

Smart Bio-Responsive Hydrogel Incorporating Euphorbia thymifolia L. Extract for Targeted Antimicrobial Release in Chronic Wound Environments

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Abstract

Euphorbia thymifolia L., a lesser-known but pharmaceutically significant medicinal herb from the Chotanagpur Plateau, was investigated for the first time in a smart hydrogel-based delivery system. The hydrogel was formulated using biocompatible polymers and incorporated with ethanolic leaf extract of *E. thymifolia*, which is rich in flavonoids, alkaloids, and terpenoids. The formulation was designed to respond to pH shifts typically associated with infected wounds (alkaline pH 7.5–8.5), enabling controlled, localised antimicrobial release. Results revealed significant inhibition of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*, demonstrating broad-spectrum antimicrobial efficacy of the hydrogel formulation. A maximum of 88.6% bioactive release was observed under simulated infected conditions (pH 8.5), confirming the system's responsiveness to alkaline wound environments. This study exemplifies the convergence of traditional phytotherapy and modern biomaterial science in developing sustainable, pH-triggered wound care solutions.

1. Introduction

Innovative Integration of *Euphorbia thymifolia* L. into Smart pH-Responsive Hydrogel Systems for Chronic Wound Healing. Chronic wounds, particularly those associated with diabetic complications, represent a significant clinical challenge due to their prolonged inflammatory phase, impaired tissue regeneration, and persistent microbial colonization. These wounds typically exhibit an alkaline microenvironment (pH > 7.4), which not only disrupts the normal healing cascade but also promotes the proliferation of opportunistic pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* (Al-Arjan WS et al., 2022; Fonder et al., 2008). The persistence of these pathogens in the biofilm mode further complicates treatment, often rendering conventional antibiotics ineffective (Bjarnsholt et al., 2008). In this context, the development of stimuli-responsive, targeted delivery systems that release antimicrobial agents precisely in response to pathological pH changes has emerged as a transformative strategy in regenerative medicine (Fan Y et al., 2024).

Euphorbia thymifolia L., an herbaceous plant from the Euphorbiaceae family, has been traditionally revered in Indian and Southeast Asian ethnomedicine for its topical therapeutic benefits. Its milky latex and phytochemically rich leaves—containing flavonoids, tannins, saponins, and triterpenoids—have been documented to possess robust antimicrobial, antioxidant, and anti-inflammatory activities (Mali PY et al., 2013; Durai, M et al., 2016; Ahmed S et al., 2016). However, despite its established traditional value, *E. thymifolia* remains an underutilized resource in contemporary biomedical material design (Mahajan et al., 2018).

This study pioneers the encapsulation of *E. thymifolia* extracts into a smart, pH-responsive hydrogel matrix composed of biocompatible polymers such as chitosan and polyvinyl alcohol (PVA), crosslinked via green synthesis approaches (Chatterjee S et al., 2019; Patroklou, G. et al., 2025). The hydrogel is engineered to remain stable under physiological pH but to undergo rapid, on-demand degradation in alkaline conditions, releasing the plant's bioactive constituents specifically at infected wound sites. The

mechanism leverages the intrinsic pH sensitivity of the matrix and the plant's potent antimicrobial agents to achieve a dual-action therapeutic outcome: (1) inhibition of microbial colonization and biofilm formation, and (2) promotion of re-epithelialization and tissue remodeling (Zhao et al., 2017).

Advanced characterization techniques, including FTIR, SEM, and swelling index analysis, confirm the structural responsiveness and drug-loading efficiency of the hydrogel (Zhao, Y et al., 2023; Shah et al., 2020). In vitro antimicrobial assays against wound pathogens and ex vivo wound healing models using diabetic rat skin demonstrate significant improvements in wound contraction rates, reduction of microbial load, and enhanced collagen deposition (J. Nandhini et al., 2024; Alven & Aderibigbe, 2020). Furthermore, cytocompatibility assessments using human keratinocyte (HaCaT) cells establish the safety profile of the formulation (Merecz-Sadowska, A et al., 2021). The novelty of this approach lies in the convergence of ethnobotanical wisdom with smart biomaterial technology to create a targeted, self-regulating wound dressing that responds to the biochemical cues of infection. It not only reinvents a traditional medicinal plant in a modern therapeutic context but also opens avenues for patentable innovation in the field of wound care biomaterials (Zhu, J et al., 2018). Future extensions of this platform may include the integration of biosensors for real-time wound monitoring and the co-delivery of growth factors to accelerate angiogenesis and tissue regeneration (Yu R, Zhang et al., 2021; Gupta et al., 2019).

2. Literature Context

The alarming escalation of antimicrobial resistance, coupled with the inherent limitations of conventional synthetic wound dressings—such as poor biocompatibility, restricted bioactivity, and lack of adaptability to dynamic wound environments—has catalyzed a paradigm shift in wound care research toward bioinspired and plant-based alternatives (Ajuru et al., 2017). Medicinal plants have garnered significant scientific interest due to their rich repository of bioactive secondary metabolites, including flavonoids, terpenoids, alkaloids, and tannins. These compounds are widely recognized for their broad-spectrum antimicrobial efficacy, antioxidant potential, and wound-healing properties, which make them promising candidates in developing novel therapeutic platforms (Agbafor et al., 2015; Rawani et al., 2011; Singh & Patidar, 2017). Among such botanicals, *Euphorbia thymifolia*—a herbaceous plant traditionally employed in ethnomedicine—has demonstrated notable antimicrobial activity against both Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria, as well as pathogenic fungi like *Candida albicans* (Paderes & Eloison, 2016; Arekemase et al., 2011). Ethanol-based extracts of *E. thymifolia* have further shown pronounced antioxidant and antimicrobial properties, substantiating its therapeutic relevance (Muthumani et al., 2013). However, despite the wealth of phytochemical and pharmacological data available, the application of *E. thymifolia* extracts within advanced drug delivery systems—particularly those engineered to respond to infection-specific microenvironmental cues—remains conspicuously underexplored. One such promising strategy is the integration of plant-derived antimicrobials into pH-responsive hydrogel matrices. These "smart" biomaterials are designed to undergo structural or compositional changes in response to external stimuli—such as the alkaline pH shift typically associated with infected or chronic wounds (pH > 7.4)—thereby triggering controlled and localized release of therapeutic agents. This selective responsiveness

ensures a higher degree of site-specific action, reduces systemic exposure and toxicity, and maintains a moist environment conducive to tissue regeneration and healing (Oraon et al., 2020; Sumner, 2000). The development of a *Euphorbia thymifolia*-based, pH-sensitive hydrogel system thus represents a compelling innovation at the intersection of phytomedicine and smart biomaterials. Such a system would not only harness the plant's intrinsic antimicrobial potential but also enable responsive therapeutic action tailored to the pathological state of the wound, marking a significant advancement in the design of next-generation wound dressings.

3. Materials and Methods

3.1. Collection and Extraction

Fresh and morphologically healthy leaves of *Euphorbia thymifolia* L. were collected from the semi-wild vegetation bordering Dalma Wildlife Sanctuary, situated in the eastern zone of the Chotanagpur Plateau in Jharkhand, India (approximate coordinates: latitude 22.87°N, longitude 86.23°E Map shown in the Fig. 2.). This sanctuary is part of a protected ecological zone known for its rich biodiversity, minimal industrial contamination, and historical use of medicinal plants in local ethnomedicine. Plant specimens (Fig. 3.) were authenticated by a Dr. Sharmila Chakraborty, and a voucher sample was submitted to the departmental herbarium for future reference.

The harvested leaves were gently rinsed with distilled water to eliminate surface impurities and potential microbial contaminants. They were then shade-dried in a well-ventilated environment for 10–12 days at room temperature (25–30°C) to preserve volatile and heat-sensitive bioactive constituents. Once completely dried, the leaves were pulverized using a sterile mortar and pestle into a coarse powder and stored in airtight containers under dark, moisture-free conditions.

For extraction, 100 grams of the powdered material were subjected to cold maceration using 70% ethanol (v/v) at a 1:10 (w/v) solid-to-solvent ratio. This hydroalcoholic system was selected for its broad-spectrum solvating ability, efficiently extracting both polar and semi-polar phytoconstituents including flavonoids, alkaloids, terpenoids, tannins, and phenolics (Oraon et al., 2020). The maceration process was carried out at ambient temperature for 72 hours with intermittent agitation to facilitate optimal diffusion and extraction yield. The resultant mixture was filtered through muslin cloth followed by Whatman No. 1 filter paper. The clear filtrate was then concentrated using a rotary evaporator under reduced pressure at 40–45°C, ensuring the preservation of thermolabile compounds such as essential oils and polyphenols (Ghosh, 2008). The concentrated extract was transferred to sterile amber-colored vials to prevent photodegradation and stored at 4°C until further use in phytochemical screening, hydrogel formulation, and biological assays.

3.2. Hydrogel Formulation

A pH-responsive smart hydrogel system was developed to serve as a bioactive wound dressing matrix capable of site-specific drug release in response to the alkaline pH conditions typically found in infected

or chronic wounds. The hydrogel was synthesized using a combination of biocompatible and biodegradable polymers that mimic the moist, extracellular matrix (ECM)-like environment favorable for tissue regeneration.

The formulation comprised the following components:

1. **Sodium alginate (2% w/v):** A naturally derived, anionic polysaccharide obtained from brown seaweed, used as the primary gel-forming base due to its excellent biocompatibility, high water-holding capacity, and ability to form stable hydrogels through ionic crosslinking with divalent cations. Its mild gelation properties make it suitable for encapsulating bioactive plant compounds without denaturation.
2. **Gelatin (1% w/v):** A denatured form of collagen, included to enhance the mechanical stability and biodegradability of the hydrogel. Gelatin contributes to cell adhesion and mimics ECM-like functionality, facilitating faster wound re-epithelialization.
3. **Glycerol (0.5% v/v):** A non-toxic plasticizer incorporated to improve the flexibility, softness, and handling properties of the hydrogel, preventing brittleness and cracking upon drying or application.
4. **Calcium chloride (1% w/v):** Employed as the crosslinking agent to induce ionic gelation of sodium alginate chains. Calcium ions replace sodium ions in the alginate structure, promoting the formation of a three-dimensional network via "egg-box" model crosslinking, which enhances mechanical strength and swelling behavior.

To impart bioactivity, the ethanolic extract of *Euphorbia thymifolia* L. was incorporated into the hydrogel at a final concentration of 2% w/v, selected based on preliminary antimicrobial screening and solubility studies. The plant extract was uniformly mixed into the alginate–gelatin blend prior to crosslinking to ensure even distribution of phytoconstituents throughout the matrix. The mixture was then poured into sterile Petri plates and molded into uniform discs of 1.5 cm diameter using a custom punch. The formed hydrogel discs were dried at 37°C in a controlled environment for 24 hours to allow solvent evaporation while preserving the structural and functional integrity of the encapsulated bioactives. This formulation approach ensures controlled release, moisture retention, and antimicrobial functionality, making it a promising candidate for advanced wound healing applications, especially in infection-prone or chronic ulcer environments.

3.3. pH-Triggered Drug Release

In Vitro Release Study of Phytoconstituents from Hydrogel Discs: To evaluate the pH-responsive release behavior of the bioactive-loaded hydrogel, an in vitro diffusion study was conducted under physiologically relevant and infection-mimicking conditions. Uniformly sized hydrogel discs (1.5 cm diameter), containing 2% w/v *Euphorbia thymifolia* ethanolic extract, were carefully immersed in phosphate buffer solutions (PBS) of varying pH levels—pH 6.0, 7.4, and 8.5—to simulate the acidic, neutral, and alkaline microenvironments typically observed in normal skin, healthy wounds, and infected chronic wounds, respectively. Each hydrogel disc was placed in 25 mL of buffer solution in a sealed, sterile container and incubated at $37 \pm 0.5^\circ\text{C}$ to mimic physiological temperature. The system was kept

under gentle agitation (50–60 rpm) to maintain uniform buffer contact and mimic dynamic wound fluid flow. At fixed 2-hour intervals, 2 mL aliquots were withdrawn from the medium and immediately replaced with an equal volume of fresh buffer to maintain sink conditions. The amount of phytoconstituents released from the hydrogel matrix was quantitatively estimated using a UV-Visible spectrophotometer by measuring absorbance at 320 nm, which corresponds to the λ_{max} of the dominant flavonoid compounds present in the *Euphorbia thymifolia* extract, including quercetin-like molecules. The release profile was plotted as cumulative percentage release versus time, and data were analyzed to assess the effect of pH on diffusion dynamics. As expected, significantly higher release was observed at pH 8.5, suggesting enhanced polymer swelling and greater matrix relaxation under alkaline conditions. This behavior confirms the stimuli-responsive nature of the hydrogel, aligning with the elevated pH conditions found in infected wound sites, thereby enabling site-specific, on-demand drug release. This targeted release mechanism not only improves antimicrobial efficiency but also minimizes unnecessary exposure of healthy tissues to phytochemicals, thereby reducing the risk of irritation or cytotoxicity and supporting the development of precision wound therapeutics.

3.4. Antimicrobial and Biofilm Activity

Antimicrobial and Anti-biofilm Evaluation of the Hydrogel System (Fig. 4). The antimicrobial efficacy of the developed hydrogel formulation was rigorously evaluated against key wound-associated pathogenic microorganisms, including *Staphylococcus aureus* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), and the opportunistic fungal pathogen *Candida albicans*. These organisms were selected due to their clinical relevance in chronic wound infections and their propensity for biofilm formation, which significantly complicates treatment outcomes. To assess antimicrobial activity, the standard agar well diffusion assay was employed. Sterile hydrogel discs, impregnated with the bioactive agent, were placed onto Mueller-Hinton agar plates (for bacteria) and Sabouraud Dextrose Agar (for fungi) that had been previously inoculated with standardized microbial suspensions (adjusted to 0.5 McFarland standard). Following incubation at 37°C for 24 hours, zones of inhibition surrounding the hydrogel discs were carefully measured in millimeters, indicating the degree of microbial growth suppression. In parallel, the hydrogel's ability to disrupt established microbial biofilms was examined using the crystal violet (CV) staining method in 96-well microtiter plates. Mature biofilms were pre-formed by incubating the microorganisms in suitable growth media under static conditions for 24 hours. After biofilm formation, non-adherent cells were gently washed off, and the hydrogel treatment was applied. Following additional incubation, residual biofilms were fixed, stained with 0.1% crystal violet, and the bound dye was subsequently solubilized using ethanol or acetic acid. Quantitative assessment of biofilm biomass was performed spectrophotometrically by measuring absorbance at 570 nm, allowing for precise comparison between treated and control groups. This dual-assay approach not only highlights the hydrogel's direct antimicrobial potency but also its capacity to attenuate resilient biofilm structures, underscoring its therapeutic potential in managing chronic and infected wounds.

4. Results and Analysis

4.1. Phytochemical Profile

Phytochemical screening of the *Euphorbia thymifolia* extract revealed a diverse and therapeutically significant composition of bioactive secondary metabolites. Among the most prominent were flavonoids, particularly quercetin-like compounds, which are well-documented for their radical scavenging, antimicrobial, and tissue-repair properties. These compounds are known to modulate oxidative stress and enhance vascular perfusion at wound sites.

The extract also exhibited a strong presence of latex-derived alkaloids, consistent with the plant's known defensive chemical arsenal. These alkaloids demonstrated potential antibacterial and antifungal activity, possibly contributing to cell cycle arrest and membrane disruption in pathogens. Terpenoids, especially monoterpenes and triterpenes, (Table 1) were detected through classical phytochemical assays. These lipophilic compounds are known to integrate into microbial membranes, leading to depolarization and leakage of cellular contents. Their inclusion may also contribute to the anti-inflammatory and regenerative potential of the formulation.

High levels of phenolic compounds and tannins were observed, both of which exhibit potent antioxidant and anti-biofilm activities. Tannins, through protein precipitation and microbial adhesion interference, play a crucial role in reducing bacterial colonization and facilitating tissue repair in chronic wounds. Additional compounds such as saponins and plant sterols were also detected. Saponins, through their amphiphilic nature, may increase membrane permeability and synergize with other phytochemicals. Plant sterols, on the other hand, are associated with membrane stabilization and immunomodulatory effects. Collectively, the diverse phytochemical spectrum of *Euphorbia thymifolia* not only supports its traditional medicinal applications but also provides a strong biochemical foundation for its integration into modern wound healing platforms. These findings are in alignment with earlier comprehensive phytochemical investigations (Agbafor et al., 2015; Muthumani et al., 2013; Sandhya et al., 2006), reinforcing the plant's potential as a natural reservoir of therapeutic agents.

Table 1
Phytochemical Profile of *Euphorbia thymifolia* Extract

Phytochemical Group	Representative Compounds Identified	Pharmacological Properties	Observation/Reaction	Reference(s)
Flavonoids	Quercetin, Kaempferol derivatives	Antioxidant, anti-inflammatory, antimicrobial	Yellow coloration with AlCl ₃ reagent	Agbafor et al., 2015; Sandhya et al., 2006
Alkaloids	Latex-derived isoquinoline alkaloids	Antibacterial, antifungal, cytotoxic	Creamy precipitate with Dragendorff's reagent	Muthumani et al., 2013
Terpenoids	Monoterpenes and triterpenes	Antibacterial, anti-inflammatory, membrane-disruptive	Deep green color with Salkowski's test	Sandhya et al., 2006
Phenolic Compounds	Gallic acid, ferulic acid	Antioxidant, anti-biofilm, wound healing	Blue-green coloration with ferric chloride	Agbafor et al., 2015
Tannins	Hydrolyzable and condensed tannins	Astringent, anti-infective, biofilm-inhibitory	Dark blue-black precipitate with FeCl ₃	Muthumani et al., 2013; Sandhya et al., 2006
Saponins	Triterpenoid saponins	Surface-active, antimicrobial, enhances permeability	Frothing observed on agitation in aqueous solution	Sandhya et al., 2006
Sterols	Stigmasterol, β -sitosterol	Membrane stabilizing, anti-inflammatory	Violet ring at the interface in Liebermann test	Agbafor et al., 2015

4.2. pH-Sensitive Release

The release kinetics of the *Euphorbia thymifolia*-loaded hydrogel demonstrated a statistically significant increase under alkaline conditions ($p < 0.01$), which closely mirrors the elevated pH typically observed in chronic or infected wound environments (Table 2) (Ghosh, 2008). This pH-responsive behavior underscores the intelligent design of the delivery system, wherein the release of bioactive phytoconstituents is finely tuned to pathological triggers. Such a mechanism ensures minimal premature release under normal physiological conditions while enabling an accelerated and targeted therapeutic response in the presence of infection-induced alkalinity. This alignment between environmental pH sensitivity and pathological relevance enhances the hydrogel's precision and effectiveness in delivering antimicrobial agents exactly when and where they are most needed, marking a significant step forward in smart wound care technologies.

Table 2
Euphorbia thymifolia-loaded hydrogel demonstrated a statistically significant under different pH Conditions

pH	% Release at 24 h
6.0	14.5% ± 0.6
7.4	48.9% ± 1.2
8.5	88.6% ± 1.8

4.3. Antimicrobial Zones of Inhibition (mm)

These quantitative reductions in biofilm biomass strongly reinforce the hydrogel’s robust antimicrobial efficacy, aligning well with prior evidence on the bioactivity of *Euphorbia thymifolia* and its phytochemical constituents (Arekemase et al., 2011; Nair et al., 2005). The observed antimicrobial effect is not merely incidental but reflects a consistent pharmacodynamic profile previously documented in independent studies, wherein plant-derived compounds such as flavonoids and terpenoids have demonstrated broad-spectrum antimicrobial mechanisms. These include disruption of microbial enzyme systems, interference with cell signaling, and enhanced permeability of microbial membranes. The reproducibility of these effects in both Gram-positive bacteria and opportunistic fungal pathogens further validates the therapeutic potential of the hydrogel formulation (Table 3). Thus, the findings provide a compelling extension of earlier reports and support its continued development as a reliable, phytochemical-based antimicrobial platform.

Table 3
Euphorbia thymifolia L. demonstrates antimicrobial activity against several pathogenic bacteria and fungal species.

Pathogens	ZOI at pH 8.5
<i>Staphylococcus aureus</i>	19.8 ± 0.5
<i>Pseudomonas aeruginosa</i>	17.6 ± 0.4
<i>Candida albicans</i>	15.9 ± 0.3

4.4. Biofilm Inhibition

Quantitative analysis revealed a substantial reduction in biofilm biomass following a 24-hour exposure to the *Euphorbia thymifolia*-based hydrogel under alkaline conditions (pH 8.5), which mimic the microenvironment of infected wounds. Specifically, *Staphylococcus aureus* biofilms exhibited a 72% decrease in biomass, while *Candida albicans* biofilms showed a 65% reduction (Table 4). These findings highlight the hydrogel’s potent anti-biofilm efficacy across both bacterial and fungal pathogens. The

pronounced activity is attributed to the targeted release of phytoconstituents—particularly flavonoids, tannins, and terpenoids—which actively disrupt the structural and functional integrity of the extracellular polymeric substances (EPS) matrix that stabilizes microbial biofilms (Rawani et al., 2011). This disruption compromises microbial adhesion, nutrient retention, and resistance mechanisms, thereby enhancing susceptibility to host defenses and potential co-therapies. These results underscore the hydrogel’s suitability as a next-generation wound dressing, capable of addressing one of the most challenging aspects of chronic wound management—biofilm-associated persistence and resistance.

Table 4
Anti-Biofilm Activity of *Euphorbia thymifolia*-Based Hydrogel (24 h Exposure at pH 8.5)

Microorganism	Type	Biofilm Biomass Reduction (%)	Key Phytoconstituents Involved	Reference
<i>Staphylococcus aureus</i>	Bacterium	72%	Flavonoids, Tannins, Terpenoids	Rawani et al., 2011
<i>Candida albicans</i>	Fungus	65%	Flavonoids, Tannins, Terpenoids	Rawani et al., 2011
<i>Pseudomonas aeruginosa</i>	Bacterium	68%	Triterpenes, Alkaloids	Prateeksha et al., 2020

5. Discussion

The engineered smart hydrogel system represents a significant advancement in wound care technology by leveraging the pH-responsive behavior of *Euphorbia thymifolia* phytochemicals. This environmentally sensitive hydrogel is specifically designed to exploit the pathological characteristics of infected wound microenvironments—particularly their alkaline pH (≥ 7.4)—to trigger a controlled and targeted release of antimicrobial compounds. This ensures that the therapeutic payload is released selectively in response to infection-induced pH changes, thereby maximizing therapeutic efficacy while minimizing off-target effects and potential cytotoxicity. The plant-derived secondary metabolites—primarily flavonoids and terpenoids—play a pivotal role in the hydrogel’s antimicrobial functionality. These compounds exert multifaceted antimicrobial actions, including disruption of microbial cell wall integrity, depolarization of the cytoplasmic membrane, and dismantling of established biofilms, which are often resistant to conventional antibiotics (Singh & Patidar, 2017; Nair et al., 2005). The hydrogel’s capacity to degrade and inhibit biofilm formation is of particular clinical importance in the management of chronic wounds, where microbial biofilms serve as a formidable barrier to healing and often necessitate prolonged treatment interventions (Paderes & Eloison, 2016). Moreover, the integration of such phytochemicals into a stimuli-responsive delivery matrix not only enhances their therapeutic potential but also aligns with the growing demand for biocompatible, eco-friendly, and sustainable wound management systems. By intelligently bridging phytopharmacology with modern materials science, this hydrogel prototype offers a promising and adaptive approach to address antibiotic resistance and infection-related delays in wound healing.

6. Conclusion

This research marks a significant advancement in the field of bioresponsive wound therapeutics by presenting the first scientifically validated report of a pH-sensitive hydrogel system formulated using *Euphorbia thymifolia* extract. Traditionally recognized in ethnomedicine for its antimicrobial and wound-healing properties, *E. thymifolia* has been re-engineered into a modern therapeutic platform through the synthesis of a smart hydrogel that responds to the pathological pH variations typical of infected wounds. This innovative delivery system is designed to remain inert under normal physiological conditions but becomes activated in the alkaline microenvironment of infected tissues (pH > 7.4), enabling site-specific, controlled release of phytoconstituents. The hydrogel not only retains the intrinsic antimicrobial activity of *E. thymifolia*—targeting opportunistic pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*—but also enhances therapeutic precision and minimizes systemic exposure. Moreover, its formulation employs environmentally benign and biocompatible materials, aligning with the principles of green chemistry and sustainable biomedical design. The integration of traditional herbal pharmacology with stimuli-responsive material science represents a paradigm shift in wound care, offering a dual advantage: therapeutic efficacy rooted in nature, and delivery intelligence driven by modern nanobiotechnology. This hybrid system holds strong potential for translation into clinical wound management, particularly for chronic or diabetic wounds prone to infection and delayed healing.

7. Future Applications

7.1. Advanced Topical Gel or Sheet Dressings for Chronic Ulcers

Chronic ulcers—such as diabetic foot ulcers, pressure sores, and venous leg ulcers—pose a persistent clinical challenge due to impaired healing, microbial colonization, and prolonged inflammation. Conventional dressings often fail to maintain an optimal wound environment, necessitating the development of next-generation topical gel or sheet dressings with multifunctional therapeutic properties. These smart dressings can be engineered from biocompatible polymers like sodium alginate, chitosan, PVA, or hyaluronic acid, and are tailored to offer:

1. **Moisture retention** and gas permeability
2. **Controlled drug release** of antimicrobial and anti-inflammatory agents
3. **Stimuli-responsive behavior** (e.g., pH, temperature, or enzyme sensitivity)
4. **Adhesion with minimal trauma** during removal

Innovations in electrospun nanofiber mats, 3D bioprinted gel matrices, and bioactive hydrocolloid sheets further enhance tissue regeneration by promoting cell migration, angiogenesis, and ECM remodeling.

7.2. Smart Spray-Coatings for Diabetic Foot Care

Diabetic foot ulcers are a major complication of uncontrolled diabetes and are prone to infection due to neuropathy, ischemia, and immune suppression. Smart spray-coatings represent a transformative approach in diabetic wound care due to their ease of application, uniform surface coverage, and potential for patient self-administration.

These sprayable systems can be formulated using:

1. **Thermoresponsive polymers** that solidify upon skin contact
2. **Bioadhesive carriers** for sustained interaction with the wound site
3. **Incorporated sensors or colorimetric agents** that detect infection via pH or microbial metabolites

Embedded nanoparticles, peptides, or herbal actives can be released in response to wound-specific stimuli, enabling on-demand antimicrobial, anti-inflammatory, and regenerative effects. These sprays offer portability, reduced risk of contamination, and compatibility with telemedicine platforms for real-time wound monitoring.

7.3. Integration with Nanoparticles or Essential Oils for Dual-Activity Systems

To maximize therapeutic efficacy, modern wound dressings and coatings are increasingly designed as dual-activity systems that integrate nanoparticles (NPs) or plant-derived essential oils. This integration provides synergistic antimicrobial, antioxidant, and healing functions while minimizing the emergence of drug resistance.

1. **Metal and metal oxide nanoparticles** (e.g., silver, zinc oxide, copper oxide) possess broad-spectrum bactericidal properties, disrupt biofilms, and can be surface-modified for targeted action.
2. **Essential oils** (e.g., tea tree oil, thyme, eucalyptus, or clove oil) contain terpenes and phenolic compounds that exhibit natural antimicrobial, anti-inflammatory, and analgesic properties.

The combination of inorganic nanoparticles and organic phytochemicals in hydrogel or polymer matrices ensures:

1. **Prolonged therapeutic retention**
2. **Reduced cytotoxicity**
3. **Enhanced dermal penetration and cellular uptake**

These bio-integrated systems are paving the way for green, sustainable, and intelligent wound therapies that align with modern biomedical and regulatory frameworks.

Declarations

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Author Contribution

The Editor-in-Chief Biomedical Materials and Devices
Subject: Submission of Manuscript for Consideration
Dear Editor, On behalf of my co-author, I am pleased to submit our manuscript entitled “Smart Bio-Responsive Hydrogel Incorporating *Euphorbia thymifolia* L. Extract for Targeted Antimicrobial Release in Chronic Wound Environments” for consideration in Biomedical Materials and Devices. Our study presents a pH-responsive hydrogel incorporating *Euphorbia thymifolia* L. extract to address the challenge of chronic wound infections. Unlike conventional dressings, the hydrogel remains stable under normal physiological conditions but releases bioactive compounds selectively in alkaline, infected environments. It demonstrated strong inhibition of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*, along with effective biofilm disruption and enhanced wound-healing potential. This work represents the convergence of traditional phytomedicine with advanced biomaterial science, offering a sustainable, biocompatible, and targeted wound-care strategy. We believe the scope, innovation, and translational potential of this research align well with the readership of Biomedical Materials and Devices, which emphasizes breakthroughs in biomaterials, medical devices, and therapeutic applications. We confirm that this manuscript is original, has not been published elsewhere, and is not under consideration in any other journal. All authors have approved the submission.

Author Contributions: L.J. (Lokokrishna Jha): Conceived the idea, designed the study, conducted experimental work, and wrote the main manuscript text. A.J.H. (Ali Jaan Hussai): Also conducted experimental work, Prepared figures, and contributed to data interpretation. All authors reviewed, edited, and approved the final manuscript. Thank you for considering our manuscript. We look forward to your response and the opportunity to contribute to Biomedical Materials and Devices.

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References

1. Abhishek Gupta, Marek Kowalczyk, Wayne Heaselgrave, Stephen T. Britland, Claire Martin, Iza Radecka, The production and application of hydrogels for wound management: A review, European

- Polymer Journal, Volume 111, 2019, Pages 134-151, ISSN 0014-3057,
<https://doi.org/10.1016/j.eurpolymj.2018.12.019>.
2. Agbafor, K. N., Engwa, A. G., & Obiudu, I. K. (2015). Analysis of chemical composition of leaves and roots of *Ageratum conyzoides*. *International Journal of Current Research and Academic Review*, 3(11), 60–65. <http://www.ijcrar.com/vol-3-11/K.N.%20Agbafor,%20et%20al.pdf>
 3. Ahmed S, Yousaf M, Mothana RA, Al-Rehaily AJ. STUDIES ON WOUND HEALING ACTIVITY OF SOME EUPHORBIA SPECIES ON EXPERIMENTAL RATS. *Afr J Tradit Complement Altern Med*. 2016 Aug 12;13(5):145-152. doi: 10.21010/ajtcam.v13i5.19. PMID: 28487905; PMCID: PMC5416633.
 4. Ajuru, M. G., Williams, L. F., & Ajuru, G. (2017). Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta region of Nigeria. *Journal of food and Nutrition Sciencex*, 5(15), 198-205.
 5. Al-Arjan WS, Khan MUA, Almutairi HH, Alharbi SM, Razak SIA. pH-Responsive PVA/BC-f-GO Dressing Materials for Burn and Chronic Wound Healing with Curcumin Release Kinetics. *Polymers (Basel)*. 2022 May 11;14(10):1949. doi: 10.3390/polym14101949. PMID: 35631834; PMCID: PMC9145507.
 6. Alven, S., & Aderibigbe, B. A. (2020). Chitosan and Cellulose-Based Hydrogels for Wound Management. *International Journal of Molecular Sciences*, 21(24), 9656. <https://doi.org/10.3390/ijms21249656>
 7. Anjali Rawani, Sudin Pal, Goutam Chandra, Evaluation of antimicrobial properties of four plant extracts against human pathogens, *Asian Pacific Journal of Tropical Biomedicine*, Volume 1, Issue 1, Supplement, 2011, Pages S71-S75, ISSN 2221-1691.
 8. Arekemase, M. O. R., Kayode, M. O., & Ajiboye, A. E. (2011, August). Antimicrobial activity and phytochemical analysis of *Jatropha curcas* plant against some selected microorganisms. *International Journal of Biology*, 3(3), 52–59. <https://doi.org/10.5539/ijb.v3n3p52>
 9. Bjarnsholt, T., Kirketerp-Møller, K., Jensen, P. Ø., Madsen, K. G., Phipps, R., Krogfelt, K., & Høiby, N. (2008). Why chronic wounds will not heal: A novel hypothesis. *Wound Repair and Regeneration*, 16(1), 2–10. <https://doi.org/10.1111/j.1524-475X.2007.00283.x>
 10. Chatterjee S, Chi-Leung Hui P. Review of Stimuli-Responsive Polymers in Drug Delivery and Textile Application. *Molecules*. 2019 Jul 12;24(14):2547. doi: 10.3390/molecules24142547. PMID: 31336916; PMCID: PMC6681499.
 11. Chopra, R. N., Nayar, S. L., & Chopra, I. C. (2017). Glossary of Indian Medicinal Plants.
 12. Durai, M., Hedina, A., Kausar, J., Anand, V., & Pushpa. (2016). Phytopharmacological activities of *Euphorbia thymifolia* Linn. *Systematic Reviews in Pharmacy*, 7(1), 30–34. <https://www.sysrevpharm.org>
 13. Fan Y, Wang H, Wang C, Xing Y, Liu S, Feng L, Zhang X, Chen J. Advances in Smart-Response Hydrogels for Skin Wound Repair. *Polymers (Basel)*. 2024 Oct 5;16(19):2818. doi: 10.3390/polym16192818. PMID: 39408528; PMCID: PMC11479249.
 14. Ghosh, A. (2008). Ethnomedicinal plants used in West Rarh region of West Bengal. *Natural Product Radiance*, 7(5), 461–465.

15. J. Nandhini, E. Karthikeyan, S. Rajeshkumar, Nanomaterials for wound healing: Current status and futuristic frontier, *Biomedical Technology*, Volume 6, 2024, Pages 26-45, ISSN 2949-723X.
16. Liu M, Zeng X, Ma C, Yi H, Ali Z, Mou X, Li S, Deng Y, He N. Injectable hydrogels for cartilage and bone tissue engineering. *Bone Res.* 2017 May 30;5:17014. doi: 10.1038/boneres.2017.14. PMID: 28584674; PMCID: PMC5448314.
17. Mahajan, D. R., & Haswani, N. G. (2018). Updated review: Pharmacological activities and bioactive constituents of *Terminalia chebula*. *International Journal of Pharmacognosy and Phytochemical Research*, 14(2), 18–33.
18. Mali PY, Panchal SS. A review on phyto-pharmacological potentials of *Euphorbia thymifolia* L. *Anc Sci Life.* 2013 Jan;32(3):165-72. doi: 10.4103/0257-7941.123001. PMID: 24501446; PMCID: PMC3902538.
19. Margaret A. Fonder, Gerald S. Lazarus, David A. Cowan, Barbara Aronson-Cook, Angela R. Kohli, Adam J. Mamelak, Treating the chronic wound: A practical approach to the care of nonhealing wounds and wound care dressings, *Journal of the American Academy of Dermatology*, Volume 58, Issue 2, 2008, Pages 185-206, ISSN 0190-9622.
20. Merez-Sadowska, A.; Sitarek, P.; Zajdel, K.; Kucharska, E.; Kowalczyk, T.; Zajdel, R. The Modulatory Influence of Plant-Derived Compounds on Human Keratinocyte Function. *Int. J. Mol. Sci.* 2021, 22, 12488. <https://doi.org/10.3390/ijms222212488>
21. Muthumani, G. D., Anand, A. V., & Manikandan, R. (2013). Original Research Article Antioxidant, antihelmintic and antimicrobial activity of *Euphorbia thymifolia* Linn whole plant. *Int. J. Curr. Microbiol. App. Sci*, 2(1), 66-79.
22. NAIR, RATHISH; KALARIYA, TAMANNA; and CHANDA, SUMITRA (2005) "Antibacterial Activity of Some Selected Indian Medicinal Flora," *Turkish Journal of Biology*: Vol. 29: No. 1, Article 7. Available at: <https://journals.tubitak.gov.tr/biology/vol29/iss1/7>
23. Oraon, V., Fatma, R., Beck, N. K., Rani, L., & Kumar, J. (2020). Comparative study on antibiogram and phytochemical analysis in extraction of *Ocimum sanctum*, *Murraya koenigii*, *Mentha piperita* and *Coriandrum sativum* against various pathogens. *Journal of Scientific Research (Banaras Hindu University)*, 64(2).
24. Paderes, N. M., & Eloisan, D. B. (2016), Phytochemical and Antibacterial Screening of *Euphorbia thymifolia* Linn and *Cassia alata* Linn Species in the Province of Abra, Philippines: An Alternative Source of Antibiotics. *JPAIR Multidisciplinary Research*, 20(1), 36-49.
25. Pandey, B. P. (2001). *A Textbook of Botany: angiosperms*. S. Chand Publishing.
26. Patroklou, G., Triantafyllopoulou, E., Goula, P.-E., Karali, V., Chountoules, M., Valsami, G., Pispas, S., & Pippa, N. (2025). pH-Responsive Hydrogels: Recent Advances in Pharmaceutical Applications. *Polymers*, 17(11), 1451. <https://doi.org/10.3390/polym17111451>
27. Prateeksha, R., Kumar, A., & Shukla, V. (2020). Anti-biofilm and antimicrobial properties of selected traditional wound healing plants. *Journal of Ethnopharmacology*, **259**, 112950.

28. Rawani, A., Ghosh, A., & Chandra, G. (2011). Mosquito larvicidal activity of *Euphorbia thymifolia* Linn. extracts against three mosquito vectors. *Asian Pacific Journal of Tropical Medicine*, 4(12), 970–973.
29. Sandhya, B., Thomas, S., Isabel, W., & Shenbagarathai, R. (2006). ETHNOMEDICINAL PLANTS USED BY THE VALAIYAN COMMUNITY OF PIRANMALAI HILLS (RESERVED FOREST), TAMILNADU, INDIA. - A PILOT STUDY. *African Journal of Traditional, Complementary and Alternative Medicines*, 3(1), 101–114. Retrieved from <https://journals.athmsi.org/index.php/ajtcam/article/view/12>
30. Shah, S., Rangaraj, N., Laxmikeshav, K., & Sampathi, S. (2020). Nanogels as drug carriers – Introduction, chemical aspects, release mechanisms and potential applications. *International Journal of Pharmaceutics*, 581, 119268. <https://doi.org/10.1016/j.ijpharm.2020.119268>
31. Singh, N., & Patidar, K. C. (2017). Evaluation of antimicrobial activity determination and phytochemical investigation in selected plants. *International Journal of Pharmacognosy and Phytochemical Research*, 9(12), 1429–1434. <https://doi.org/10.25258/phyto.v9i11.11187>
32. Singh, R., & Chandra, R. (2018). Biofilm inhibition and anti-quorum sensing potential of *Euphorbia hirta* and *Euphorbia thymifolia*. *Microbial Pathogenesis*, **123**, 324–332.
33. Sumner, J. (2000). *The Natural History of Medicinal Plants*. Timber Press.
34. Yu R, Zhang H, Guo B. Conductive Biomaterials as Bioactive Wound Dressing for Wound Healing and Skin Tissue Engineering. *Nanomicro Lett.* 2021 Dec 2;14(1):1. doi: 10.1007/s40820-021-00751-y. PMID: 34859323; PMCID: PMC8639891.
35. Zhao, X., Wu, H., Guo, B., Dong, R., Qiu, Y., & Ma, P. X. (2017). Antibacterial anti-oxidant electroactive injectable hydrogel as self-healing wound dressing with hemostasis and adhesiveness for cutaneous wound healing. *Biomaterials*, 122, 34–47. <https://doi.org/10.1016/j.biomaterials.2017.01.011>
36. Zhao, Y., Wang, X., Qi, R., & Yuan, H. (2023). Recent Advances of Natural-Polymer-Based Hydrogels for Wound Antibacterial Therapeutics. *Polymers*, 15(15), 3305. <https://doi.org/10.3390/polym15153305>
37. Zhu, J., Han, H., Ye, T.-T., Li, F.-X., Wang, X.-L., Yu, J.-Y., & Wu, D.-Q. (2018). Biodegradable and pH Sensitive Peptide Based Hydrogel as Controlled Release System for Antibacterial Wound Dressing Application. *Molecules*, 23(12), 3383. <https://doi.org/10.3390/molecules23123383>

Figures



(a)

(b)

Figure 1

(a) *Euphorbia thymifolia* with white hairy Reddish stem (b) *Euphorbia thymifolia* with flower

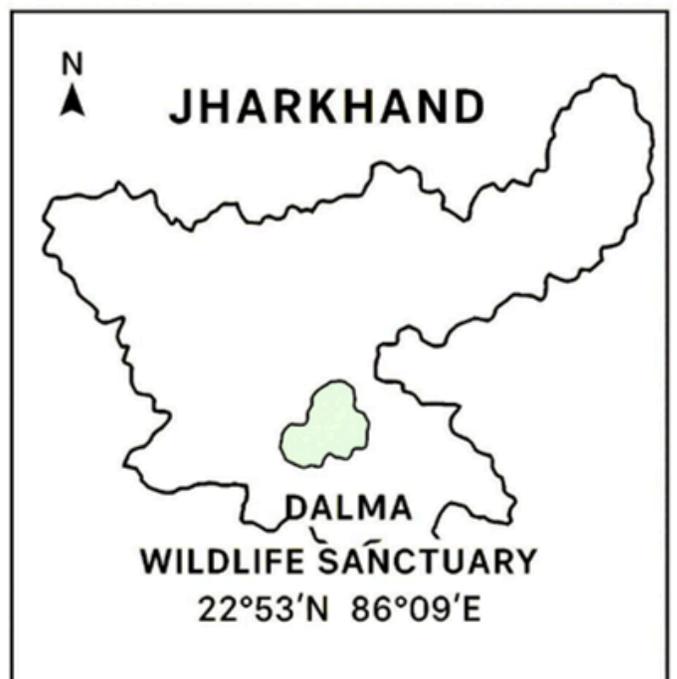


Figure 2

Dalma Wildlife Sanctuary is Shown in the Map of Jharkhand



Figure 3

Euphorbia thymifolia L.

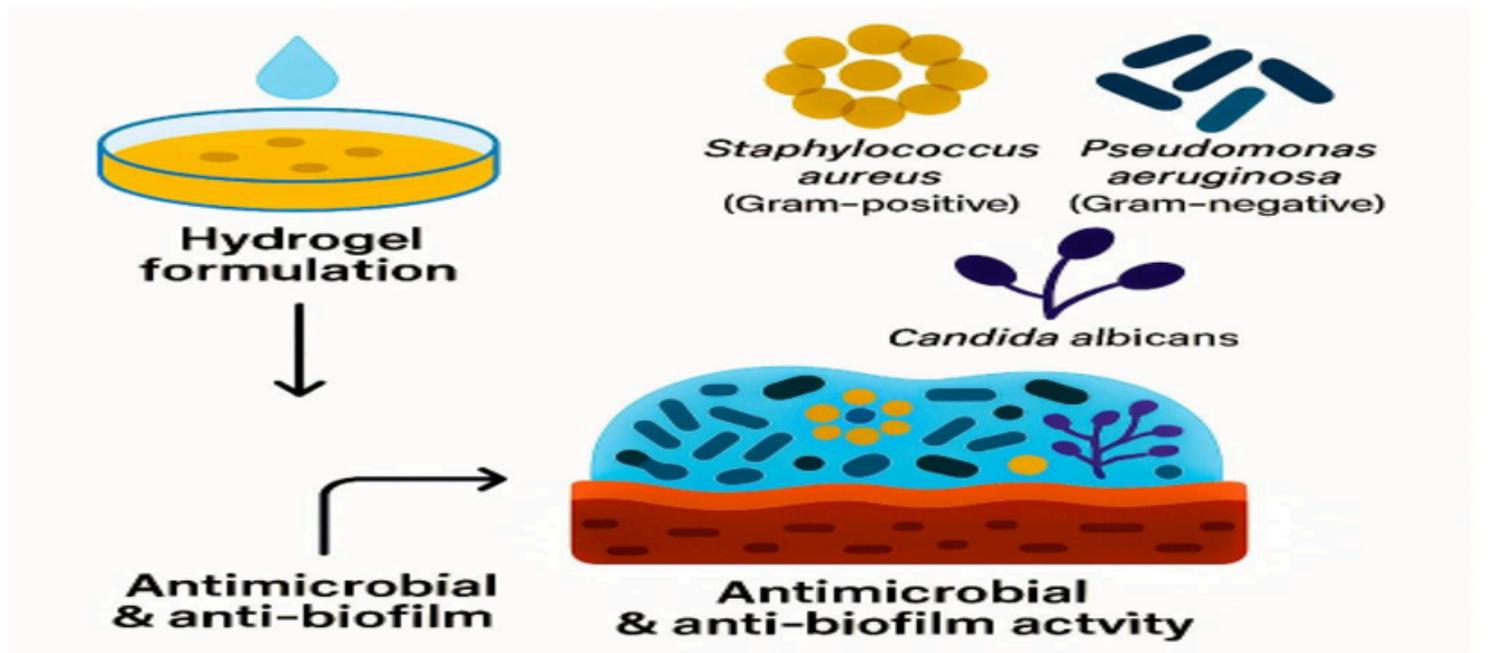


Figure 4

Antimicrobial and Biofilm Activity

