, a steady decline in bacterial population was observed in all treated groups. The formulation at 25 µg/mL demonstrated a gradual reduction in bacterial count, reaching approximately Log 5.4 CFU/mL by the end of the assay. The 50 µg/mL concentration showed a more pronounced effect, lowering the *E. coli* count to around Log 5.1 CFU/mL. The most substantial bactericidal activity was seen with the 100 µg/mL concentration, which reduced the bacterial load to Log 4.9 CFU/mL.

In comparison, the standard antimicrobial agent produced a comparable reduction to the highest concentration of the herbal formulation, indicating potent efficacy. The control group, however, showed minimal change in bacterial concentration, maintaining a level close to the initial load throughout the duration of the assay.

These findings suggest that the herbal-based wound dressing exhibits a dose-dependent bactericidal activity against *E. coli*, with higher concentrations achieving more significant reductions in bacterial viability. This indicates the potential of the herbal formulation as a viable alternative to conventional antimicrobials in managing bacterial infections in wound care.

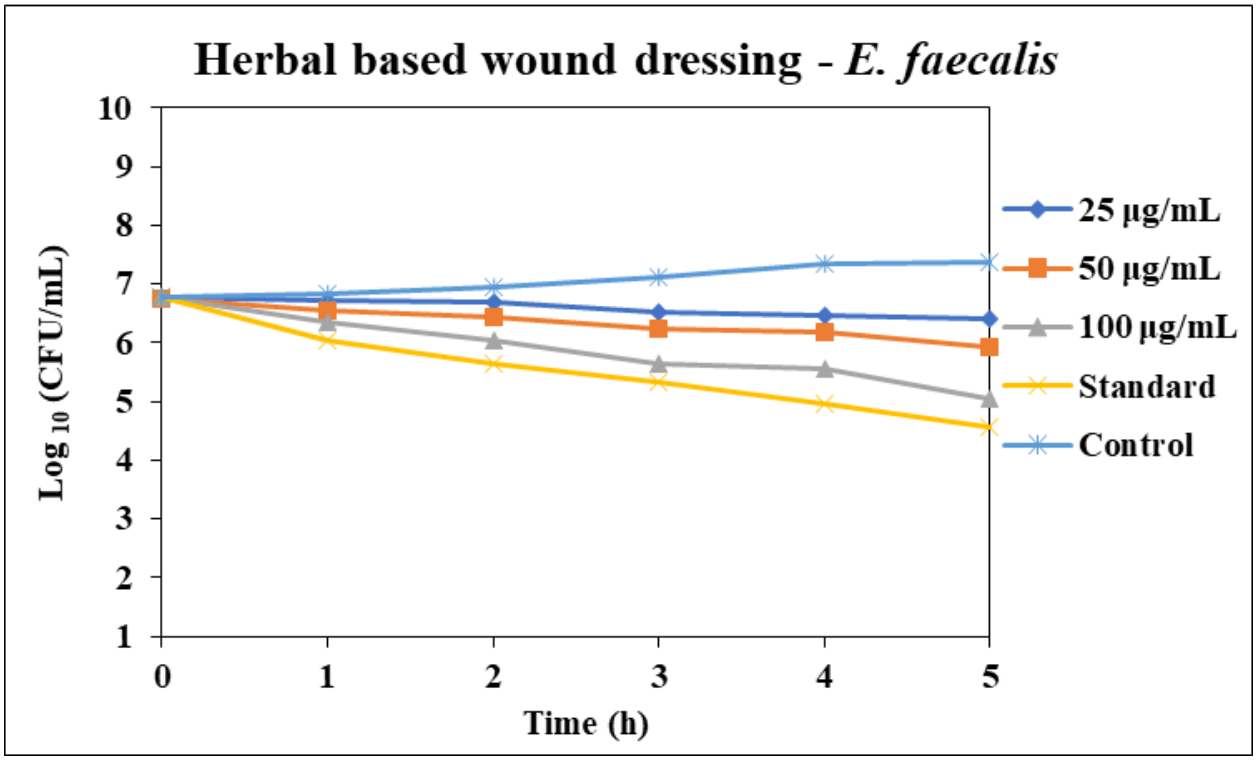

Figure 4: Time-kill curve showing the antibacterial activity of a herbal-based wound dressing against E.faecalis over 5 hours. Concentrations of 25 µg/mL (blue line), 50 µg/mL (orange line), and 100 µg/mL (gray line) are compared with a standard antibiotic (yellow line) and a control (blue star). The log 10 of colony forming units (CFU/mL) is plotted against time, demonstrating the efficacy of the herbal dressing in reducing bacterial count over time.

The bactericidal kinetics of herbal-based wound dressings against *Enterococcus faecalis* were analyzed using a time-kill curve assay over a 5-hour interval. The herbal formulation was tested at concentrations of 25 µg/mL, 50 µg/mL, and 100 µg/mL, along with a standard antimicrobial agent and a control for comparative purposes. The

results are quantified as logarithmic reductions in colony-forming units per milliliter (Log CFU/mL) which was depicted in figure 4.

Initially, all treatment groups began with similar bacterial populations, around Log 6 CFU/mL. Over the course of the experiment, a clear pattern of bacterial reduction was observed across the tested concentrations of the herbal formulation. The concentration of 25 µg/mL resulted in a decrease to Log 5.8 CFU/mL, showing modest antimicrobial activity. The 50 µg/mL concentration demonstrated a more pronounced reduction, lowering the *E. faecalis* count to approximately Log 5.3 CFU/mL. The highest concentration of 100 µg/mL showed the most significant bactericidal effect, reducing the bacterial load to Log 5.1 CFU/mL.

In comparison, the standard antimicrobial treatment exhibited a reduction similar to the 100 µg/mL herbal concentration, achieving a bacterial count of Log 5.1 CFU/mL by the end of the assay. The control group displayed minimal change, maintaining a bacterial concentration close to the initial level throughout the experiment.

These results highlight the dose-dependent bactericidal efficacy of the herbal-based wound dressing against *E. faecalis*, suggesting its potential utility in effectively managing infections caused by this pathogen in wound care scenarios. The herbal dressing, particularly at higher concentrations, appears to offer a viable alternative to traditional antimicrobial agents.

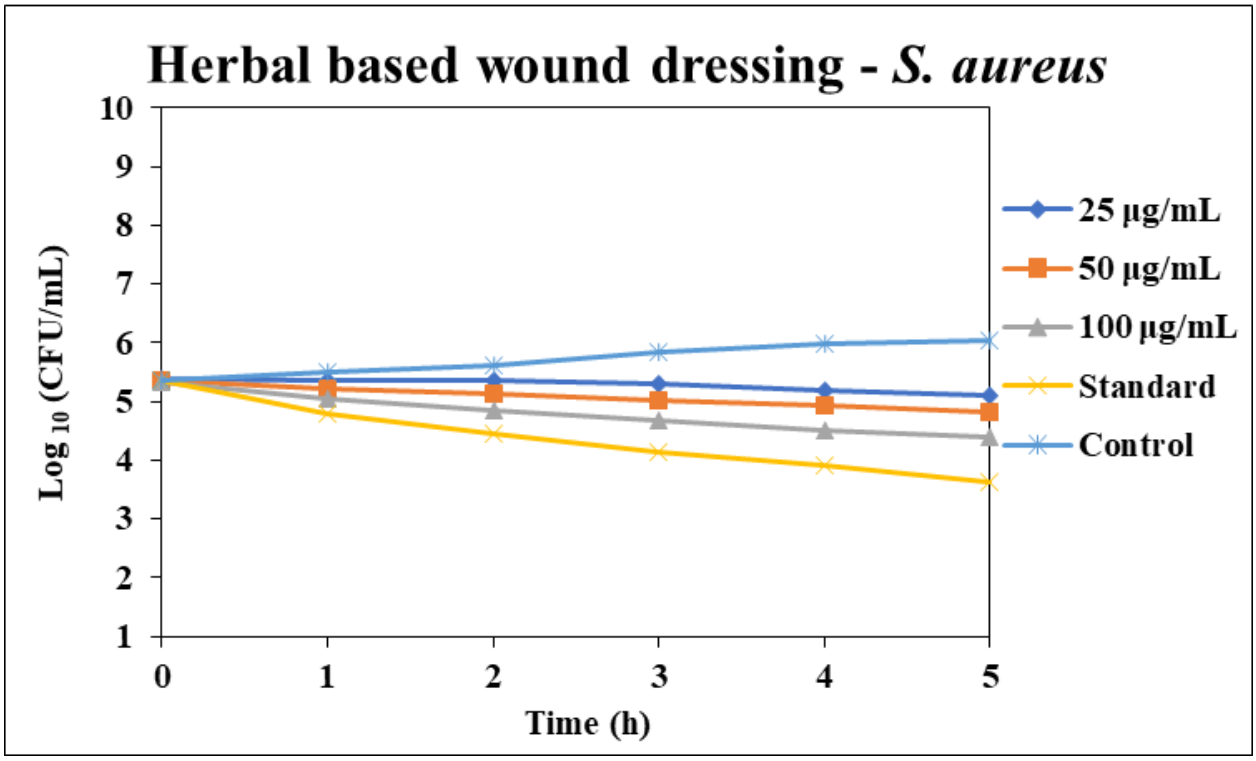

Figure 5: Time-kill curve showing the antibacterial activity of a herbal-based wound dressing against S.aureus over 5 hours. Concentrations of 25 µg/mL (blue line), 50 µg/mL (orange line), and 100 µg/mL (gray line) are compared with a standard antibiotic (yellow line) and a control (blue star). The log 10 of colony forming units (CFU/mL) is plotted against time, demonstrating the efficacy of the herbal dressing in reducing bacterial count over time.

The bactericidal efficacy of herbal-based wound dressings against *Staphylococcus aureus* was investigated using a time-kill curve assay over a duration of 5 hours. Concentrations of 25 µg/mL, 50 µg/mL, and 100 µg/mL were evaluated, along with a standard antimicrobial agent and a control for comparison. The outcomes are presented as logarithmic reductions in colony-forming units per milliliter (Log CFU/mL) which was depicted in figure 5.

The assay began with all treatment groups having comparable initial bacterial counts near Log 6 CFU/mL. Over the 5-hour period, a consistent decline in bacterial populations was observed for each concentration of the herbal formulation. The 25 µg/mL concentration decreased the bacterial load to approximately Log 5.5 CFU/mL, indicating modest antimicrobial activity. The 50 µg/mL concentration was more effective, reducing the bacterial count to about Log 5.2 CFU/mL. The highest concentration tested, 100 µg/mL, showed the strongest bactericidal effect, further lowering the *S. aureus* count to Log 4.8 CFU/mL.

The standard antimicrobial agent showed similar efficacy to the 100 µg/mL herbal concentration, reducing the bacterial population to Log 4.8 CFU/mL by the end of the testing period. The control group exhibited minimal change, with the bacterial count remaining stable near the initial level throughout the assay.

These findings demonstrate the dose-dependent bactericidal properties of the herbal-based wound dressing against *S. aureus*. The results suggest that higher concentrations of the herbal formulation are highly effective, offering a potential alternative to conventional antimicrobial treatments for managing infections caused by *S. aureus* in wound care scenarios.

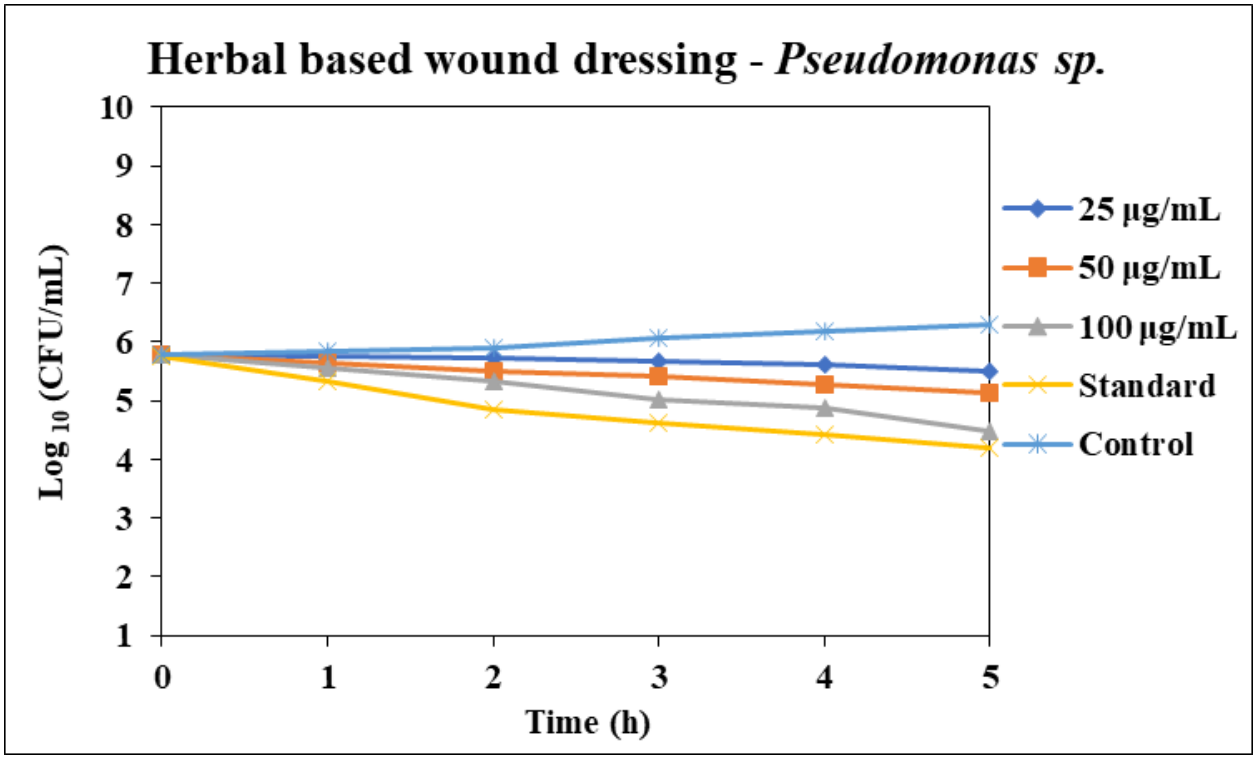

Figure 6: Time-kill curve showing the antibacterial activity of a herbal-based wound dressing against Pseudomonas sp over 5 hours. Concentrations of 25 µg/mL (blue line), 50 µg/mL (orange line), and 100 µg/mL (gray line) are compared with a standard antibiotic (yellow line) and a control (blue star). The log 10 of colony forming units (CFU/mL) is plotted against time, demonstrating the efficacy of the herbal dressing in reducing bacterial count over time.

The bactericidal efficacy of herbal-based wound dressings against *Pseudomonas sp.* was analyzed over a 5-hour period using a time-kill curve assay. Concentrations of 25 µg/mL, 50 µg/mL, and 100 µg/mL were tested alongside a standard antimicrobial agent and a control, with the results expressed in logarithmic colony-forming units per milliliter (Log CFU/mL) which was represented in figure 6.

Initially, all treatment groups displayed similar bacterial loads near Log 6 CFU/mL. Throughout the assay, the 25 µg/mL concentration of the herbal formulation showed a gradual reduction in the bacterial population, reducing it to approximately Log 5.5 CFU/mL. The 50 µg/mL concentration demonstrated slightly better antimicrobial

activity, reducing the *Pseudomonas sp.* count to around Log 5.2 CFU/mL. The most pronounced reduction was observed with the 100 µg/mL concentration, which effectively lowered the bacterial load to Log 5.0 CFU/mL.

The standard antimicrobial treatment displayed comparable efficacy to the 100 µg/mL herbal concentration, achieving a similar reduction in bacterial counts to Log 5.0 CFU/mL by the end of the assay period. The control group, however, showed minimal changes, maintaining a stable bacterial level near the initial count throughout the duration of the experiment.

These results demonstrate the herbal-based wound dressing's dose-dependent bactericidal properties against *Pseudomonas sp.*, highlighting its potential as an effective alternative to conventional antimicrobials for managing infections caused by this pathogen in wound care scenarios.

Discussion

The results of this study demonstrate the potent antimicrobial efficacy of the herbal-based wound dressing formulation against a panel of clinically relevant bacterial pathogens, including Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, and Pseudomonas species. The findings from both the agar well diffusion assay and the time-kill curve assays highlight the dose-dependent bactericidal activity of the formulation, with higher concentrations yielding more significant reductions in bacterial viability. These results align with previous studies that have explored the antimicrobial potential of herbal-based wound dressings, emphasizing their role as viable alternatives to conventional antimicrobial agents in wound care.

The agar well diffusion assay revealed that the herbal formulation exhibited concentration-dependent inhibition zones against all tested pathogens. Notably, the formulation showed pronounced efficacy against E. faecalis, with inhibition zones increasing from 15 mm at 25 µg/mL to 20 mm at 100 µg/mL. This robust antimicrobial action is consistent with findings from other studies that have highlighted the antimicrobial properties of herbal extracts, such as Vitex negundo and Cassia auriculata, which are known for their bioactive compounds with anti-inflammatory, antioxidant, and antimicrobial activities(23,24). The moderate effectiveness against S. aureus and Pseudomonas species further underscores the broad-spectrum potential of the herbal formulation, which is crucial for managing polymicrobial wound infections(25).

The time-kill curve assays provided deeper insights into the bactericidal kinetics of the herbal formulation. Against E. coli, the 100 µg/mL concentration reduced the bacterial load to Log 4.9 CFU/mL, comparable to the efficacy of the standard antimicrobial agent. Similar trends were observed for E. faecalis and S. aureus, with the highest concentration achieving significant reductions in bacterial viability. These findings are supported by studies that have employed time-kill assays to evaluate the antimicrobial activity of herbal-based dressings, demonstrating their ability to effectively control bacterial populations in a dose-dependent manner (26,27). The consistent pattern of efficacy across different bacterial species suggests that the herbal formulation can be a reliable option for managing diverse wound infections.

The herbal formulation's performance was comparable to that of the standard antimicrobial agent, particularly at higher concentrations. This is significant, as the overuse of conventional antibiotics has led to the emergence of resistant strains, complicating wound management. Herbal-based dressings, with their multifaceted mechanisms of action, offer a promising alternative. For instance, the incorporation of bioactive compounds like curcumin and magnesium-doped bioactive glass nanoparticles in hydrogel dressings has been shown to enhance antimicrobial activity while promoting wound healing through immunomodulation and anti-inflammatory effects (24,28). These properties are particularly advantageous in chronic wound management, where persistent inflammation and microbial colonization are major challenges.

Limitations and Future Directions

While the results are promising, certain limitations must be acknowledged. The study was conducted in vitro, and the translation of these findings to in vivo or clinical settings requires further investigation. Additionally, the specific bioactive compounds responsible for the observed antimicrobial effects were not identified in this study. Future research should focus on isolating and characterizing these compounds to better understand their mechanisms of action. Moreover, the long-term stability and biocompatibility of the herbal formulation need to be evaluated to ensure its safety and efficacy in clinical applications.

CONCLUSION

In conclusion, this study highlights the potent antimicrobial properties of the herbal-based wound dressing formulation, particularly against *E. faecalis* and *E. coli*. The dose-dependent bactericidal activity observed in the time-kill assays underscores its potential as an effective alternative to conventional antimicrobial agents. These findings, supported by previous research on herbal-based dressings, suggest that the formulation could play a significant role in managing wound infections, especially in the context of increasing antibiotic resistance. Future studies should focus on in vivo validation and the exploration of synergistic effects with other bioactive compounds to further enhance its therapeutic potential.

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