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A Biocompatible Bacterial Cellulose Patch for Enhanced Wound Healing Applications

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ABSTRACT: The present invention describes a biodegradable wound healing patch made from bacterial cellulose produced through static aerobic fermentation using coconut water as a sustainable growth medium. The cellulose is synthesized using Acetobacter xylinum with sucrose and optional yeast extract to enhance yield. The obtained cellulose pellicle is purified using sodium hydroxide and rinsed to form a sterile and flexible matrix. The purified cellulose is then infused with aqueous extracts of Centella asiatica , Azadirachta indica (neem), and Curcuma longa (turmeric), which contain antimicrobial and anti-inflammatory phytochemicals. The composite patch shows antibacterial activity against Gram-positive and Gram-negative bacteria, including Staphylococcus aureus and Escherichia coli . It also demonstrates high water absorbency, moisture retention, and tensile flexibility suitable for wound dressing. The invention provides a sustainable, cost-effective, and scalable natural alternative for managing acute and chronic wounds without synthetic polymers or antibiotics.

KEYWORDS: Wound healing, Bacterial cellulose, Acetobacter Xylinum , Centella asiatica, Neem, Turmeric, Biodegradable patch

I. INTRODUCTION

In this paper, a bioactive wound dressing based on herbal-infused bacterial cellulose is developed, in which wound repair is facilitated through a biocompatible cellulose matrix incorporated with medicinal plant extracts. The bacterial cellulose patch functions as an active wound dressing material that provides moisture retention, antimicrobial protection, and enhanced tissue regeneration. The project focuses on the production of bacterial cellulose using coconut water as a fermentation medium, its incorporation with selected herbal extracts, and the evaluation of its wound healing potential in comparison with conventional wound dressings.

[1].Wound healing is a complex physiological process involving coordinated phases of hemostasis, inflammation, proliferation, and tissue remodeling. Impaired healing and wound-associated infections remain major clinical concerns, particularly in resource-limited settings. Conventional wound dressings such as cotton gauze primarily function as passive barriers and often lack adequate moisture retention, antimicrobial protection, and biocompatibility, resulting in delayed healing and increased infection risk (Singh et al., 2020).

[2].In recent years, biopolymer-based wound dressings have gained attention as advanced alternatives to traditional materials. Among them, bacterial cellulose (BC) has emerged as a promising biomaterial due to its high purity, excellent water-holding capacity, mechanical strength, biodegradability, and non-toxicity (Ullah et al., 2019). Produced extracellularly by Acetobacter xylinum, BC possesses a three-dimensional nanofibrillar network that closely resembles the extracellular matrix, making it particularly suitable for wound healing applications.

[3]. Conventionally, bacterial cellulose is synthesized using synthetic media such as Hestrin–Schramm medium, which significantly increases production costs and limits scalability (Hestrin & Schramm, 1954). To address these limitations, low-cost and sustainable substrates have been explored. Coconut water, an agricultural byproduct rich in sugars, amino acids, and essential minerals, has been identified as an efficient alternative fermentation medium. Its nutrient composition supports bacterial growth and cellulose synthesis while reducing environmental impact and production expenses (Pradeep et al., 2022).

[4].Although bacterial cellulose exhibits favorable physical characteristics, its therapeutic performance can be further enhanced by incorporating bioactive agents. Medicinal plant extracts offer a natural and effective strategy to improve wound healing outcomes. In this study,

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Centella asiatica, Azadirachta indica (Neem), and Curcuma longa (Turmeric) were selected due to their well-established wound healing, antimicrobial, anti-inflammatory, and antioxidant properties (Shukla et al., 1999; Patel et al., 2020; Gupta et al., 2021; Rani et al., 2023). Centella asiatica promotes collagen synthesis and epithelialization, Neem provides broad-spectrum antimicrobial activity, and Turmeric reduces oxidative stress and inflammation, collectively supporting accelerated tissue repair.

[5].The integration of these herbal extracts into bacterial cellulose matrices has been shown to improve moisture retention, flexibility, biocompatibility, and antimicrobial efficacy (Santos et al., 2016). However, studies focusing on the incorporation of Centella asiatica, Neem, and Turmeric into coconut water–derived bacterial cellulose for wound dressing applications remain limited.

Therefore, the present study aims to develop and evaluate a herbal-infused bacterial cellulose wound dressing produced using coconut water as a cost-effective and sustainable fermentation medium. This work highlights a novel, eco-friendly approach to designing bioactive wound dressings with enhanced healing potential and antibacterial efficiency, contributing to sustainable biomaterials development and improved wound care strategies

II. MATERIALS AND METHODOLOGY

MATERIALS

Fresh coconut water was collected from fully matured coconuts procured from local markets and used as the primary fermentation medium. The coconut water was filtered through sterile muslin cloth to remove particulate matter and other impurities before use. The bacterial strain Acetobacter xylinum, known for its cellulose-producing ability, was obtained from a recognized microbiology culture collection and maintained under aseptic laboratory conditions throughout the study.

Coconut water

Sucrose was employed as an additional carbon source, while yeast extract served as a nitrogen source to enhance bacterial growth and bacterial cellulose production. These nutrients were selected based on their effectiveness in supporting microbial metabolism and cellulose synthesis.

Fresh plant materials, including Centella asiatica leaves, Azadirachta indica (neem) leaves, and Curcuma longa (turmeric) rhizomes, were collected locally. The plant materials were washed thoroughly with running water followed by distilled water to remove dirt and contaminants before being processed for herbal extract preparation. The selected medicinal plants are well known for their anti-inflammatory, antimicrobial, and wound-healing properties.

All chemicals, solvents, and reagents used in the study were of analytical grade and were procured from standard commercial suppliers. Distilled water was used throughout the experimental procedures.

PREPARATION OF COCONUT WATER MEDIUM

Fresh coconut water was initially filtered through sterile muslin cloth to remove suspended impurities. The pH of the filtrate was then adjusted to approximately 5.0 using dilute acetic acid in order to provide optimal acidic conditions for the growth and metabolic activity of Acetobacter xylinum. To enhance bacterial cellulose production, sucrose (2–5% w/v) was incorporated as an additional carbon source, while yeast extract (0.2–1% w/v) was added as a nitrogen source and to supply essential growth factors.

The prepared culture medium was transferred into suitable containers and sterilized by autoclaving at 121 °C under 15 psi pressure for 15 minutes to ensure complete elimination of microbial contaminants. After sterilization, the medium was allowed to cool naturally to room temperature under aseptic conditions before being used for bacterial inoculation.

PRODUCTION OF BACTERIAL CELLULOSE

Acetobacter xylinum was initially cultured on Hestrin–Schramm (HS) medium and incubated at 28–30 °C for 24 hours to obtain an actively growing starter culture. The freshly prepared inoculum was then aseptically transferred into the previously sterilized coconut water–based fermentation medium under laminar airflow conditions.

The inoculated media were incubated under static conditions at 28–30 °C for a period of 7–10

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days to facilitate bacterial cellulose production. Static incubation was maintained to promote the formation of a uniform cellulose layer. During theincubation period, the gradual formation of a thick, gelatinous bacterial cellulose pellicle at the air–liquid interface was observed, confirming successful cellulose synthesis by Acetobacter xylinum.

Inoculated Medium

HARVESTING AND PURIFICATION OF BACTERIAL CELLULOSE

The formed bacterial cellulose pellicle was carefully harvested from the culture medium using sterile forceps and washed thoroughly with distilled water to remove residual culture medium components. Purification of the pellicle was carried out by boiling it in 0.1 M sodium hydroxide solution for 30–60 minutes in order to eliminate entrapped bacterial cells, proteins, and other impurities.
Following alkaline treatment, the bacterial cellulose was repeatedly washed with distilled water until the washings reached neutral pH, confirming complete removal of residual alkali. The purified bacterial cellulose pellicle was then used for further processing and characterization.

Harvested Bacterial Cellulose

PREPARATION OF HERBAL EXTRACTS

Fresh leaves of Centella asiatica and Azadirachta indica were thoroughly washed with running tap water followed by distilled water to remove adhering dust and contaminants. The leaves were then chopped into small pieces and boiled separately in distilled water for 30 minutes to facilitate the extraction of water-soluble phytoconstituents. After boiling, the extracts were allowed to cool and were filtered using muslin cloth followed by Whatman No. 1 filter paper to obtain clear aqueous extracts.


Turmeric extract Neem Extract

Centellaasiatica Extract

Turmeric (Curcuma longa) rhizome powder was boiled in distilled water for 15–20 minutes to extract its bioactive components, after which the extract was filtered in a similar manner. All the aqueous herbal extracts were cooled to room temperature and stored under sterile conditions until further use.

PREPARATION OF COMBINED HERBAL EXTRACTS

The individual aqueous extracts of Centella asiatica, Azadirachta indica (neem), and Curcuma longa (turmeric) were mixed in equal proportions (1:1:1) under aseptic conditions to obtain a combined herbal extract solution. The mixture was stirred gently to ensure uniform distribution of the

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bioactive phytoconstituents and was maintained under sterile conditions until further use.

INFUSION OF HERBAL EXTRACTS INTO BACTERIAL CELLULOSE

Purified bacterial cellulose mats were immersed in the combined herbal extract solution for 12–24 hours to facilitate effective absorption of bioactive compounds. The infused cellulose mats were subsequently treated with 10% glycerol for 1 hour to improve flexibility.

Infused Bacterial Cellulose

DRYING AND STORAGE

The purified bacterial cellulose mats were immersed in the combined herbal extract solution and incubated for a period of 12–24 hours to facilitate effective absorption and uniform impregnation of the bioactive compounds into the cellulose matrix. The infusion process was carried out under sterile conditions to prevent microbial contamination.

Following herbal infusion, the cellulose mats were treated with 10% (v/v) glycerol solution for 1 hour to enhance flexibility and reduce brittleness of the material. After glycerol treatment, the infused bacterial cellulose mats were removed and allowed to drain excess solution prior to further processing and evaluation.

Dried Bacterial Cellulose

III. CHARACTERIZATION

I.Phytochemical analysis of herbal extract:

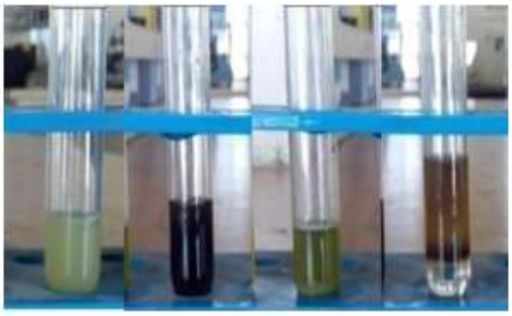
Conducted qualitative phytochemical tests for all four extracts to identify presence of key bioactive compounds such as flavonoids, alkaloids, tannins, saponins, terpenoids, and phenols.

I.**For Neem:**

PHYTO CHEMICALS	TEST	RESULT
Alkaloids	Mayer's test	Positive
Tannins	Ferricchloride test	Positive
Phenols	Ferricchloride test	Positive
Terpenoids	Salkowski test	Positive

Phytochemical analysis of neem

Neem Extract Test



For Turmeric:

PHYTO CHEMICALS	TEST	RESULT
Flavanoids	Alkalinereagent test	Positive
Tannins	Ferricchloride test	Positive
Phenols	Ferricchloride test	Positive
Terpenoids	Salkowski test	Positive
Curcuminoids	Alcohol+Boric acid	Positive

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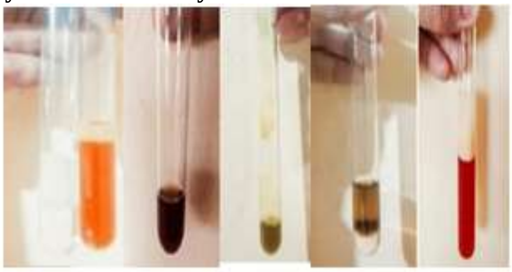
Phytochemical analysis of turmeric

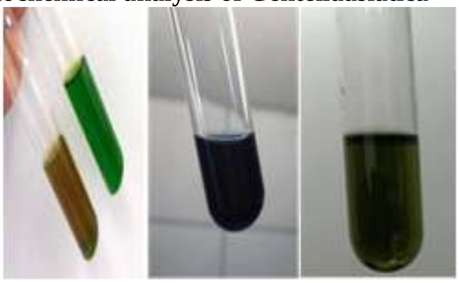


Turmeric Extract Test

For Centellaasiatica:

PHYTO CHEMICALS	TEST	RESULT
Saponins	Foam test	positive
Tannins	Ferricchloride test	Positive
Phenols	Ferricchloride test	Positive
Terpenoids	Salkowski test	Positive

Phytochemical analysis of Centellaasiatica



Centella asiatica extract Test

MOISTURERETENTION:

The moisture retention capacity of the bacterial cellulose patch was evaluated by measuring the weight loss over time under controlled conditions (37°C and 50% relative humidity). The freshly prepared BC patch exhibited an initial moisture content of 92.4% ± 1.8% (w/w).After 24 hours of incubation, the retained moisture was found to be 68.2%, which gradually decreased to 52.6% after 48 hours and 41.3% after **72 hours.**

TIME(HOURS)	MOISTURERETAINED
0	100
24	68.2
48	52.6
72	42.3

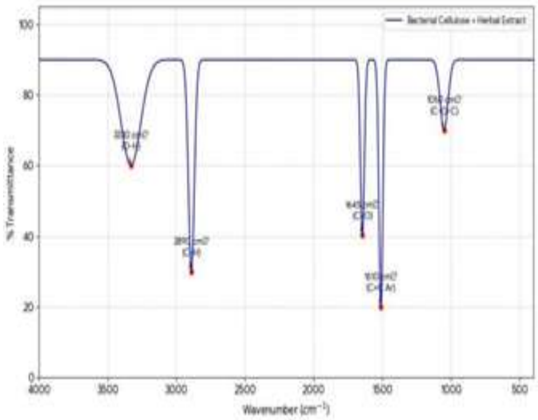
Moisture retention result

FTIR:

- FTIR spectroscopy was performed in the range of 4000–400cm⁻¹ to confirm chemical interactions between cellulose and herbal extracts.
- FTIR analysis confirmed the successful incorporation of herbal bioactive without altering cellulose chemistry, ensuring a stable composite with functional bioactivity.

PEAK (cm-1)	FUNCTIONALGROUP	OBSERVATION
3330	O-H stretching	Broadpeakdue to hydrogenbonding
2890	C-H stretching	Cellulose backbone
1645	C=O stretching	Phytochemical carbonyl groups
1510	C=C aromaticstretching	Presence of phenolic compounds
1050	C-O-C stretching	Glycosidiclinkage of cellulose retained

FTIR result



FTIR graph

SEM:

Surface morphology was studied using Scanning Electron Microscopy (SEM) to observe the fiber network and porosity of the BC patches. SEM images revealed highly porous nanofibrous structures facilitating oxygen diffusion and moisture retention. Herbal extract integration improved surface texture without disrupting structural uniformity.

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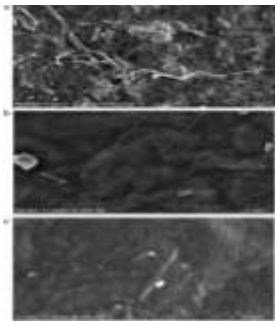
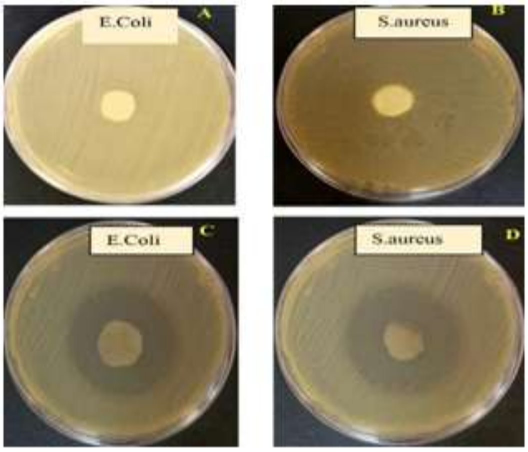


SEM Result

ANTIBACTERIAL ASSAY:

The antibacterial activity of the bacterial cellulose (BC) patch was evaluated using the agar well diffusion method to determine its inhibitory effect against pathogenic bacteria. The test organisms selected were Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative). These findings confirm the potential of the BC–herbal composite patch as an effective wound dressing material capable of preventing microbial infection and promoting faster healing

Anti-Bacterial Assay



IV. CONCLUSION

Bacterial cellulose (BC) was successfully produced using Acetobacter xylinum with coconut water as a sustainable and cost-effective medium. The BC was functionalized by incorporating herbal extracts with therapeutic properties. Characterization revealed a porous, fibrous structure suitable for biomedical use. The infused BC shows potential for applications such as wound dressings, tissue engineering, and drug delivery. The herbal extracts may provide antimicrobial and anti-inflammatory benefits that support tissue repair. Further studies on biocompatibility and stability are needed. Overall, the BC–herbal composite is a promising novel biomaterial for biomedical applications.

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