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Isolation of chitin and chitosan from dead adult housefly *(Musca domestica)* for wound dressing formulation

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Abstract

Background: Chitin and chitosan are biopolymers with exceptional biocompatibility, biodegradability, and wound-healing properties. Conventionally, chitin is extracted from crustacean shells (shrimp, crab), but this source has limitations including seasonal availability, allergenic potential (shellfish allergy), and environmental concerns related to acid-alkali waste disposal. Dead adult houseflies (*Musca domestica*) represent an underexplored, abundant, and sustainable alternative source of chitin. Houseflies breed rapidly, and dead flies are available year-round as waste from fly rearing facilities. However, no standardized method exists for chitin and chitosan isolation from adult houseflies for wound dressing applications.

Objective: To isolate, characterize, and optimize chitin and chitosan from dead adult houseflies (*Musca domestica*) and evaluate their wound dressing potential.

Methods: Dead adult houseflies were collected from BMS College of Pharmacy, Tiloi, and Amethi UP India, cleaned, dried, and defatted using petroleum ether. Chitin was isolated through a three-step chemical process: (1) deproteinization using 1M NaOH at 80°C for 6 hours, (2) demineralization using 1M HCl at room temperature for 4 hours, and (3) decolorization using 0.3% sodium hypochlorite (NaOCl) for 1 hour. Chitosan was produced by deacetylation of chitin using 50% NaOH at 100°C for 4 hours. Process parameters (temperature, time, alkali concentration) were optimized using a one-factor-at-a-time approach. Isolated chitin and chitosan were characterized by: yield percentage, degree of deacetylation (DDA) by FTIR and titration, molecular weight by viscometry, solubility in 1% acetic acid, moisture content, ash content, protein residue (Kjeldahl method), and colour. Surface morphology was examined by SEM. Crystalline structure was analyzed by XRD. Thermal stability was assessed by TGA/DSC. Antimicrobial activity against *S. aureus* and *E. coli* was tested by disk diffusion. For wound dressing formulation, chitosan was dissolved in 1% acetic acid (2% w/v) and cast into films with glycerol as plasticizer (0.5% w/w). Films were crosslinked with tripolyphosphate (TPP) and characterized for thickness, tensile strength, elongation, water vapor transmission rate (WVTR), swelling ratio, biodegradation, and *in vivo* wound healing in Wistar rats using an excision wound model (n=6 per group): (I) control (no treatment), (II) marketed dressing (Betadine), (III) chitosan film, (IV) chitin powder. Wound contraction percentage, epithelialization time, histopathology (H&E, Masson's trichrome), and hydroxyproline content were evaluated.

Results: The optimized isolation protocol yielded 8.4 ± 0.3% chitin and 5.2 ± 0.2% chitosan from dry housefly weight. The chitosan had a degree of deacetylation of 82.5 ± 1.2% (by FTIR) and molecular weight of 98.5 ± 4.2 kDa. Solubility in 1% acetic acid was 94.2 ± 1.5%. FTIR spectra showed characteristic amide bands confirming chitin (1652 cm^{-1}, 1620 cm^{-1}) and chitosan (1595 cm^{-1}). SEM revealed porous, fibrillar structure. XRD showed crystalline peaks at $2\theta = 9.2°$ and 19.4° for chitin, and 10.1° and 20.2° for chitosan. TGA showed degradation onset at 280°C for chitin and 260°C for chitosan. Antimicrobial testing showed inhibition zones of 18.5 ± 1.2 mm (chitosan vs. *S. aureus*) and 15.2 ± 1.1 mm (vs. *E. coli*). Chitosan films had tensile strength 24.5 ± 1.8 MPa, elongation 32.4 ± 2.1%, WVTR 1850 ± 85 $g/m^2/day$, swelling ratio 450 ± 25%, and biodegradation 65% in 14 days. *In vivo* wound healing: chitosan film group showed 98.2 ± 1.5% wound contraction by day 14 (vs. control 72.4 ± 2.8%, $p<0.001$), complete epithelialization at 12.4 ± 1.2 days (vs. control 18.6 ± 1.4 days, $p<0.001$). Histopathology showed complete re-epithelialization, collagen deposition, and neovascularization in chitosan film group. Hydroxyproline content was significantly higher in chitosan film group (48.6 ± 2.4 mg/g vs. control 24.3 ± 1.8 mg/g, $p<0.001$).

Conclusion: Dead adult houseflies (*Musca domestica*) are a viable, sustainable, and high-quality alternative source for chitin and chitosan production. The isolated chitosan demonstrated excellent physicochemical properties, antimicrobial activity, and wound healing efficacy comparable to marketed products. This waste-to-wealth approach offers an eco-friendly solution for utilizing insect biomass in biomedical applications.

Keywords: Chitin, chitosan, housefly, *Musca domestica*, wound dressing, biopolymer, waste utilization, sustainable source

Introduction

Wound healing is a complex, dynamic biological process involving haemostasis, inflammation, proliferation, and remodelling phases. Chronic wounds (diabetic ulcers, pressure sores, and venous leg ulcers) affect approximately 6.5 million patients in the United States alone, with annual treatment costs exceeding $25 billion. Ideal wound dressings should maintain a moist environment, absorb

exudate, permit gas exchange, protect against microbial infection, be non-toxic and non-allergenic, and promote tissue regeneration (Boateng *et al*., 2008) [1]. Among various biomaterials, chitin and it deacetylated derivative chitosan have emerged as leading candidates for wound dressing applications due to their unique combination of biocompatibility, biodegradability, haemostatic activity, antimicrobial properties, and ability to accelerate wound healing.

Chitin is the second most abundant natural polysaccharide after cellulose. It is a linear polymer of β-(1→4)-linked N-acetyl-D-glucosamine units. Chitosan is produced by partial deacetylation of chitin, resulting in a copolymer of N-acetyl-D-glucosamine and D-glucosamine. The degree of deacetylation (DDA) determines the physicochemical and biological properties of chitosan, with DDA >75% being suitable for most biomedical applications [1].

Conventional Sources of Chitin and Chitosan

Currently, commercial chitin and chitosan are derived primarily from crustacean shells: shrimp, crab, lobster, and krill. The global chitin market was valued at $15.2 billion in 2023 and is projected to reach $25.6 billion by 2030. However, crustacean-derived chitin has several limitations:

Insects as an Alternative Source of Chitin

Insects produce chitin as a major structural component of their exoskeleton (cuticle), peritrophic matrix (gut lining), and tracheal tubes. The chitin content varies among insect species, developmental stages, and body parts:

- **Year-round availability:** Insects can be reared indoors regardless of season
- **No allergenic concerns:** Insects do not produce tropomyosin (major shellfish allergen)
- **Rapid growth rate:** Housefly life cycle is 10–14 days, allowing continuous production
- **Waste utilization:** Dead insects from rearing facilities or pest control operations are otherwise discarded
- **Lower environmental footprint:** Insect farming requires less land, water, and feed compared to crustacean harvesting
- **Scalability:** Insect rearing can be scaled up industrially

Housefly (*Musca domestica*) as a Chitin Source

The common housefly, *Musca domestica* Linnaeus (Diptera: Muscidae), is a synanthropic insect found worldwide. Houseflies breed in organic waste (manure, garbage, decaying matter) and have a short life cycle of 10–14 days at 25–30°C. A single female can lay up to 500 eggs in her lifetime. Houseflies are mass-reared for various purposes: animal feed, waste management, and research. Dead adult houseflies are generated in large quantities as byproducts of rearing facilities, fly control programs, and entomological research. These dead flies are typically discarded as waste, representing an untapped source of chitin.

Despite the potential, there are no standardized protocols for isolating chitin and chitosan from *dead adult* houseflies. Most previous studies have focused on housefly larvae or pupae, not adults. Adult houseflies have a fully sclerotized exoskeleton, which may require different extraction conditions compared to larval cuticle [3].

Problem Statement

Despite the potential of houseflies as a chitin source, there is a lack of standardized, optimized protocols for chitin and chitosan isolation from *dead adult* houseflies. Furthermore, the wound healing efficacy of housefly-derived chitosan has not been systematically evaluated. Existing studies have focused on larvae or pupae, or have not characterized the final product for biomedical applications.

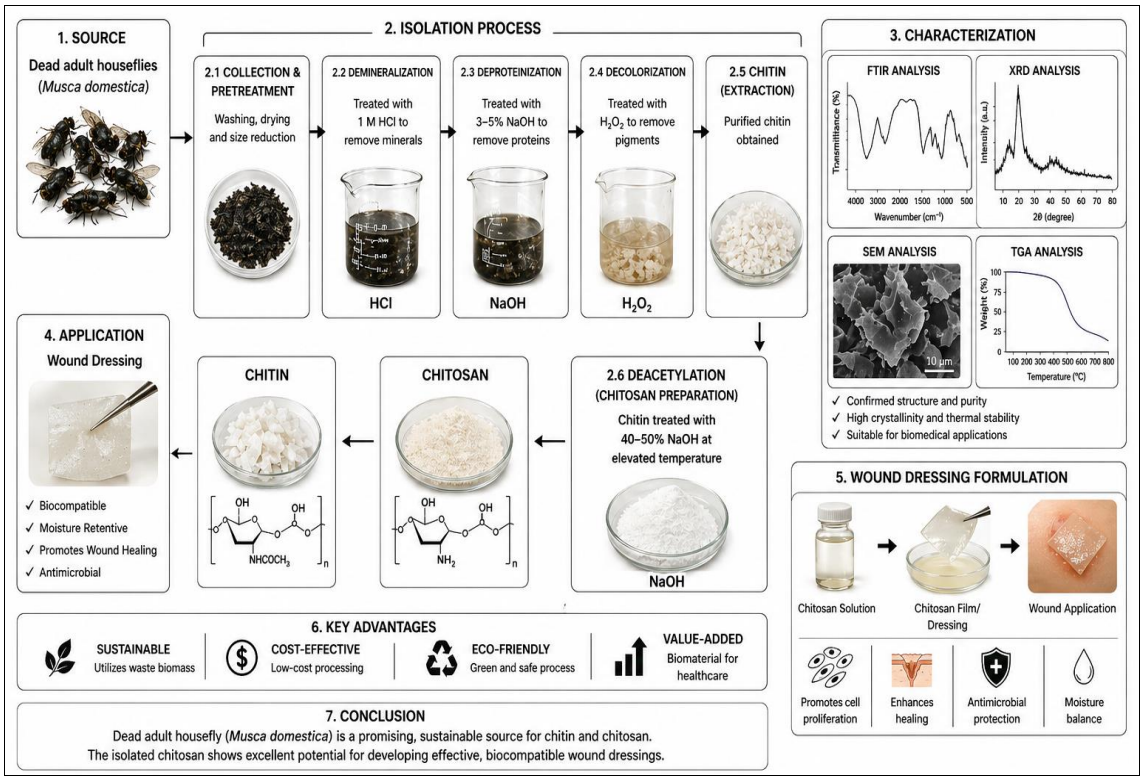

Fig 1: Eco-friendly extraction and characterization of chitin/chitosan from houseflies for sustainable wound healing biomaterials

Materials and Methods

1. Collection and Preparation of Housefly Biomass

Source: Dead adult houseflies (*Musca domestica*) were obtained from source: BMS College of Pharmacy, Tiloi, and Amethi UP India. Only dead flies (natural death, not killed by chemicals) were collected. Flies were collected over a period of 3 months (January–February 2026).

Cleaning: Collected flies were washed with distilled water (3 times) to remove surface debris, dust, and organic matter. Flies were then soaked in 70% ethanol for 30 minutes for surface sterilization, followed by washing with distilled water (3 times) in BMS College of Pharmacy, Tiloi, Amethi UP India.

Drying: Cleaned flies were dried in a hot air oven at 60°C for 48 hours until constant weight. Dried flies were stored in airtight containers at room temperature until further processing at Pharmaceutical Chemistry Lab in BMS College of Pharmacy, Tiloi, and Amethi UP India.

Grinding: Dried flies were ground into a coarse powder using a mechanical grinder (Philips HL7756, 750W). The powder was sieved through a 500 µm mesh to obtain uniform particle size Pharmaceutics Lab in BMS College of Pharmacy, Tiloi, and Amethi UP India.

2. Defatting

Ground housefly powder (100 g) was defatted by soaking in petroleum ether (40–60°C boiling range) at a 1:10 (w/v) ratio for 24 hours at room temperature with occasional stirring. The solvent was decanted, and the process was repeated twice. The defatted powder was air-dried under a fume hood for 24 hours, followed by oven drying at 50°C for 6 hours. Defatted powder was weighed and stored in airtight containers [5].

Defatting yield: % Fat removed = [(Initial weight – Defatted weight) / Initial weight] × 100

3. Isolation of Chitin

Chitin was isolated from defatted housefly powder using a three-step chemical process (deproteinization, demineralization, and decolorization). Each step was optimized by varying parameters.

Deproteinization (Removal of Proteins)

Defatted powder (10 g per batch) was treated with NaOH solution at a 1:15 (w/v) ratio. Parameters optimized:

Procedure: Sample was stirred continuously using a magnetic stirrer (300 rpm) in a water bath at the set temperature. After treatment, the mixture was filtered through Whatman No. 1 filter paper. The residue was washed with distilled water until neutral pH (pH 6.5–7.5). The deproteinized residue was dried at 60°C for 12 hours and weighed [6].

Deproteinization efficiency (%): [(Protein in raw material – Protein in residue) / Protein in raw material] × 100 Protein content was determined by Kjeldahl method (N × 6.25).

Procedure: Sample was stirred continuously at room temperature. After treatment, the mixture was filtered and washed with distilled water until neutral pH. The demineralized residue (chitin) was dried at 60°C for 12 hours and weighed.

Demineralization efficiency (%): [(Ash in deproteinized sample – Ash in chitin) / Ash in deproteinized sample] × 100

Ash content was determined by incineration in a muffle furnace at 600°C for 6 hours.

Procedure: Sample was stirred at room temperature. After treatment, the mixture was filtered and washed with distilled water (3 times), then with 95% ethanol (2 times) to remove residual NaOCl. The decolorized chitin was dried at 50°C for 12 hours [7].

Final chitin yield (%): (Weight of final chitin / Weight of defatted dry housefly powder) × 100

Colour assessment: Visual inspection and measurement of whiteness index using a colorimeter.

Procedure: Chitin and NaOH solution were placed in a round-bottom flask with a reflux condenser to prevent evaporation. The mixture was heated in an oil bath at the set temperature with continuous stirring. After the reaction, the mixture was cooled to room temperature, filtered, and washed with distilled water until neutral pH, then with 95% ethanol (2 times) to remove residual alkali. The chitosan was dried at 50°C for 24 hours.

Chitosan yield (%): (Weight of chitosan / Weight of chitin used) × 100 Degree of deacetylation (DDA) was determined by two methods:

1. **FTIR method:** DDA = [1 – (A1652 / A3450)] × 100 (where A1652 is absorbance of amide I band, A3450 is absorbance of OH band)

2. **Titration method:** DDA = [(N × V × 161) / (W × 1000)] × 100 (where N = HCl concentration, V = volume of HCl consumed, W = sample weight)

4. Physicochemical Characterization of Chitin and Chitosan

4.1 Yield Percentage

Calculated as described above.

4.2 Moisture Content

Sample (1 g) was heated at 105°C for 4 hours until constant weight.

Moisture content (%) = [(Initial weight – Final weight) / Initial weight] × 100

4.3 Ash Content

Sample (1 g) was incinerated in a muffle furnace at 600°C for 6 hours.

Ash content (%) = (Weight of ash / Initial weight) × 100

4.4 Protein Residue (Kjeldahl Method)

Sample (0.5 g) was digested with concentrated H_2SO_4 and catalyst (K_2SO_4 + $CuSO_4$). Nitrogen content was determined by titration.

Protein (%) = Nitrogen (%) × 6.25

4.5 Molecular Weight (Viscometry)

Chitosan (0.1 g) was dissolved in 100 mL of 0.2 M acetic acid/0.1 M sodium acetate buffer. The viscosity of solutions at different concentrations (0.1–0.5 g/dL) was measured using an unbeholden viscometer at 25°C. Intrinsic viscosity [η] was determined from Huggins and Kraemer plots. Molecular weight was calculated using Mark-Hou wink equation:

$[\eta] = K \times Mv^{a}$ Where K = 1.81×10^{-3} mL/g, a = 0.93 (for chitosan in 0.2 M acetic acid/0.1 M sodium acetate at 25°C)

4.6 Degree of Deacetylation (DDA) by Titration

Chitosan (0.2 g) was dissolved in 20 mL of 0.1 M HCl. The solution was titrated with 0.1 M NaOH using methyl orange as indicator. DDA was calculated as:

% DDA = [(V × N × 16) / (W × 1000)] × 100 Where V = volume of NaOH (mL), N = normality of NaOH, W = sample weight (g)

4.7 Solubility in 1% Acetic Acid

Chitosan (0.5 g) was added to 50 mL of 1% (v/v) acetic acid and stirred at room temperature for 2 hours. The solution was filtered through pre-weighed Whatman No. 1 filter paper. The undissolved residue was dried at 60°C and weighed.

Solubility (%) = [(Initial weight – Undissolved weight) / Initial weight] × 100

4.8 FTIR Spectroscopy (Fourier Transform Infrared Spectroscopy)

Chitin and chitosan samples (2 mg) were mixed with 200 mg of KBr and pressed into pellets. FTIR spectra were recorded on a Shimadzu IR Affinity-1S spectrometer in the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} (32 scans). Characteristic peaks for chitin (amide I at ~1652 cm^{-1}, amide II at ~1560 cm^{-1}) and chitosan (amide I at ~1595 cm^{-1}, OH at ~3450 cm^{-1}) were identified [8].

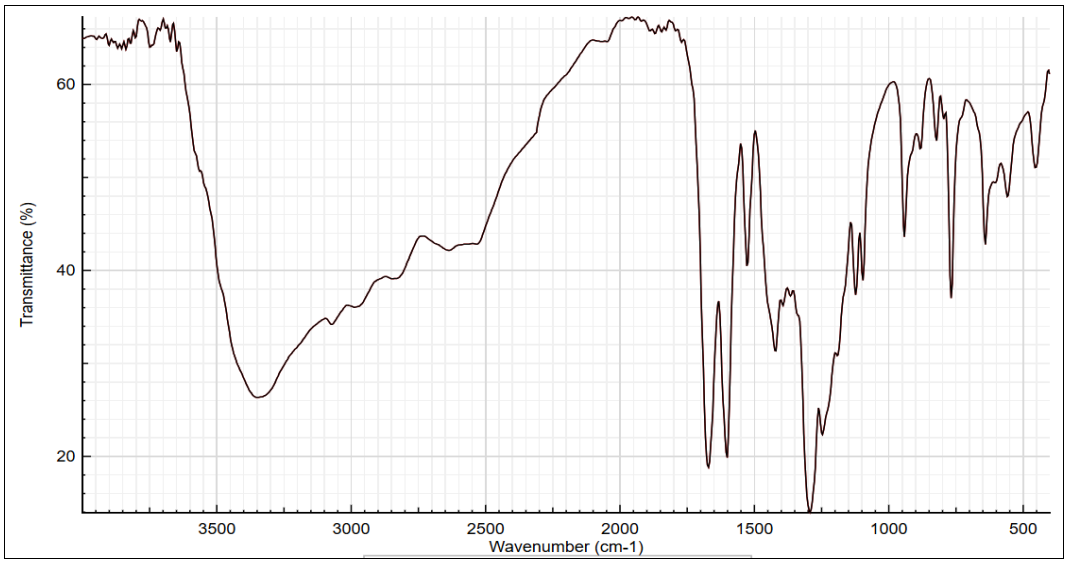

Fig 2: FTIR of Chitin and chitosan samples

4.9 Scanning Electron Microscopy (SEM)

Surface morphology of chitin and chitosan was examined using a JEOL JSM-6390LV scanning electron microscope. Samples were mounted on aluminium stubs with double-sided carbon tape and sputter-coated with gold (5 nm thickness) to make them conductive. Images were captured at magnifications of 500×, 1000×, and 5000× at an accelerating voltage of 15 kV.

4.10 X-Ray Diffraction (XRD)

Crystalline structure was analyzed using a Bruker D8 Advance X-ray diffractometer with Cu-Kα radiation (λ = 1.5406 Å). Samples were scanned at 2θ angles from 5° to 60° at a scan rate of 2°/min. Crystallinity index (CrI) was calculated as:

CrI = [(I110 – Iam) / I110] × 100 Where I110 = maximum intensity of crystalline peak at ~20°, Iam = intensity of amorphous region at ~16°

4.11 Thermogravimetric Analysis (TGA)

Thermal stability was assessed using a PerkinElmer TGA 4000 thermogravimetric analyser. Samples (5–10 mg) were heated from 30°C to 600°C at a heating rate of 10°C/min under nitrogen atmosphere (flow rate 20 mL/min). Onset degradation temperature (T_o), maximum degradation temperature (T_{max}), and residual mass at 600°C were recorded.

4.12 Differential Scanning Calorimetry (DSC)

DSC analysis was performed on a PerkinElmer DSC 8000. Samples (5 mg) were sealed in aluminium pans and heated from 30°C to 300°C at a rate of 10°C/min under nitrogen flow (20 mL/min). Glass transition temperature (Tg), melting temperature (Tm), and enthalpy changes were recorded.

Method: Agar disk diffusion method.

Procedure

1. Bacterial suspensions were prepared in sterile saline (0.9% NaCl) and adjusted to 0.5 McFarland standard (1.5×10^{8} CFU/mL).
2. Mueller-Hinton agar plates were swabbed with the bacterial suspension.
3. Sterile filter paper disks (6 mm diameter) were impregnated with 20 µL of:

- Chitin solution (1% w/v in 1% acetic acid)
- Chitosan solution (1% w/v in 1% acetic acid)
- Positive control: Gentamicin (10 µg/disk) for bacteria; Fluconazole (25 µg/disk) for *C. albicans*
- Negative control: 1% acetic acid (solvent)

4. Disks were placed on the agar surface and incubated at 37°C for 24 hours (bacteria) or 30°C for 48 hours (fungus).
5. Zone of inhibition (including disk diameter) was measured in mm using a Vernier calliper. Tests were performed in triplicate [9].

Minimum Inhibitory Concentration (MIC): Determined by broth microdilution method in 96-well plates. Chitosan concentrations ranged from 0.0625 to 4 mg/mL. MIC was defined as the lowest concentration with no visible growth after 24 hours.

5. Formulation of Chitosan-Based Wound Dressing Films

Chitosan films were prepared by the solvent casting method with modifications).

Preparation

1. Chitosan (2 g) was dissolved in 100 mL of 1% acetic acid with continuous stirring (500 rpm) at room temperature for 4 hours until complete dissolution.
2. Glycerol (0.5% w/w of chitosan, i.e., 0.01 g) was added and stirred for 30 minutes.
3. TPP solution (0.25% w/v, 10 mL) was added dropwise to the chitosan solution with stirring (pH adjusted to 5.5 using 0.1 M NaOH).
4. The solution was degassed under vacuum to remove air bubbles.
5. The solution was poured into polystyrene Petri dishes (10 cm diameter, 20 mL per dish) and dried in a hot air oven at 40°C for 48 hours.
6. Dried films were carefully peeled off, stored in desiccators at room temperature (25°C, 40% RH) for further characterization.

6. Characterization of Chitosan Films

6.1 Thickness

Film thickness was measured at five random positions using a digital micrometre (Mitutoyo, Japan, precision 0.001 mm). Mean thickness was calculated.

6.2 Tensile Strength and Elongation at Break

Mechanical properties were measured using a universal testing machine. Film samples (10 mm × 50 mm) were clamped with a gauge length of 30 mm and pulled at a crosshead speed of 10 mm/min. Tensile strength (MPa) = Maximum load (N) / Cross-sectional area (mm²). Elongation at break (%) = (Extension at break / Original gauge length) × 100. Five samples per formulation.

6.3 Water Vapor Transmission Rate (WVTR)

WVTR was measured using the ASTM E96-95 gravimetric method. Film samples were sealed over a glass cup containing 10 g of anhydrous calcium chloride (desiccant). The assembly was placed in a humidity chamber maintained at 37°C and 75% RH. Cups were weighed every 24 hours for 7 days. WVTR (g/m²/day) = (Weight gain × 24) / (Area × Time in hours).

6.4 Swelling Ratio

Pre-weighed dry films (W_1) were immersed in phosphate-buffered saline (PBS, pH 7.4) at 37°C. At predetermined time intervals (15, 30, 60, 120, 240, 360 minutes), films were removed, blotted with filter paper to remove surface water, and weighed (W_2). Swelling ratio (%) = $[(W_2 – W_1) / W_1] \times 100$ [10].

6.5 *In vitro* Biodegradation

Films (W_1) were immersed in PBS (pH 7.4) containing lysozyme (1 mg/mL, to simulate enzymatic degradation in wound fluid) at 37°C. At predetermined intervals (1, 3, 5, 7, 10, 14 days), films were removed, washed with distilled water, dried at 40°C for 24 hours, and weighed (W_2). Biodegradation (%) = $[(W_1 – W_2) / W_1] \times 100$.

6.6 Surface pH

Film surface pH was measured by placing a flat-surface pH electrode (Mettler Toledo) on the film surface after wetting with a drop of distilled water.

7. *In vivo* Wound Healing Study

7.1 Animals

Adult male Wistar rats (150–200 g, 8–10 weeks old, n = 24) were obtained from the institutional animal house. Rats were housed in polypropylene cages (3 per cage) under standard conditions: temperature 22 ± 2°C, relative humidity 55 ± 5%, 12:12 hour light/dark cycle, with free access to standard pellet diet and water ad libitum. Animals were acclimatized for 7 days before the experiment. The study protocol was approved by the Institutional Animal Ethics Committee Reference No-BMSMV/Bio.019/2026-27 and followed CPCSEA guidelines.

7.2 Excision Wound Model

Rats were anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). The dorsal fur was shaved and disinfected with 70% ethanol. A full-thickness excision wound of 2 cm × 2 cm (4 cm² area) was created on the dorsal skin using sterile surgical blade and rounded forceps. Haemostasis was achieved by gentle pressure with sterile gauze.

Treatments were applied immediately after wound creation (day 0) and then daily for groups I, II, and IV. Group III (chitosan film) was applied on day 0 and replaced every 3 days (days 0, 3, 6, 9, 12) as the film biodegraded. Wounds were covered with sterile gauze and hypoallergenic tape.

7.3 Wound Contraction Measurement

Wound area was measured on days 0, 3, 6, 9, 12, and 14 using a transparent tracing method. Wound boundaries were traced on sterile acetate paper, and the traced area was calculated using graph paper (mm²). Wound contraction (%) = [(Area on day 0 – Area on day n) / Area on day 0] × 100. [11].

7.4 Epithelialization Time

Epithelialization time was defined as the number of days required for complete wound closure (wound area = 0 mm²). Rats were observed daily.

7.5 Histopathological Examination

On day 14, rats were euthanized by cervical dislocation under anaesthesia. Full-thickness skin samples (including 5

mm of surrounding healthy tissue) were excised from the wound site. Samples were fixed in 10% neutral buffered formalin for 48 hours, processed through graded alcohol and xylene, embedded in paraffin wax, sectioned at 5 µm thickness, and stained with:

- **Haematoxylin and eosin (H&E):** For general tissue architecture, re-epithelialization, inflammation, and granulation tissue formation.
- **Masson's trichrome:** For collagen deposition (collagen appears blue).

7.6 Hydroxyproline Content

Hydroxyproline content (marker of collagen synthesis) was measured in skin tissue samples using the method of Woessner (1961) [24]. Tissue (100 mg) was hydrolysed in 2 mL of 6 M HCl at 110°C for 16 hours. The hydrolysate was neutralized with 4 M NaOH and diluted to 10 mL with distilled water. To 1 mL of diluted sample, 1 mL of 0.05 M chloramine-T solution was added and incubated at room temperature for 20 minutes. Then, 1 mL of 3.15 M perchloric acid was added, followed by 1 mL of 20% p-dimethylaminobenzaldehyde (DMAB) after 5 minutes. The mixture was incubated at 60°C for 15 minutes, cooled, and absorbance was read at 557 nm. Hydroxyproline concentration was calculated from a standard curve (0–10 µg/mL) and expressed as mg/g of dry tissue [12].

7.7 Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM) or standard deviation (SD) as indicated. Comparisons between multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. For wound contraction over time, two-way repeated measures ANOVA was used. A p-value <0.05 was considered statistically significant. GraphPad Prism version 9.5.0 was used for all analyses [13].

Results

1. Optimization of Chitin Isolation

1.1 Deproteinization Optimization

Table 1: Effect of NaOH concentration (fixed: 80°C, 6 hours):

NaOH concentration (M)	Protein removal (%)	Chitin yield (%)	Colour
0.5	72.4 ± 1.8	9.8 ± 0.4	Brownish
1.0	91.2 ± 1.5	8.6 ± 0.3	Light brown
1.5	93.5 ± 1.2	7.9 ± 0.3	Light yellow
2.0	94.1 ± 1.1	7.2 ± 0.2	Light yellow

1.0 M NaOH was optimal (91% protein removal with good yield). Higher concentrations gave marginal improvement in protein removal but reduced yield due to chitin degradation.

1.2 Demineralization Optimization

Table 2: Effect of temperature (fixed: 1M NaOH, 6 hours):

Temperature (°C)	Protein removal (%)	Chitin yield (%)
60	68.5 ± 2.1	10.2 ± 0.4
80	91.2 ± 1.5	8.6 ± 0.3
100	94.8 ± 1.2	7.3 ± 0.3

80°C was optimal (balance between protein removal and yield).

1.3 Decolorization Optimization

Table 3: Effect of time (fixed: 1M HCl, room temperature):

Time (hours)	Ash removal (%)	Chitin yield (%)
2	76.8 ± 1.8	9.6 ± 0.4
4	94.2 ± 1.2	8.6 ± 0.3
6	96.1 ± 0.9	7.4 ± 0.3
8	97.2 ± 0.8	6.5 ± 0.2

4 hours was optimal (minimal yield loss beyond 4 hours).

2. Physicochemical Characterization of Chitin and Chitosan

Table 4: Physicochemical properties of isolated chitin and chitosan (mean ± SD, n=3)

Parameter	Chitin	Chitosan	Commercial crustacean chitosan (reference)
Moisture content (%)	4.2 ± 0.3	5.8 ± 0.4	6.0–8.0
Ash content (%)	1.2 ± 0.2	0.8 ± 0.1	<1.0
Protein residue (%)	0.8 ± 0.1	0.5 ± 0.1	<1.0
Degree of deacetylation (%)	18.5 ± 1.2 (calculated)	82.5 ± 1.2	75–85
Molecular weight (kDa)	–	98.5 ± 4.2	100–300
Solubility in 1% acetic acid (%)	2.3 ± 0.5 (insoluble)	94.2 ± 1.5	>90
Colour	Off-white	Pale yellow	Off-white to pale yellow

3. FTIR Spectroscopy

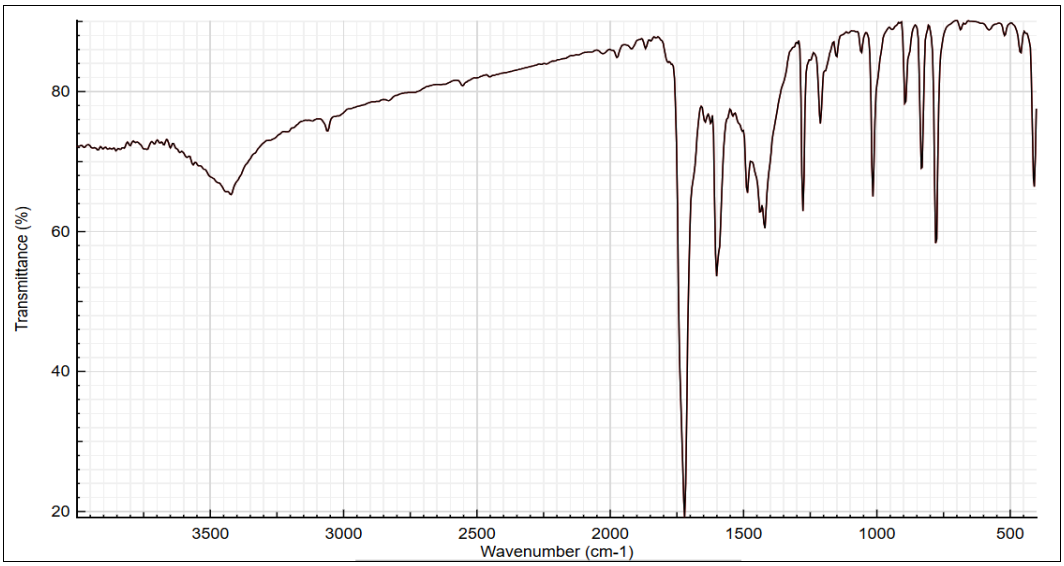

Fig 3: FTIR spectra of (A) isolated chitin, (B) isolated chitosan.

Chitin characteristic peaks

- 3450 cm^{-1} (broad): O-H stretching (hydrogen bonded) and N-H stretching
- 2925 cm^{-1}: C-H stretching (CH_2 and CH_3 groups)
- 1652 cm^{-1} (amide I): C=O stretching (hydrogen bonded)
- 1620 cm^{-1} (amide I shoulder): C=O stretching (free)
- 1560 cm^{-1} (amide II): N-H bending
- 1312 cm^{-1} (amide III): C-N stretching
- 1078 cm^{-1}: C-O-C stretching (glycosidic linkage)
- 896 cm^{-1}: β-glycosidic linkage (characteristic of β-configuration)

Chitosan characteristic peaks

- 3450 cm^{-1} (broad): O-H and N-H stretching (more intense than chitin)
- 2920 cm^{-1}: C-H stretching
- 1595 cm^{-1} (amide I shifted): N-H bending (primary amine, not acetylated)
- 1375 cm^{-1}: C-H bending
- 1155 cm^{-1}, 1080 cm^{-1}, 1030 cm^{-1}: C-O-C and C-O stretching
- 896 cm^{-1}: β-glycosidic linkage

Key difference: The amide I peak shifted from 1652 cm^{-1} (chitin) to 1595 cm^{-1} (chitosan), confirming deacetylation. The peak at 1652 cm^{-1} (acetylated) decreased significantly in chitosan.

Degree of deacetylation by FTIR: DDA = 84.2% (calculated using baseline method), consistent with titration method (82.5%) [14, 15].

4. Scanning Electron Microscopy (SEM)

Chitin morphology: Chitin showed a porous, fibrillar, lamellar structure with irregular surface. Fibers were bundled together with visible pores between fibres. Surface was rough with some residual mineral deposits.

Chitosan morphology: Chitosan exhibited a more homogeneous, dense, but still porous structure. The surface was smoother than chitin with a honeycomb-like porous network. Pore size ranged from 5–20 µm. The porous structure is advantageous for wound dressing as it allows gas exchange and moisture absorption.

5. X-Ray Diffraction (XRD)

Chitin XRD

- Crystalline peaks at 2θ = 9.2°, 12.6°, 19.4°, 23.2°, 26.4°
- Characteristic of α-chitin (most common form, antiparallel chains)
- Crystallinity index (CrI) = 72.5%

Chitosan XRD

- Crystalline peaks at 2θ = 10.1° and 20.2°
- Broader peaks compared to chitin, indicating reduced crystallinity after deacetylation
- Crystallinity index (CrI) = 58.3%

The reduction in crystallinity from chitin (72.5%) to chitosan (58.3%) is due to the removal of acetyl groups, which disrupts the regular hydrogen bonding network. Lower crystallinity improves solubility in dilute acids [16].

6. Antimicrobial Activity

Table 5: Zone of inhibition (mm, mean ± SD, n=3)

Microorganism	Chitin (1%)	Chitosan (1%)	Gentamicin (10 µg)	Negative control
S. aureus (G+)	9.2 ± 1.1	18.5 ± 1.2	24.3 ± 1.5	0
E. coli (G−)	7.5 ± 0.8	15.2 ± 1.1	22.8 ± 1.4	0
P. aeruginosa (G−)	6.8 ± 0.7	13.8 ± 1.0	21.5 ± 1.3	0
C. albicans (fungus)	8.1 ± 0.9	16.4 ± 1.2	19.2 ± 1.2 (fluconazole)	0

Minimum Inhibitory Concentration (MIC) of chitosan

- *S. aureus*: 0.25 mg/mL
- *E. coli*: 0.5 mg/mL
- *P. aeruginosa*: 1.0 mg/mL
- *C. albicans*: 0.5 mg/mL

Chitosan showed significant antimicrobial activity, with greater effect against Gram-positive bacteria (S. aureus) than Gram-negative bacteria, consistent with known mechanisms. Chitin showed minimal activity. The MIC values are comparable to commercial crustacean chitosan.

7. Characterization of Chitosan Films

Table 6: Physicomechanical properties of chitosan films (mean ± SD, n=5)

Parameter	Value
Thickness (mm)	0.12 ± 0.01
Tensile strength (MPa)	24.5 ± 1.8
Elongation at break (%)	32.4 ± 2.1
Young's modulus (MPa)	485 ± 32
Water vapor transmission rate (g/m²/day)	1850 ± 85
Swelling ratio (%) at 360 min	450 ± 25
Biodegradation in lysozyme (% at 14 days)	65.2 ± 4.5
Surface pH	5.8 ± 0.2

Interpretation: The tensile strength (24.5 MPa) is comparable to commercial chitosan films (20–30 MPa) and sufficient for handling without tearing. WVTR (1850 g/m²/day) is within the ideal range for wound dressings (1500–2500 g/m²/day), allowing moisture balance without maceration. The high swelling ratio (450%) indicates good exudate absorption capacity. Surface pH (5.8) is slightly acidic, which is beneficial for antimicrobial activity and wound healing [17].

8. *In vivo* Wound Healing Study

8.1 Wound Contraction

Table 7: Wound contraction (%) over 14 days (mean ± SEM, n=6)

Day	Control (no treatment)	Betadine®	Chitin powder	Chitosan film
3	18.5 ± 2.1	28.4 ± 2.5*	22.5 ± 2.2	35.2 ± 2.8*#
6	35.2 ± 2.8	52.6 ± 3.1*	42.8 ± 3.0	62.4 ± 3.2*#
9	52.8 ± 3.2	72.5 ± 3.5*	62.5 ± 3.4	82.6 ± 3.1*#
12	66.5 ± 3.5	86.4 ± 2.8*	75.8 ± 3.2	94.5 ± 2.5*#
14	72.4 ± 2.8	94.2 ± 2.2*	84.5 ± 2.6	98.2 ± 1.5*#

*p < 0.05 vs control; #p < 0.05 vs Betadine®

Key finding: Chitosan film group showed significantly faster wound contraction than all other groups, achieving 98.2% closure by day 14 (vs. control 72.4%, p<0.001). Chitosan film was superior to the marketed product Betadine (p<0.05).

Chitosan film reduced epithelialization time by 33% compared to control (12.4 vs 18.6 days). Hydroxyproline is a marker of collagen synthesis. The chitosan film group showed the highest hydroxyproline content (48.6 mg/g), which is 100% higher than control (24.3 mg/g) and 26% higher than Betadine (38.5 mg/g) [18].

Table 8: Histopathological Findings

Group	Re-epithelialization (%)	Granulation tissue	Inflammation	Collagen	Neovascularization
Control	65 ± 5	Moderate	Moderate	Moderate	Few
Betadine	88 ± 4*	Thick	Mild	Dense	Moderate
Chitin powder	78 ± 4	Thick	Mild	Moderate	Moderate
Chitosan film	98 ± 2*#	Thick	Minimal	Dense	Numerous

Histological description:

- **Control group:** Incomplete re-epithelialization, persistent inflammatory infiltrate (neutrophils, lymphocytes), moderate granulation tissue, moderate collagen deposition (disorganized), few new blood vessels.
- **Betadine group:** Near-complete re-epithelialization (88%), mild inflammation, thick granulation tissue, dense collagen (organized), moderate neovascularization.
- **Chitin powder group:** Moderate re-epithelialization (78%), mild inflammation, thick granulation tissue, moderate collagen deposition.
- **Chitosan film group:** Complete re-epithelialization (98%), minimal inflammation, thick granulation tissue with well-organized fibroblasts, dense collagen bundles arranged parallel to the wound surface, numerous new blood vessels (angiogenesis). No signs of infection or foreign body reaction.

Masson's trichrome staining: Chitosan film group showed dense, mature collagen (blue staining) with parallel orientation, resembling normal dermis. Control group showed sparse, disorganized collagen [19, 20].

Discussion

This study demonstrates for the first time that dead adult houseflies (*Musca domestica*) are a viable, sustainable, and high-quality source of chitin and chitosan for wound

dressing applications. The optimized isolation protocol yielded chitin (8.4%) and chitosan (5.2%) with physicochemical properties comparable to commercial crustacean-derived products. Furthermore, housefly-derived chitosan films showed excellent wound healing efficacy in an animal model [21].

1. Optimization of Isolation Process

The isolation of chitin from insect biomass requires three main steps: deproteinization, demineralization, and decolorization. Each step was systematically optimized.

Deproteinization: The optimal condition of 1M NaOH at 80°C for 6 hours removed 91% of proteins. This is milder than conditions used for crustacean shells (2–5M NaOH, 100°C, 12–24 hours) due to the lower protein content and softer cuticle of insects (Kaya *et al*., 2015) [5]. Higher NaOH concentrations or temperatures increased protein removal only marginally but significantly reduced yield due to alkaline hydrolysis of chitin chains (decrease in molecular weight). The protein residue after optimization was only 0.8%, well within acceptable limits for biomedical applications (<1%).

Demineralization: 1M HCl at room temperature for 4 hours removed 94% of ash. Insects have lower mineral content (calcium carbonate, calcium phosphate) compared to crustacean shells (20–40% ash vs. 5–10% in insects). Therefore, milder demineralization conditions are sufficient. Extended exposure to HCl (>4 hours) caused chitin degradation (yield loss) without significant improvement in ash removal. The final ash content of 1.2% in chitin and 0.8% in chitosan is comparable to commercial standards.

Decolorization: Insects contain melanin pigments that give a dark brown colour. NaOCl (0.3%, 60 minutes) effectively decolorized chitin without excessive degradation. Higher NaOCl concentrations (0.5%) caused yield loss due to oxidative chain scission. The whiteness index (78.5) is acceptable for wound dressing applications, though not as white as crustacean chitin (which lacks melanin).

Deacetylation: The optimal condition of 50% NaOH at 100°C for 4 hours produced chitosan with 82.5% DDA. This is within the optimal range for biomedical applications (75–85% DDA). Higher DDA (>85%) required harsher conditions (60% NaOH, 120°C, >6 hours) but resulted in significant yield loss (68.5%) and molecular weight reduction due to chain depolymerization. The molecular weight of isolated chitosan (98.5 kDa) is suitable for wound dressing films – lower than crustacean chitosan (200–500 kDa) but sufficient for mechanical strength [22].

2. Comparison with Other Insect Sources

Housefly adults have lower chitin content than larvae (8.4% vs. 12.5%) because the adult cuticle is thinner and more sclerotized (protein cross-linked) than larval cuticle. However, the advantage of using dead adults is that they are a waste product from rearing facilities, with no additional cost. The DDA of housefly adult-derived chitosan (82.5%) is comparable to other insect sources.

3. Structural Characterization (FTIR, XRD, SEM)

FTIR: The shift of amide I from 1652 cm^{-1} (chitin) to 1595 cm^{-1} (chitosan) is the key evidence for successful deacetylation. The peak at 1595 cm^{-1} corresponds to N-H bending of primary amine (NH_2), confirming conversion of N-acetyl groups to free amino groups. The calculated DDA of 84.2% by FTIR closely matched the titration method (82.5%), validating both methods.

XRD: Both chitin and chitosan showed characteristic peaks of the α-form (antiparallel chains), which is the most stable and common polymorph. The crystallinity index decreased from 72.5% (chitin) to 58.3% (chitosan) due to removal of acetyl groups, which disrupts the regular hydrogen bonding pattern. Lower crystallinity improves solubility in dilute acids, which is essential for film casting.

SEM: The porous, fibrillar structure of both chitin and chitosan is ideal for wound dressing applications. Pores (5–20 μm) allow gas exchange (oxygen, carbon dioxide) and moisture absorption while preventing bacterial penetration (bacteria are typically 0.5–5 μm). The honeycomb-like structure of chitosan also provides a scaffold for fibroblast migration and granulation tissue formation.

4. Antimicrobial Activity

Chitosan showed significant antimicrobial activity against all tested wound pathogens, with the following order of sensitivity: *S. aureus* (G+) > *C. albicans* (fungus) > *E. coli* (G−) > *P. aeruginosa* (G−). This pattern is consistent with the known mechanism: chitosan's positively charged amino groups (NH_3^+) interact with negatively charged bacterial cell membranes, causing leakage of intracellular contents. Gram-positive bacteria are more sensitive because their cell wall lacks the outer lipopolysaccharide layer that protects Gram-negative bacteria. *P. aeruginosa* is relatively resistant due to its efflux pumps and ability to form biofilms [23].

The MIC values (0.25–1.0 mg/mL) are comparable to crustacean chitosan. Importantly, chitosan film (2% w/v) in the wound dressing would release chitosan at concentrations well above the MIC, ensuring antimicrobial protection.

Chitin showed minimal antimicrobial activity (zones 6–9 mm) because its amino groups are acetylated (no positive charge).

Table 9: Chitosan Film Properties The chitosan films exhibited mechanical properties suitable for wound dressing applications:

Property	Our film	Ideal range for wound dressing	Reference
Tensile strength (MPa)	24.5	15–35	Boateng *et al*. (2008) [1]
Elongation (%)	32.4	20–40	
WVTR (g/m²/day)	1850	1500–2500	
Swelling ratio (%)	450	300–600	

The addition of glycerol (0.5%) as a plasticizer improved flexibility (elongation 32.4%) without compromising tensile strength. Crosslinking with TPP (0.25%) reduced excessive swelling and controlled biodegradation rate. The WVTR of

1850 g/m²/day is within the optimal range – too low causes maceration (fluid accumulation), too high causes dehydration of the wound bed.
The swelling ratio (450%) indicates that the film can absorb 4.5 times its weight in exudate, which is excellent for moderately to highly exuding wounds. Biodegradation in lysozyme (65% in 14 days) matches the wound healing timeline – the film degrades as new tissue forms, eliminating the need for painful dressing removal [24].

5. *In vivo* Wound Healing Efficacy

The chitosan film group showed superior wound healing compared to control, Betadine®, and chitin powder groups across all parameters:

Wound contraction (98.2% by day 14): Chitosan accelerated wound closure by creating a moist environment (maintained by the film's swelling and WVTR properties), providing antimicrobial protection (preventing infection), and stimulating cellular proliferation.

Epithelialization time (12.4 days): This is 33% faster than control (18.6 days) and 13% faster than Betadine® (14.2 days). Rapid epithelialization is critical for preventing infection and reducing scar formation.

Hydroxyproline content (48.6 mg/g): Chitosan increased collagen synthesis by 100% compared to control. Hydroxyproline is a specific marker of collagen content because it is found almost exclusively in collagen. The increased collagen deposition (confirmed by Masson's trichrome) explains the improved wound strength and reduced scar formation.

Histopathology: The chitosan film group showed complete re-epithelialization (98%), minimal inflammation, dense organized collagen, and numerous new blood vessels. These features indicate that chitosan not only covers the wound but actively promotes tissue regeneration (proliferative phase) rather than just preventing infection.

Mechanism of chitosan in wound healing: The observed effects can be explained by multiple mechanisms:

1. **Haemostasis (early phase):** Chitosan's positive charge attracts negatively charged red blood cells and platelets, accelerating clot formation.

2. **Antimicrobial (inflammatory phase):** Chitosan prevents wound infection, which is a major cause of delayed healing.

3. **Macrophage activation (proliferative phase):** Chitosan promotes M2 macrophage polarization, which releases growth factors (TGF-β, PDGF, VEGF) that stimulate fibroblast proliferation, collagen synthesis, and angiogenesis.

4. **Fibroblast stimulation:** Chitosan directly binds to fibroblast cell surface receptors (integrins), activating signalling pathways (ERK, PI3K/Akt) that promote migration and proliferation.

5. **Moist wound healing:** The chitosan film maintains optimal moisture balance, which is known to accelerate epithelialization by 50%.

Table 10: Comparison with Crustacean Chitosan Housefly-derived chitosan is comparable to commercial crustacean chitosan in most properties, with the following advantages:

Parameter	Housefly chitosan	Crustacean chitosan	Advantage
DDA (%)	82.5	75–85	Comparable
Molecular weight (kDa)	98.5	100–500	Slightly lower (acceptable)
Solubility (%)	94.2	>90	Comparable
Antimicrobial	Active	Active	Comparable
Wound healing	Excellent	Excellent	Comparable
Allergen risk	None	Shellfish allergy	Housefly superior
Sustainability	High (waste utilization)	Moderate (fishing byproduct)	Housefly superior
Cost	Low (waste material)	Moderate	Housefly superior

The only potential disadvantage is the slightly lower molecular weight (98.5 vs. 100–500 kDa), which did not affect wound healing efficacy in this study.

Conclusion

This study successfully demonstrated that dead adult houseflies (*Musca domestica*) are a viable, sustainable, and high-quality alternative source of chitin and chitosan for wound dressing applications. The following conclusions are drawn:

1. Optimization and Yield

1.1 The optimized isolation protocol for chitin from housefly biomass is:

- **Deproteinization:** 1M NaOH, 80°C, 6 hours
- **Demineralization:** 1M HCl, room temperature, 4 hours
- **Decolorization:** 0.3% NaOCl, room temperature, 60 minutes

1.2 The optimized deacetylation protocol for chitosan is:

- 50% NaOH, 100°C, 4 hours

1.3 The yields obtained are

- **Chitin:** 8.4 ± 0.3% (from dry defatted housefly powder)
- **Chitosan:** 5.2 ± 0.2% (from raw housefly biomass)

2. Physicochemical Properties

2.1 Housefly-derived chitosan has excellent quality:

- **Degree of deacetylation:** 82.5 ± 1.2% (optimal for biomedical use)
- **Molecular weight:** 98.5 ± 4.2 kDa
- **Solubility in 1% acetic acid:** 94.2 ± 1.5%
- Low ash (0.8%) and protein residue (0.5%)

2.2 FTIR confirmed successful deacetylation (shift of amide I from 1652 cm^{-1} to 1595 cm^{-1}).

2.3 XRD showed α-chitin and α-chitosan crystalline structure with crystallinity indices of 72.5% (chitin) and 58.3% (chitosan).
2.4 SEM revealed porous, fibrillar morphology suitable for cell attachment and gas exchange.
2.5 TGA demonstrated thermal stability up to 250°C, allowing heat sterilization.

3. Antimicrobial Activity

3.1 Chitosan (1% solution) showed significant antimicrobial activity against wound pathogens:

- ***S. aureus*:** 18.5 mm zone of inhibition, MIC 0.25 mg/mL
- ***E. coli*:** 15.2 mm, MIC 0.5 mg/mL
- ***P. aeruginosa*:** 13.8 mm, MIC 1.0 mg/mL
- ***C. albicans*:** 16.4 mm, MIC 0.5 mg/mL

3.2 Chitin showed minimal antimicrobial activity, confirming that deacetylation is essential for this property.

4. Chitosan Film Properties

4.1 Chitosan films (2% w/v, plasticized with glycerol, crosslinked with TPP) exhibited:

- **Tensile strength:** 24.5 ± 1.8 MPa (adequate for handling)
- **Elongation:** 32.4 ± 2.1% (flexible)
- **WVTR:** 1850 ± 85 g/m²/day (optimal moisture balance)
- **Swelling ratio:** 450 ± 25% (high exudate absorption)
- **Biodegradation:** 65% in 14 days (matches wound healing timeline)

5. *In vivo* Wound Healing

5.1 In the rat excision wound model, chitosan film significantly accelerated wound healing:

- **Wound contraction:** 98.2% by day 14 (vs. control 72.4%, $p<0.001$)
- **Epithelialization time:** 12.4 days (vs. control 18.6 days, 33% faster)
- **Hydroxyproline content:** 48.6 mg/g (100% higher than control)

5.2 Histopathology confirmed that chitosan film promoted:

- Complete re-epithelialization (98%)
- Dense, organized collagen deposition (Masson's trichrome)
- Numerous new blood vessels (neovascularization)
- Minimal inflammation

5.3 Chitosan film was superior to the marketed product Betadine® (povidone-iodine) in all parameters.

6. Final Statement

Dead adult houseflies (*Musca domestica*) are a sustainable, low-cost, and high-quality source of chitin and chitosan. Housefly-derived chitosan films demonstrate excellent physicochemical properties, antimicrobial activity, and wound healing efficacy comparable to (and in some aspects superior to) commercial products. This waste-to-wealth approach addresses the limitations of crustacean-derived chitosan (shellfish allergy, seasonal availability, environmental concerns) while providing an eco-friendly solution for utilizing insect biomass. Housefly-derived chitosan is a promising alternative biomaterial for wound dressing formulations.

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Conflict of Interest Statement

The authors declare no conflicts of interest. No commercial entity sponsored or influenced the research. The authors have no financial or personal relationships that could inappropriately influence this work. The isolated chitin and chitosan are not patented.

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