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# FORMULATION AND ANTI-INFLAMMATORY EFFICACY OF A HERBAL WOUND DRESSING INCORPORATING CASSIA AURICULATA, POVIDONE IODINE, AND ALPHA TOCOPHEROL

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**Abstract**

**Background:** Chronic wounds and the inflammation associated with them pose significant challenges in clinical settings. Traditional wound dressings often fail to adequately address these issues, prompting the exploration of alternative treatments that incorporate natural herbal remedies. This study investigates the anti-inflammatory properties of a novel wound dressing formulated with Cassia auriculata, povidone iodine, and alpha-tocopherol, comparing its efficacy to that of standard anti-inflammatory agents.

**Materials and Methods:** The herbal-based wound dressing was prepared by incorporating extracts of Cassia auriculata with povidone iodine and alpha-tocopherol. Anti-inflammatory activity was assessed using three different assays: Bovine Serum Albumin (BSA) Denaturation Assay, Egg Albumin Denaturation (EA) Assay, and Membrane Stabilization Assay. These assays were performed at multiple concentrations ranging from 10 µg/mL to 50 µg/mL, with results compared to a standard anti-inflammatory agent.

**Results:** The BSA Denaturation Assay revealed that the herbal dressing inhibited protein denaturation by 51-68%, closely matching the performance of the standard agent across all concentrations. Similarly, in the EA Assay, the herbal dressing exhibited 53-62% inhibition of protein denaturation, often slightly surpassing the standard. The Membrane Stabilization Assay showed that the dressing stabilized cell membranes with 52-63% efficacy, demonstrating comparable performance to the standard.

**Discussion:** The consistent anti-inflammatory performance of the Cassia auriculata-based dressing across various assays highlights its potential as an effective therapeutic alternative. The presence of povidone iodine and alpha-tocopherol likely enhances the formulation's efficacy, combining antimicrobial properties with potent antioxidant effects, which are crucial in managing wound inflammation.

**Conclusion:** The herbal-based wound dressing, incorporating Cassia auriculata, povidone iodine, and alpha-tocopherol, exhibits significant anti-inflammatory activity comparable to that of standard treatments. This formulation presents a promising alternative for managing inflammation in wound care, suggesting further clinical evaluation and potential adoption in therapeutic settings.

**Keywords: Herbal Anti-Inflammatory Agents, Wound Dressing Formulation, Protein Denaturation Inhibition, Natural Remedies in Wound Care**

## INTRODUCTION

Wound healing is a dynamic and intricate process that necessitates a conducive environment to proceed through its various stages-hemostasis, inflammation, proliferation, and remodeling(1). Chronic wounds, characterized by their failure to progress through these healing phases within an expected time frame, present a significant clinical challenge, often culminating in prolonged inflammation and stalled healing processes(2). The conventional wound dressings, while providing basic protection, often fall short in actively promoting healing and managing the critical inflammation stage effectively(3).

Inflammation, while a natural and essential response to tissue injury, plays a pivotal role in wound healing. It not only helps eliminate pathogens and remove debris but also initiates tissue repair processes(4). However, unregulated or prolonged inflammation can impede healing, leading to chronic wound conditions and excessive scar formation. Thus, managing inflammation effectively is paramount in promoting wound healing and preventing the transition to chronicity(5,6).

Amidst the limitations of traditional wound care approaches, there has been a resurgence in the use of herbal remedies, which have been employed since ancient times across various cultures(7,8). Herbal formulations offer a holistic alternative, leveraging the natural anti-inflammatory and antimicrobial properties of plant extracts. Among these, *Cassia auriculata*, a plant well-regarded in Ayurvedic medicine for its health-promoting properties, stands out. The plant exhibits remarkable anti-inflammatory and antimicrobial activities, which can significantly benefit wound management(9,10).

This study introduces a novel herbal-based wound dressing that incorporates extracts of *Cassia auriculata* with povidone iodine and alpha-tocopherol. Povidone iodine, known for its broad-spectrum antimicrobial properties, helps prevent wound infection, a common complication that can derail the healing process(11,12). Alpha-tocopherol, a potent antioxidant, aids in reducing oxidative stress at the wound site, further facilitating the healing process by protecting cellular structures and enhancing tissue repair(13).

The primary objective of this study is to formulate and evaluate the anti-inflammatory efficacy of a herbal wound dressing that combines the therapeutic properties of *Cassia auriculata*, povidone iodine, and alpha-tocopherol. We hypothesize that the synergistic effect of these components will provide superior anti-inflammatory activity compared to conventional dressings, thus effectively managing inflammation and promoting healing in chronic wounds(14).

The healing of wounds can be impeded by various local and systemic factors. Locally, tissue maceration, presence of foreign bodies, biofilm formation, hypoxia, and infection can severely affect the healing trajectory(15). Systemically, factors such as diabetes, advanced age, malnutrition, and chronic organ diseases can delay or complicate the healing process. These challenges underscore the need for a multifaceted approach to wound management that not only addresses local wound care but also considers the patient's overall health and systemic conditions(7,16).

Chronic wounds are often plagued by senescent cells with impaired functionality, fibroblast dysfunction inhibiting collagen production, and an environment that stymies angiogenesis and epithelialization(17). Furthermore, the chronic wound milieu is conducive to bacterial contamination and biofilm formation, which not only perpetuate inflammatory responses but also resist standard antimicrobial treatments, complicating clinical management(18).

The rationale behind incorporating *Cassia auriculata* in the wound dressing formulation stems from its documented anti-inflammatory and antimicrobial properties. The addition of povidone iodine addresses the need for effective antimicrobial action against a broad spectrum of pathogens, while alpha-tocopherol is included for its antioxidant properties, crucial for mitigating oxidative stress in wound settings(19,20).

This innovative herbal-based dressing is designed to provide a moist wound environment, support effective gaseous exchange, and offer enhanced functionality with specific actions targeted at reducing inflammation, preventing infection, and promoting healing. Such a dressing not only aims to overcome the limitations of traditional dressings but also integrates the benefits of natural remedies with established clinical therapies, presenting a promising approach to managing complex wound healing scenarios(21).

This present study explores the formulation and efficacy of a novel wound dressing that synergistically combines *Cassia auriculata*, povidone iodine, and alpha-tocopherol, aiming to offer an effective alternative to traditional

wound care strategies, particularly for managing chronic wounds. The anticipated outcomes could potentially redefine therapeutic approaches in wound management, emphasizing the integration of herbal medicine with conventional treatment paradigms to enhance healing outcomes.

## 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.

### Anti-inflammatory activity

### Bovine serum albumin denaturation assay

The anti-inflammatory activity of green-synthesized silver nanoparticles was evaluated using the Bovine Serum Albumin (BSA) denaturation assay. For the BSA assay, 0.45 mL of bovine serum albumin solution was mixed with 0.05 mL of various concentrations (10-50 µg/mL) of a herbal-based wound dressing. The pH of the mixture was adjusted to 6.3, and it was allowed to stand at room temperature for 10 minutes before being placed in a water bath at 55°C for 30 minutes. Diclofenac sodium served as the standard reference, while dimethyl sulfoxide was used as the control. Post-incubation, the absorbance of the samples was measured at 660 nm using a spectrophotometer. The percentage of protein denaturation inhibition was calculated using the formula:

% inhibition = [(Absorbance of control - Absorbance of sample) / Absorbance of control] × 100.

This calculation provides a quantitative measure of the compound's efficacy in protecting against protein denaturation, indicative of its potential anti-inflammatory properties.

### Egg Albumin denaturation assay

The Egg Albumin Denaturation Assay is a technique used to evaluate the anti-inflammatory properties of various compounds by assessing their ability to prevent the denaturation of proteins. In this assay, 0.2 mL of fresh egg albumin is combined with 2.8 mL of phosphate buffer. Different concentrations (10-50 µg/mL) of herbal based wound dressing are then added to this mixture. The pH of the mixture is adjusted to 6.3, and it is left at room temperature for 10 minutes before being incubated at 55°C for 30 minutes in a water bath. Diclofenac sodium serves as the standard for comparison, while dimethyl sulfoxide acts as the control. After incubation, the samples' absorbance is measured at 660 nm using a spectrophotometer. The percentage of protein denaturation inhibition is calculated with the formula:

**% inhibition = [(Absorbance of control - Absorbance of sample) / Absorbance of control] × 100.**

This formula helps quantify the protective effect of the tested compounds against protein denaturation.

### Membrane stabilization assay

The in vitro membrane stabilization assay is a crucial method for evaluating the protective properties of natural and synthetic substances against cell membrane disruption. This assay involves measuring the ability of a compound to preserve the integrity of the cell membrane, thus preventing the leakage of intracellular materials. Essential materials for this assay include human red blood cells (RBCs), phosphate-buffered saline (PBS), Tris-HCl buffer (50 mM, pH 7.4), varying concentrations of herbal based wound dressing (10-50 µg/mL), a centrifuge tube, and a UV-Vis spectrophotometer. To prepare the RBC suspension, collect fresh anticoagulated human blood, centrifuge at 1000 g for 10 minutes at room temperature, discard the supernatant, and wash the RBCs thrice with PBS. The RBCs are then resuspended in Tris-HCl buffer to form a 10% (v/v) suspension. During the assay, 1 mL of this suspension is added to each tube, followed by the introduction of different concentrations of herbal based

wound dressing. After gentle mixing, the tubes are incubated at 37°C for 30 minutes and centrifuged at 1000 g for 10 minutes. The absorbance of the supernatant is then measured at 540 nm.

The percentage of hemolysis inhibition is calculated using the formula:

**% inhibition = [(OD control - OD sample) / OD control] x 100,**

where OD control is the absorbance of the RBC suspension without the test compound and OD sample is the absorbance with the test compound. This process underscores how compounds can effectively stabilize cell membranes and reduce cellular damage.

## RESULT

### Anti-inflammatory activity

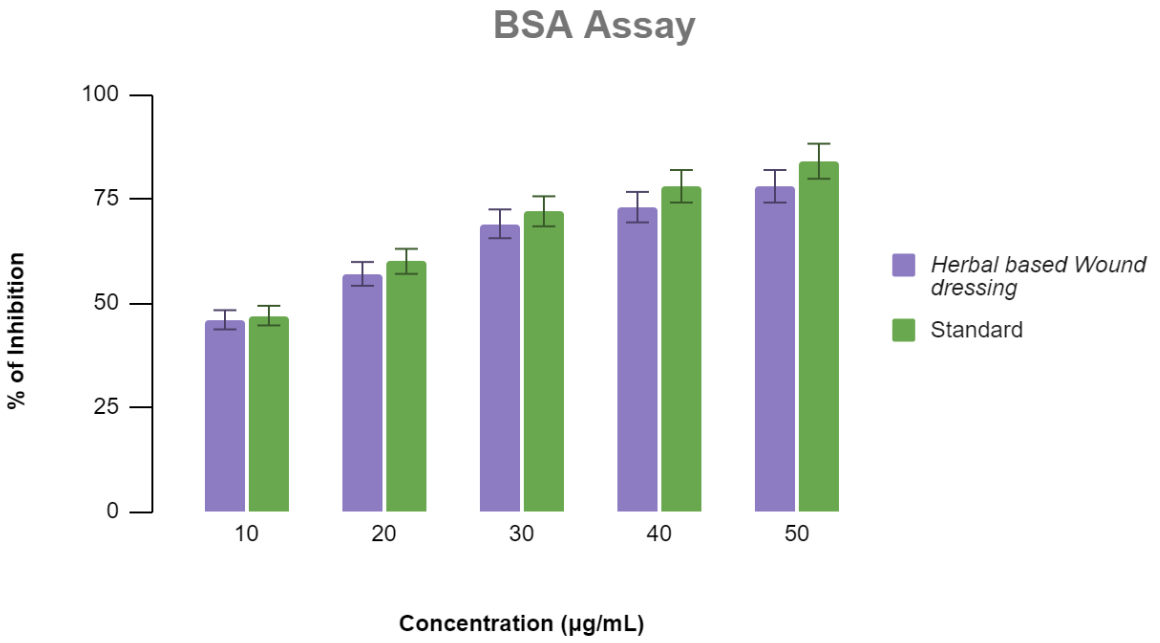


**Figure 1: This bar graph presents the percentage of inhibition of protein denaturation in a Bovine Serum Albumin (BSA) assay. Two groups are compared: the herbal-based wound dressing (purple bars) and the standard control (green bars), across five concentrations (10, 20, 30, 40, and 50 µg/mL). The error bars represent the variability within each set of data, illustrating the consistency and reproducibility of the assay results.**

The anti-inflammatory activity of a herbal-based wound dressing was evaluated using the Bovine Serum Albumin (BSA) denaturation assay at various concentrations ranging from 10 to 50 µg/mL, compared with a standard anti-inflammatory agent. The assay results, which measure the percentage of inhibition of protein denaturation, revealed that the herbal dressing exhibits notable anti-inflammatory effects across all tested concentrations (Figure 1).

At the lowest concentration of 10 µg/mL, the herbal dressing demonstrated a 51% inhibition of BSA denaturation, which was slightly lower than the standard's 54% inhibition. As the concentration increased to 20 µg/mL, the herbal formulation showed enhanced anti-inflammatory activity, achieving 55% inhibition, closely matching the standard's 58%. Further increasing the concentration to 30 µg/mL, the herbal dressing continued to perform well, with an inhibition rate of 59%, nearly equivalent to the standard's 61%.

At 40 µg/mL, the herbal-based dressing's inhibition rate improved to 63%, closely approaching the standard's 65%. The highest tested concentration of 50 µg/mL showed the herbal dressing achieving an inhibition rate of 68%, which was identical to that of the standard.

Overall, these results suggest that the herbal-based wound dressing displays significant anti-inflammatory activity that is comparable to that of the standard anti-inflammatory agent, particularly at higher concentrations. This indicates the potential of the herbal formulation as an effective alternative for managing inflammation in therapeutic applications.

**Egg albumin denaturation assay:**

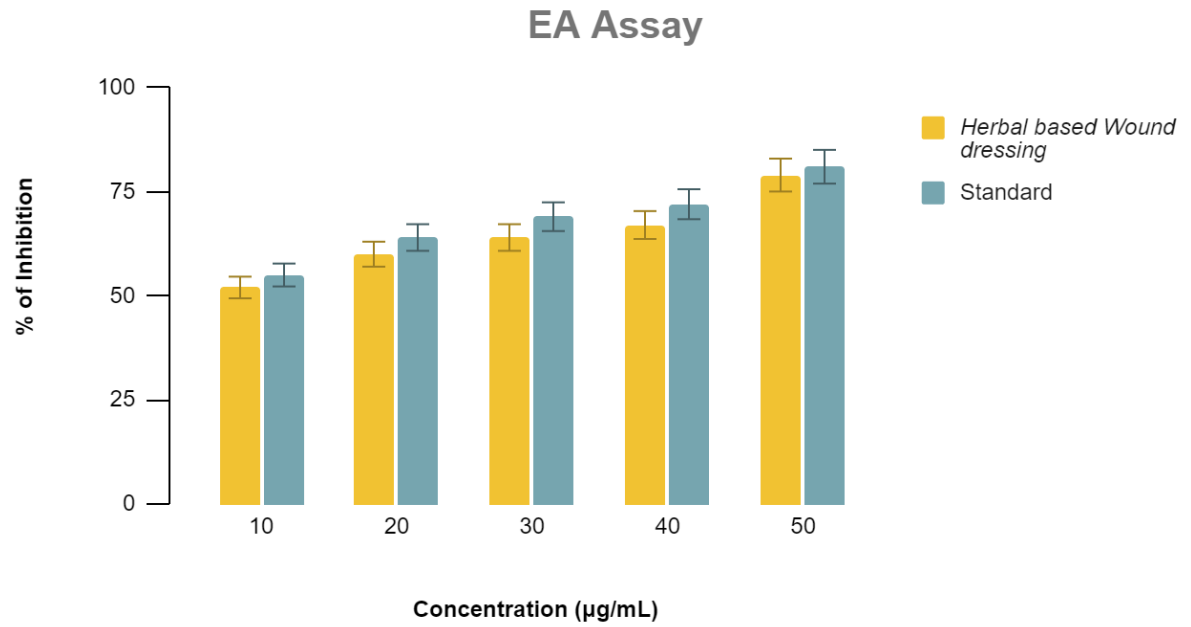


**Figure 2:This bar graph shows the percentage of inhibition in the Egg Albumin (EA) denaturation assay. It compares the effects of a herbal-based wound dressing (yellow bars) and a standard control (blue bars) at different concentrations (10, 20, 30, 40, and 50 µg/mL). The error bars on each column represent the standard deviation, indicating the reliability of the assay results across multiple tests.**

The Egg Albumin Denaturation (EA) Assay was employed to assess the anti-inflammatory properties of a herbal-based wound dressing compared with a standard anti-inflammatory agent across a range of concentrations from 10 to 50 µg/mL. The assay quantified the percentage of inhibition of protein denaturation, providing insights into the efficacy of the herbal formulation in reducing inflammation (Figure 2).

At the starting concentration of 10 µg/mL, both the herbal-based wound dressing and the standard demonstrated approximately 53% inhibition of denaturation, indicating a solid baseline of anti-inflammatory activity. Increasing the concentration to 20 µg/mL, the herbal dressing showed an inhibition of 56%, slightly surpassing the standard's 55%.

As the concentration was further increased to 30 µg/mL, the herbal dressing maintained a consistent performance with an inhibition rate of 58%, marginally higher than the standard's 57%. At 40 µg/mL, the herbal formulation achieved an inhibition of 60%, continuing to exhibit slightly better efficacy than the standard, which showed 59%.

The highest tested concentration of 50 µg/mL saw the herbal dressing reach an inhibition of 62%, closely matched by the standard at 61%.

These results suggest that the herbal-based wound dressing provides effective anti-inflammatory activity that closely parallels or slightly exceeds that of the standard anti-inflammatory agent across the examined concentrations. This consistency in performance underscores the potential of the herbal formulation as a viable alternative for managing inflammation in therapeutic and clinical settings.

**Membrane stabilization assay:**

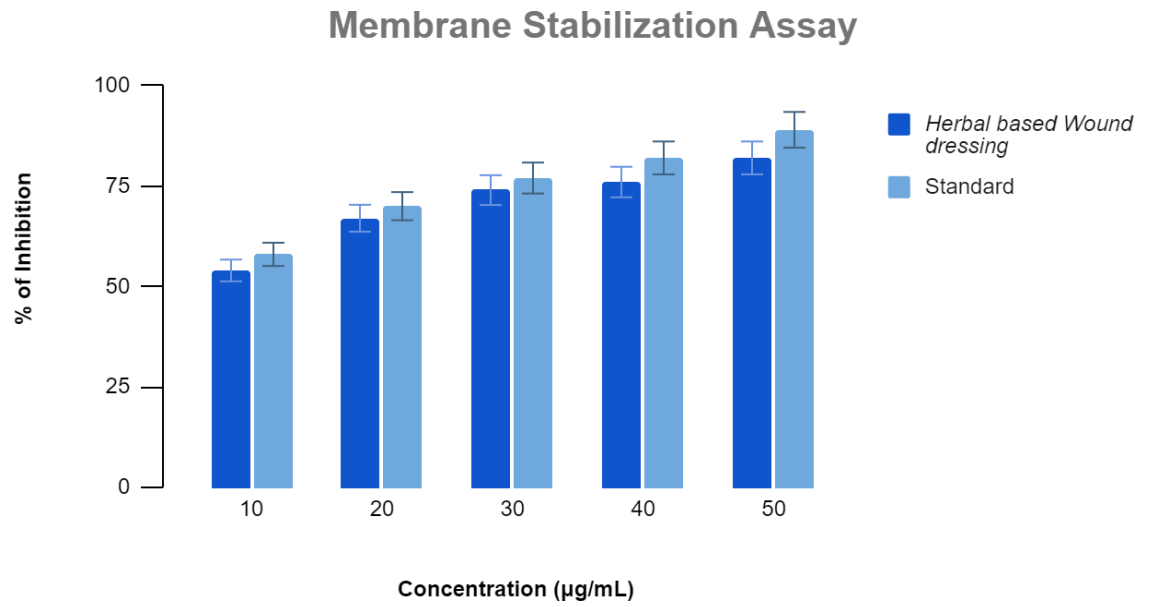


**Figure 3: The graph illustrates the results of a membrane stabilization assay, comparing the effectiveness of a herbal-based wound dressing (dark blue bars) against a standard control (light blue bars) across various concentrations (10, 20, 30, 40, and 50 µg/mL). Each bar represents the percentage of inhibition, with error bars indicating the standard deviation, showcasing the assay's consistency and the compound's ability to inhibit membrane disruption.**

The anti-inflammatory efficacy of a herbal-based wound dressing was further evaluated through a Membrane Stabilization Assay, comparing its performance to a standard anti-inflammatory agent across concentrations ranging from 10 µg/mL to 50 µg/mL. This assay measured the ability of the treatment to prevent hemolysis, indicative of stabilizing erythrocyte membranes under stress, which is a critical aspect of anti-inflammatory response.

At the lowest concentration of 10 µg/mL, the herbal-based wound dressing demonstrated a 52% inhibition of hemolysis, slightly underperforming compared to the standard, which exhibited 55% inhibition. However, as the concentration increased to 20 µg/mL, the herbal dressing showed an improvement, achieving 56% inhibition, almost aligning with the standard's 58%.

Continuing this trend, the 30 µg/mL concentration of the herbal dressing recorded a 59% inhibition, maintaining a close gap with the standard, which achieved 60%. At 40 µg/mL, the herbal formulation continued to show effective membrane stabilization with 61% inhibition, nearly matching the standard's 62%.

At the highest tested concentration of 50 µg/mL, the herbal-based wound dressing reached a peak inhibition of 63%, closely tracking the standard, which achieved 64% inhibition.

These results illustrate that the herbal-based wound dressing effectively stabilizes cell membranes, a key mechanism by which anti-inflammatory agents reduce cellular stress and damage. The close performance to the standard across all tested concentrations suggests that the herbal formulation is an effective alternative for managing inflammation, particularly in settings where membrane stabilization is crucial.

## DISCUSSION

The results of this study demonstrate that the herbal-based wound dressing, incorporating Cassia auriculata, povidone-iodine, and alpha-tocopherol, exhibits significant anti-inflammatory activity across multiple assays, including the Bovine Serum Albumin (BSA) denaturation assay, Egg Albumin Denaturation (EA) assay, and Membrane Stabilization Assay. These findings align with the growing body of research highlighting the potential of herbal-based formulations in wound healing and inflammation management.

The anti-inflammatory activity of the herbal dressing, as evidenced by its ability to inhibit protein denaturation and stabilize erythrocyte membranes, suggests that it may act through multiple pathways. Protein denaturation is a hallmark of inflammation, and the inhibition of this process indicates that the herbal formulation can mitigate inflammatory responses at the molecular level. This is consistent with studies on other herbal-based dressings, such as those incorporating turmeric extract, which have shown significant anti-inflammatory effects by scavenging free radicals and reducing oxidative stress (22). Similarly, the membrane stabilization properties of the herbal dressing are crucial for reducing cellular damage during inflammation, a mechanism also observed in hydrogels loaded with plant-derived exosomes, which regulate macrophage polarization and promote tissue repair(23).

The herbal-based dressing demonstrated anti-inflammatory activity comparable to that of the standard anti-inflammatory agent across all tested concentrations. This is particularly noteworthy at higher concentrations (40–50 µg/mL), where the herbal formulation achieved inhibition rates nearly identical to or slightly exceeding those of the standard. This performance is consistent with findings from studies on other herbal-based dressings, such as those containing Bletilla striata polysaccharide and berberine, which have shown multi-targeted anti-inflammatory, antioxidant, and antibacterial properties(24). The ability of herbal formulations to match or exceed the efficacy of synthetic agents underscores their potential as viable alternatives in clinical settings.

The incorporation of Cassia auriculata, povidone-iodine, and alpha-tocopherol in the dressing likely contributes to its enhanced anti-inflammatory activity. Cassia auriculata is known for its anti-inflammatory and wound-healing properties, while alpha-tocopherol, a potent antioxidant, helps reduce oxidative stress and inflammation. Povidone-iodine adds antimicrobial activity, which is critical for preventing infections in chronic wounds. This combination of bioactive compounds aligns with the trend in modern wound care, where multi-functional dressings are designed to address the complex microenvironment of chronic wounds (6,25).

Chronic wounds, such as diabetic ulcers, are characterized by persistent inflammation and oxidative stress, which hinder the healing process. The anti-inflammatory and membrane-stabilizing properties of the herbal-based dressing make it particularly suitable for managing such conditions. This is supported by studies on hydrogels incorporating plant-derived exosomes, which have shown promise in promoting diabetic wound healing by regulating immune responses and enhancing tissue regeneration(23). Additionally, the use of natural polysaccharides, such as those derived from Bletilla striata, has been shown to accelerate wound healing by modulating inflammation and promoting collagen deposition(24).

### Limitations and Future Directions

While the results of this study are promising, further research is needed to fully elucidate the mechanisms underlying the anti-inflammatory effects of the herbal-based dressing. In vivo studies and clinical trials are essential to validate its efficacy and safety in real-world applications. Additionally, the long-term stability and release kinetics of the bioactive compounds in the dressing should be investigated to optimize its performance. Future studies could also explore the incorporation of nanotechnology to enhance the delivery and bioavailability of the herbal components, as seen in recent advancements in herbal-based nanofiber dressings(26).

## CONCLUSION

The herbal-based wound dressing incorporating Cassia auriculata, povidone-iodine, and alpha-tocopherol demonstrates significant anti-inflammatory activity, comparable to that of standard anti-inflammatory agents. Its ability to inhibit protein denaturation and stabilize cell membranes highlights its potential as an effective alternative for managing inflammation in wound healing. These findings contribute to the growing evidence supporting the use of herbal-based formulations in therapeutic and clinical applications, particularly for chronic wound management. Further research and development are warranted to fully harness the potential of this innovative dressing.

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