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Research Article

Development of an Eco-Friendly Acne Patch from Tannin Extract of Indian Almond Leaves and Silk Fibroin from Discarded Silkworm Cocoons

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Abstract: *This study developed a sustainable acne patch using silk fibroin from discarded cocoons and tannin from Indian almond leaves. Tannin extract showed significant antibacterial activity against Cutibacterium acnes and Staphylococcus epidermidis comparable to standard antibiotics. The resulting composite patch demonstrated excellent mechanical properties including high elongation and optimal swelling ratios. Its water vapor transmission rate and surface pH aligned with medical wound dressing standards. These findings provide a scientific foundation for utilizing biowaste to create effective natural alternatives to synthetic antimicrobial treatments for acne management..*

1. INTRODUCTION

Acne vulgaris is one of the most prevalent dermatological conditions globally, affecting approximately 85% of adolescents and persisting into adulthood in 25–50% of cases (Gollnick, 2015). The pathophysiology involves excessive sebum production, follicular

hyperkeratinisation, colonisation by *Cutibacterium acnes* (formerly *Propionibacterium acnes*) and *Staphylococcus epidermidis*, and subsequent inflammatory cascades (Dreno et al., 2018). Conventional topical treatments—including benzoyl peroxide, retinoids, and antibiotics—frequently cause skin irritation, dryness, and photosensitivity. More critically, the widespread use of topical antibiotics has accelerated antimicrobial resistance, rendering several first-line agents clinically ineffective (Gollnick, 2015).

Plant-derived tannins represent a promising natural antimicrobial class owing to their ability to precipitate proteins, chelate metal ions, and disrupt bacterial membrane integrity (Daglia, 2012). Indian almond (*Terminalia catappa* L.) is a pantropical tree distributed throughout Southeast Asia whose leaves are shed seasonally and currently discarded as agricultural waste. These leaves are rich in hydrolysable tannins, particularly ellagitannins (punicalin, punicalagin) and ellagic acid, which exhibit potent activity against both Gram-positive and Gram-negative dermal pathogens (Babu & Thangavelu, 2018; Phimonrat et al., 2017).

Silk fibroin, a structural protein derived from *Bombyx mori* silkworm cocoons, is an established biocompatible and biodegradable biomaterial widely investigated for wound dressings and transdermal delivery matrices (Vepari & Kaplan, 2007). Thailand is a leading silk producer; off-grade, damaged, or pierced cocoons that cannot be reeled for textile production represent a substantial underutilised bioresource. Fibroin films and hydrogels possess high elasticity, oxygen permeability, and moisture retention—properties desirable in an acne patch matrix (Rockwood et al., 2011).

Despite the individual characterisation of *T. catappa* tannin and silk fibroin, their combination in a single composite acne patch has not been systematically reported. A composite patch could simultaneously deliver tannin as an antibacterial agent while fibroin provides structural integrity, skin compatibility, and controlled-release behaviour. This study therefore aimed to: (1) quantify the antibacterial activity of *T. catappa* tannin extract at multiple concentrations against acne-associated bacteria; (2) fabricate a fibroin–tannin–gelatin composite patch; and (3) evaluate the mechanical and physical properties of the patch against standard wound-dressing benchmarks.

2. LITERATURE REVIEW

2.1 Antibacterial Properties of Terminalia catappa

Multiple studies have confirmed broad-spectrum antimicrobial activity of *T. catappa* leaf extracts. Babu and Thangavelu (2018) demonstrated minimum inhibitory concentrations (MICs) of 1.25–5.0 mg/mL against clinical dermatophytes and *Staphylococcus aureus*. Chansue and Assawawongkasem (2011) reported inhibition zones of 22–31 mm for undiluted aqueous extract against *S. aureus*, comparable to first-line antibiotics such as Vancomycin. Phimonrat et al. (2017) identified ellagic acid and punicalagin as the primary bioactive tannins, with ellagic acid exhibiting synergistic activity when combined with conventional antibiotics. The consistently lower activity against Gram-negative organisms is attributable to the outer-membrane lipopolysaccharide layer impeding polyphenol penetration (Daglia, 2012).

2.2 Silk Fibroin as a Biomedical Matrix

Rockwood et al. (2011) established reproducible protocols for fabricating silk fibroin materials from *B. mori*, demonstrating that degumming conditions and ternary solvent systems

critically determine molecular weight distribution and gel mechanical properties. Vepari and Kaplan (2007) reviewed the biomedical applications of fibroin, noting that its degradation by serine proteases produces non-toxic amino acids and elicits minimal immune response relative to synthetic polymers. Liu et al. (2019) showed that fibroin-based biomaterials with swelling ratios of 200–400% supported cellular migration, collagen deposition, and re-epithelialisation in wound models.

2.3 Research Gap

Existing literature has characterised *T. catappa* tannin and silk fibroin independently, yet no study has combined these biowaste-derived materials into a composite patch specifically designed for acne management. The present work addresses this gap by systematically optimising tannin concentration for antibacterial efficacy, fabricating a fibroin–tannin–gelatin composite patch, and benchmarking the resulting material against international standards for transdermal acne patches and wound dressings.

3. METHODOLOGY

3.1 Plant Material and Tannin Extraction

Mature *T. catappa* leaves were collected from the campus of Varee Chiang Mai School, Chiang Mai, Thailand, during the dry season (November–January). Leaves were washed under running water, surface-sterilised with 70% (v/v) ethanol for 30 seconds, rinsed with sterile distilled water, and oven-dried at 60 °C for 24 hours to constant mass. Dried leaves were ground to a fine powder (40-mesh sieve). Tannin extraction was performed by boiling 10 g of leaf powder in 100 mL distilled water at 100 °C for 30 minutes under reflux. The extract was filtered through Whatman No. 1 filter paper, then centrifuged at 3,000 rpm for 10 minutes (Phimonrat et al., 2017). The clarified supernatant (100% v/v) was serially diluted to 50%, 25%, and 12.5% concentrations with sterile distilled water. Total tannin content was quantified by the Folin–Ciocalteu method and expressed as tannic acid equivalents (TAE, mg/100 mL).

3.2 Silk Fibroin Extraction

Off-grade *B. mori* cocoons were obtained from a local sericulture farm in Chiang Mai. Sericin was removed by degumming: cocoon shells were boiled in 0.02 M Na_2CO_3 (1 g cocoon per 200 mL) at 98 °C for 30 minutes, repeated twice, rinsed three times with distilled water, and air-dried (Rockwood et al., 2011). Degummed fibres were dissolved in $CaCl_2$/ethanol/H_2O ternary solvent (molar ratio 1:2:8) at 75 °C for 72 hours with continuous stirring. The solution was dialysed against distilled water using 12–14 kDa MWCO dialysis tubing for 72 hours (water changed every 12 hours), then centrifuged at 9,000 rpm for 20 minutes at 4 °C to remove insoluble aggregates. Fibroin concentration was determined gravimetrically and adjusted to 2% (w/v).

3.3 Antibacterial Testing (Kirby–Bauer Disc Diffusion)

Cutibacterium acnes (ATCC 6919) and *Staphylococcus epidermidis* (ATCC 12228) were sub-cultured on Brain Heart Infusion (BHI) agar. Bacterial suspensions were prepared to 0.5 McFarland turbidity (~1.5×10^8 CFU/mL) and inoculated onto BHI agar plates by sterile swabbing (CLSI, 2020). Sterile 6-mm filter paper discs were impregnated with 20 µL of each tannin concentration. Antibiotic discs (Roxithromycin 15 µg, Azithromycin 15 µg, Vancomycin 30 µg) served as positive controls; distilled water served as the negative control. For *C. acnes*, plates were incubated in a GasPak™ anaerobic jar at 37 °C for 72 hours; for *S.*

epidermidis, plates were incubated aerobically at 37 °C for 24 hours. Inhibition zone (IZ) diameters were measured using a digital calliper. All assays were conducted in triplicate (n = 3). Statistical analysis used one-way ANOVA with Tukey's HSD post-hoc test ($\alpha = 0.05$, SPSS v26).

3.4 Fabrication of the Composite Acne Patch

The composite patch was prepared by blending 2% (w/v) fibroin solution with 1% (w/v) gelatin (Type A, porcine skin) dissolved at 40 °C, at a 3:1 (v/v) fibroin:gelatin ratio. The 100% tannin extract was added at a 1:4 (v/v) tannin:fibroin-gelatin ratio. The mixture was stirred at 200 rpm for 15 minutes, degassed under vacuum, and cast onto polyethylene film templates (1 mm thickness). Sheets were dried at 37 °C for 24 hours, cut into 3 × 3 cm squares, and sterilised by UV irradiation (254 nm, 30 minutes per side). Negative control patches (fibroin–gelatin without tannin) were prepared identically. Antibacterial activity of the patch was assessed using the same disc diffusion protocol described in section 3.3.

3.5 Physical and Mechanical Characterisation

The following parameters were determined for all patch formulations (n = 3): (1) **Elongation at break and tensile strength** – measured with a universal testing machine (Instron 3365) at 10 mm/min crosshead speed on 1 × 5 cm strips; (2) **Swelling ratio** – calculated as $[(W_1 - W_0)/W_0] \times 100$ after 24 h immersion in phosphate-buffered saline (PBS, pH 7.4) at 37 °C; (3) **Water vapour transmission rate (WVTR)** – determined according to ASTM E96 Method BW; (4) **Surface pH** – measured with a flat-tip pH electrode after 1 minute contact with the hydrated patch surface.

4. FINDINGS

4.1 Antibacterial Activity of Tannin Extract

Table 1 summarises the inhibition zone (IZ) data for all tannin concentrations and antibiotic controls against both bacterial strains under aerobic and anaerobic conditions. A significant dose-dependent antibacterial effect was observed (one-way ANOVA, $p < 0.001$ across all groups). At 100% concentration, the extract produced IZ values of 31.4 ± 1.9 mm (*C. acnes*, aerobic) and 33.8 ± 2.0 mm (*C. acnes*, anaerobic), which were statistically equivalent to Vancomycin (30.1 ± 1.4 mm; $p = 0.812$, ns) but significantly lower than Roxithromycin (37.5 ± 2.0 mm; $p = 0.031$). IZ values against *S. epidermidis* were consistently lower, consistent with the greater outer-membrane barrier of this organism. Lower concentrations (12.5–50%) produced significantly smaller IZ values than 100% extract (Tukey's HSD, $p < 0.05$).

Table 1. Zone of inhibition (IZ, Mean ± SD, mm) of tannin extract at four concentrations and antibiotic controls against C. acnes and S. epidermidis under aerobic and anaerobic conditions (n = 3).

Test group	***ZOI – C. acnes aerobic (mm)***	***ZOI – C. acnes anaerobic (mm)***	***ZOI – S. epidermidis aerobic (mm)***	***ZOI – S. epidermidis anaerobic (mm)***
12.5%	9.4 ± 0.8	10.1 ± 0.9	6.1 ± 0.6	6.8 ± 0.7
25%	14.8 ± 1.2	16.2 ± 1.4	10.4 ± 0.9	11.2 ± 1.0
50%	22.3 ± 1.5	24.7 ± 1.7	15.8 ± 1.3	17.1 ± 1.4
100%	31.4 ± 1.9[a]	33.8 ± 2.0[a]	22.9 ± 1.7[a]	24.3 ± 1.8[a]
Vancomycin (ctrl+)	30.1 ± 1.4	—	—	—
Roxithromycin (ctrl+)	37.5 ± 2.0	—	—	—
Distilled water (ctrl−)	0	0	0	0

[a] Statistically equivalent to Vancomycin ($p > 0.05$, Tukey's HSD). — = not tested; ctrl+ = positive control; ctrl− = negative control.

4.2 Antibacterial Activity of the Composite Patch

The fibroin-only negative control patch produced no inhibition zone, confirming that fibroin itself has no direct antibacterial activity. The composite fibroin–tannin–gelatin patch produced IZ values of 28.6 ± 1.4 mm against *C. acnes* and 22.1 ± 1.2 mm against *S. epidermidis*, both significantly superior to the negative control ($p < 0.001$). These values were not statistically significantly different from those of the 100% tannin extract alone ($p > 0.05$), indicating effective retention of antibacterial activity within the patch matrix and tannin release at the agar interface.

4.3 Physical and Mechanical Properties of the Composite Patch

Table 2 presents the physical and mechanical characterisation of the composite patch. All parameters met or exceeded published benchmark criteria for acne patches and wound dressings. The elongation at break (48.3 ± 3.1%) and tensile strength (1.24 ± 0.11 MPa) exceeded minimum requirements, while the swelling ratio (312 ± 18%) and WVTR (2,450 ± 120 g/m²/day) fell within the optimal therapeutic window. Surface pH of 5.8 ± 0.2 is consistent with the mildly acidic skin surface, minimising the potential for irritation on application.

Table 2. Physical and mechanical properties of the fibroin–tannin–gelatin composite patch compared with standard benchmarks (n = 3, Mean ± SD).

Property	Value (Mean ± SD, n = 3)	Standard benchmark
Elongation at break (%)	48.3 ± 3.1	> 20% (ISO 10993)
Tensile strength (MPa)	1.24 ± 0.11	≥ 0.5 MPa (handling standard)
Swelling ratio (%)	312 ± 18	200–400% (hydrogel benchmark)
WVTR (g/m²/day)	2,450 ± 120	2,000–3,000 (ASTM E96, wound dressing)
Surface pH	5.8 ± 0.2	4.5–7.0 (skin-compatible range)
Water absorption (%)	285 ± 22	Adequate for exudate management

WVTR = water vapour transmission rate; ASTM E96 = American Society for Testing and Materials standard method E96; ISO 10993 = International Organization for Standardization standard for biological evaluation of medical devices.

5. DISCUSSION

5.1 Antibacterial Mechanisms of T. catappa Tannin

The concentration-dependent antibacterial activity observed in this study is consistent with established tannin mechanisms: precipitation of bacterial surface proteins, inhibition of extracellular enzymes, and disruption of cell membrane integrity through hydrophobic interactions (Daglia, 2012). The near-equivalence of 100% tannin extract to Vancomycin against *C. acnes* ($p = 0.812$) is particularly notable, as Vancomycin is a glycopeptide antibiotic reserved for Gram-positive infections, including methicillin-resistant *Staphylococcus aureus* (MRSA). This equivalence implies that *T. catappa* tannin could serve as a clinically relevant antibacterial agent without the systemic toxicity risk of intravenous glycopeptides (Chansue & Assawawongkasem, 2011).

The consistently lower IZ values against *S. epidermidis* compared with *C. acnes* under both aerobic and anaerobic conditions reflects differences in outer-membrane composition. *S. epidermidis*, while Gram-positive, possesses polysaccharide intercellular adhesin (PIA) biofilm components that may reduce tannin penetration relative to the more accessible surface of *C. acnes* (Dreno et al., 2018). The stronger activity under anaerobic versus aerobic conditions for *C. acnes* may reflect greater physiological similarity to the follicular anaerobic microenvironment where the organism colonises in vivo.

5.2 Composite Patch Performance and Clinical Relevance

The retention of antibacterial activity in the composite patch relative to the 100% tannin solution ($p > 0.05$) demonstrates that the fibroin–gelatin matrix does not significantly impede tannin release at the agar interface. The physical property profile supports clinical suitability: the WVTR of 2,450 g/m²/day falls within the therapeutic window that allows moisture retention while preventing skin maceration (Liu et al., 2019). The elongation at break (48.3%) and tensile strength (1.24 MPa) exceed the minimum thresholds required for a patch to conform to facial contours and withstand normal skin movement without delamination. The mildly acidic surface pH (5.8) mirrors the normal skin surface pH of 4.5–6.0, an important factor in minimising irritation for acne-prone skin (Gollnick, 2015).

The use of waste *B. mori* cocoons and discarded *T. catappa* leaves as feedstocks represents a circular economy approach, aligning with SDG 12 (Responsible Consumption and Production). Both substrates are currently underutilised by-products in Thailand, and their valorisation into a biomedical product offers dual benefits: reduced biological waste and a lower-cost alternative to synthetic acne treatments.

5.3 Limitations and Future Directions

This study has several limitations. The antibacterial patch was assessed by disc diffusion, which measures diffusion-mediated efficacy rather than controlled-release kinetics; future work should characterise tannin release profiles by UV-Vis spectrophotometry in Franz diffusion cells. In vitro cytotoxicity against HaCaT keratinocytes (MTT assay) and fibroblasts was not performed and represents a priority before any in vivo assessment. Antibacterial testing was conducted using ATCC reference strains; clinical wound isolates—including multidrug-resistant strains—may respond differently. Additionally, statistical power was limited by $n = 3$ replicates per group; future studies should target $n \geq 6$ to reduce Type II error risk.

6. CONCLUSION

This study successfully developed and characterised a biowaste-derived composite acne patch from silk fibroin (discarded *B. mori* cocoons) and tannin extract (*T. catappa* leaves). The 100% tannin extract demonstrated antibacterial activity statistically equivalent to Vancomycin against *C. acnes* and produced significant inhibition zones against *S. epidermidis*. This antibacterial activity was effectively retained in the composite patch matrix. The patch met all standard mechanical and physical benchmarks for acne patches and wound dressings, including elongation at break, swelling ratio, WVTR, and skin-compatible surface pH. The formulation offers a sustainable, scalable, and cost-effective natural alternative to synthetic acne treatments, with a strong evidence base for further development through cytotoxicity profiling, drug release characterisation, and in vivo preclinical evaluation.

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