Research Article

Formulation of Insect Chitosan Stabilized Silver Nanoparticles with Propolis Extract as Potent Antimicrobial and Wound Healing Composites

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Skin wounds are frequently influenced with microbial infections and inflammation, which need innovative agents for disputing them. Chitosan (Csn) was extracted from larvae of BSF “black soldier fly, *Hermetia illucens*” and ethanolic propolis extract (Pro) was employed for synthesizing silver nanoparticles (Ag-NPs), using facile biogenic protocol. The BSF-Csn was acquired with a yield of 1.56%, 91.3% deacetylation degree, and 88.600 Dalton molecular weight. The Ag-NPs were effectually biosynthesized using Pro, with a mean diameter of 8.73 nm and zeta potential of -21.34 mV. The antimicrobial activities assessment of insect Csn, Pro, synthesized Ag-NPs with Pro, and their composite against skin pathogens (*Staphylococcus aureus* and *Candida albicans*) revealed the elevated efficiency of the individual agents and the superior action of their composite (Csn/Pro/Ag-NPs), with 26.3 and 23.4 mm inhibition zones and inhibitory concentrations of 35.0 and 45.0 $\mu\text{g/mL}$ from the composite toward *S. aureus* and *C. albicans*, respectively, which exceeded the actions of commercial antibiotics. The treatment of rat’s wounds with this composite promisingly led to faster healing of wounds and absence of inflammation and infection signs. The powerful actions of Csn/Pro/Ag-NPs as antimicrobial and wound healing composite strongly advocate their applications for skin protection, disinfection, and regeneration.

1. Introduction

Chitosan (Csn), the derived amino-polysaccharide from chitin deacetylation, is a superior biopolymer that has numerous applications in the biological and medical fields. Accordingly, more Csn extraction processes were greatly required from any potential source, including crustacean wastes, fungi, plants, or insects [1]. Crustacean are the foremost sources for Csn acquiring, but due to their seasonal limited supply, the Csn extractions from fungi and insects were explored as promising alternative sources [2]. The estimation of insect species number is ranging from 6 to 10 million, which exceeds all other animal species together [3]. The BSF could be cultivated for utilization as rich protein sources in animal, poultry, and fish feeding; these insects have also remarkable amounts of chitin in their exoskeleton, which are usable for

Csn extraction [4, 5]. The Csn extraction was exploited from many insects, including *Apis mellifera*, *Calosoma rugosa*, *Schistocerca gregaria*, and *Hermetia illucens* “BSF, Black soldier fly” [2, 5–7]. The Csn bioactivities were recurrently verified as promising antimicrobial, clarifying, biochelating, antioxidant, antifungal, and healing accelerator agent [7–12]. The conjugating of Csn with other bioactive compounds and nanomaterials was proposed to augment its biological activity, e.g., antifungal and anticancerous activity [13, 14].

The nanotechnology and nanomaterials’ applications in human daily life became an actuality, including their increasing usage in biomedical and health-related fields [15]. The nanomaterials’ wide-ranging applications enforced the investigations for more efficient and ecofriendly methods to synthesized/manipulate these nanoforms [16]. The conventional synthesizing protocols (physical and chemical) have

several limitations in metal nanoparticles preparation, e.g., toxic chemicals, high-energy requirements, and expensive downstream processes [17].

The “Green synthesis/biosynthesis” strategies involve the employment of environmentally compatible agents/compounds from biological sources (plants, bacteria, biopolymers, fungi, etc.) for nanoparticles’ synthesizing [16]; these auspicious strategies have many advantages over conventional methods, i.e., they produce speedy, eco-friendly, cost-effective, high yield, and safer nanoparticles [15].

Silver nanoparticles (Ag-NPs) are form the leading studied nanometals that have elevated potentialities for applying as powerful antimicrobial, anticancer, antioxidant, and wound-healing accelerator agents; they could be effectually synthesized via biogenic protocols, which augment their eco-friendly characteristics, biocompatibility, and safety toward human cells [18–24].

Numerous reports employed plant extracts/derivatives for synthesizing and stabilizing Ag-NPs, with elevated microbial-inhibition properties [17, 19, 25, 26]; the plants phytochemicals were serving as reducing/capping agents while reacting with AgNO_3 (the commonly exercised precursor for Ag-NPs synthesis), which can effectually increase the functionality, bioactivity, and eco-friendly attributes of the protocol.

Propolis (Pro) is the natural beehive resinous composite that is collected from exudates and buds of diverse plants by honeybees, amalgamated with wax, pollen and bees’ enzyme. Pro exhibited numerous bioactive compounds and bioactivities and was effectually employed in plentiful pharmacological and biomedical applications [27], including its powerful antimicrobial action, inflammatory, antioxidant, and wound healing potentiality [28–30]. The bee Pro could efficiently synthesize metals’ NPs (e.g., silver, gold, and selenium), stimulated by Pro content from biomolecules like flavonoids, alkaloids, phenols, terpenoids, and steroids [31–33].

Wound healing comprises complicated multistep processes affected with varied external and internal factors; skin injury is regularly accompanied by microbial infections and apparent inflammation [34]; these added aspects direct to further required therapies with an extended duration that include antimicrobial and anti-inflammatory agents. The misuse/overuse of antibiotics instigated the emergence of multiple resistant microbial pathogens that require innovative antimicrobial agent/compounds for their fighting [30].

The healing process of wounds comprises six steps: [1] inflammation, [2] cell’s migration, [3] angiogenesis, [4] provisional matrix synthesis, [5] collagen deposition, and [6] reepithelization [35]. The Pro-based formulation proved its efficacy in reducing wounded areas, accelerating reepithelization, stimulating cellular proliferative, and decreasing healing time of cutaneous wounds [36, 37].

The current plan involved the extract insect Csn from BSF, to synthesized Ag-NPs using Pro extract, to evaluate their potentiality as antimicrobial candidates against skin pathogens and their capability for accelerating wound healing in rats.

2. Materials and Methods

2.1. Chitosan Extraction and Characterization. BSF “*Hermetia illucens* larvae” were achieved at their fifth instar from the “Experimental insect farm, University of King Abdulaziz, Jeddah, KSA.” The majority of BSF oils and proteins were eliminated by oil-press process, and the insects’ residues were washed with deionized water (DW) and lyophilized [3, 6]. The Csn extraction processes involved washing with DW and air drying after each step, which included (1) defatting with 10 folds (v/w) from methanol-chloroform mixture (3 : 7, respectively) and stirring for 4.5 h at $25 \pm 2^\circ\text{C}$; (2) demineralization, using 2% HCl solution (12 folds, v/w) at $25 \pm 2^\circ\text{C}$ for 125 min; (3) deproteinization, with 10 folds (v/w) from NaOH solution (1.1 M) at $50 \pm 2^\circ\text{C}$ for 150 min; and lastly, (4) deacetylation, with 15 folds (v/w) from concentrated NaOH solution (60% w/v), at $25 \pm 2^\circ\text{C}$ for 35 min then heating to 110°C for 130 min. The resulted Csn was neutralized via repeated washing with DW then lyophilized.

2.2. Propolis Extraction. Collected propolis from apiaries in the Saudi Arabia southwestern regions was employed for extraction, which was conducted with 10 folds (v/w) from 70% methanol at $27 \pm 2^\circ\text{C}$ for 40 h, under shaking (220 x g). The extracted solution was statically kept at 4°C for further 24 h to precipitate propolis wax [27]. The solution was filtered, centrifuged (to eliminate the precipitated materials), and subjected to vacuum evaporation (Buchi, Flavil, Switzerland) at 42°C until dryness. The dried propolis extract (Pro) was redissolved in aqueous ethanol (40%) to have 10% concentration (w/v) [38].

2.3. Ag Nanoparticles Synthesis with Propolis Extract. Pro was diluted to 0.1% (w/v) using DW, and the solution pH was adjusted to 9.6 using NaOH solution (1.0 M). The AgNO_3 solution (0.1 M) was prepared in DW and 500 μL from it was slowly dropped into 10 mL of Pro solution with speed stirring at $40 \pm 2^\circ\text{C}$. The color change to deep brown rapidly initiated (within 5 min of combination), which indicated Ag-NPs formation [31]. The produced Pro/Ag-NPs solution was then lyophilized and subjected for analysis.

2.4. Products’ Physiognomies Characterization

2.4.1. UV-Visible (UV-vis) Analysis. The absorbance spectrum of Pro/Ag-NPs solution was spectrophotometrically measured (UV-2450, Shimadzu, Japan), at 200–800 nm range.

2.4.2. FTIR “Fourier-transform infrared spectroscopy” Analysis. The FTIR spectra of produced Pro, insect Csn and their composite were screened using FTIR spectrophotometer (JASCO 4100, Japan), at $450\text{--}4000\text{ cm}^{-1}$ wavenumber range. Homogeneous powdered materials, blended with KBr (potassium bromide), were examined and their transmittance spectra were plotted.

2.4.3. TEM “Transmission electron microscopy” Imaging. The size, structure, and morphology of the Pro-biosynthesized Ag-NPs were depicted using TEM “JEM-2100, JEOL, Japan”, operated at 180 kV accelerating voltage and after sonication

of NPs solution in DW for 15 min, mounting onto grids of carbon-coated copper vacuum drying.

2.4.4. XRD “X-Ray Diffraction” Analysis. XRD analysis for Pro-biosynthesized Ag-NPs for purity measurements was made through a diffractometer (XRD-6000, Shimadzu, Japan), applying Cu- k_{α} radiation ($\lambda = 1.541 \text{ \AA}$) at 30 mA and 40 Kv in $10\text{--}80^{\circ}$ range of 2θ .

2.4.5. Particle Size (Ps) Distribution and Zeta Potential (ζ) Determination. The surface charges (zeta potential, ζ) of Pro-biosynthesized Ag-NPs and their Ps distribution were analyzed using zeta plus (Brookhaven, USA).

2.5. Antimicrobial Potentiality Evaluation. The antimicrobial potentialities of insect Csn, Pro, Pro/Ag-NPs, and their composites were qualitatively/quantitatively evaluated against the challenged skin pathogens, i.e., *Candida albicans* (ATCC-10231) and *Staphylococcus aureus* (ATCC-25923), as they are from the most invasive microbial pathogens to skin. The used media for maintaining and challenging microorganisms were Sabouraud Dextrose Broth/Agar (SDB and SDA, Merck, Germany) for the mycotic strain (*C. albicans*) and Nutrient Broth/Agar (NB and NA, Sigma-Aldrich, St. Louis, MO) for the bacteria strain *S. aureus*, respectively. The microorganisms were propagated and challenged aerobically at $37 \pm 1^{\circ}\text{C}$.

2.5.1. Disc Diffusion. The qualitative assay, measuring appeared inhibition zones (IZ) following disc diffusion method, was conducted via plating microbial cultures onto appropriate solid media and positioning filter paper discs (6 mm diameter) that impregnated with $30 \mu\text{L}$ from each agent solution (with a concentration of 10%, w/v) onto the surface of inoculated plates. After incubation for 18–24 h, the appeared IZ around the discs was measured. Nystatin and Vancomycin were employed as standard antibiotics for comparison, using the same challenging conditions.

2.5.2. MIC “Minimum inhibitory concentration” Assay. The elucidated microdilution technique was followed for assessing the MICs of Csn, Pro, Pro/Ag-NPs, and their composites toward tested skin pathogens [8], using TTC “Triphenyl tetrazolium chloride, Merck, Germany” as an indicator for microbial survival. The successive concentration of 10–200 $\mu\text{g/mL}$ from each agent in broth media (in 96 well microplates) was fixed and inoculated with $\sim 10^7$ CFU/mL from microbial cells, with the addition of 0.5% TTC. Samples from the colorless wells were subsequently plated onto agar media and incubated to verify the microbicidal action. The MICs were specified as the minimum concentration of a particular agent that prohibited microbial survival in microplates and on agar plates.

2.5.3. SEM “Scanning electron microscopy” Imaging. The SEM (Hitachi S-500, Japan) micrographs were captured for the exposed microbial cells, *C. albicans* and *S. aureus*, to Pro/Ag-NPs (at a concentration of $45.0 \mu\text{g/mL}$ in broth media), to elucidate the structural alterations in cells after treatment for 0, 4, and 8 h.

2.6. Wound Healing Potentiality of Compositated Materials. Young Wistar healthy rats (average weight between 141 and 164 g) were individually housed under maintained conditions “ $25 \pm 2^{\circ}\text{C}$; 12 h of light-dark cycle; $65 \pm 3\%$ relative humidity” in polyethylene clean cages. Animal’s handling and practical experiments were implemented following the guidelines of “Saudi Ethical Committee for the care and use of laboratory animals” with the aid of two veterinarian technicians. The experiment period lasted for 14 days, with rats feeding on a customary pellet diet and free water access. After anesthesia induction via ketamine intramuscular injection (100 mg/kg body weight), wounds with semicircular areas of $\sim 65 \text{ mm}^2$ were made on shaved rats’ thoracic area. From the wounding day (day 0), the experimented composite [Csn (1%) + Pro/Ag – NPs (0.1%), dissolved in DW] was topically smeared every 12 h until complete epithelization (the Ag-NPs color intense became lesser after conjugating with Csn and Pro solutions). Wounds were digitally photographed daily, after precise cleansing of the wounded area with sterile saline solution (0.9% NaOH); the reductions and manifestation of wounded areas were appraised from the captured photographs.

2.7. Statistical Analysis. Trials were mostly triplicated; their means and SD “standard deviation” were computed (by Microsoft Excel 2016). T-test and One-way ANOVA were applied for statistical significance computation using the MedCalc software (V. 18.2.1, Mariakerke, Belgium) at $p \leq 0.05$.

3. Results and Discussion

Csn was promisingly extracted from BSF larvae with a final yield of 1.56%. Table 1 illustrated the obtained yields after each process in Csn extraction. The physiochemical attributes of BSF-Csn were the DD of 91.3%, a MW of 88.600 Dalton. Produced Csn had 97.8% solubility in 1% AC “acetic acid solution”, without heating.

The BSF-derived chitin and Csn were reported as novel sources for these valuable compounds [4]; BSF requires specific extraction processes, e.g., defatting, due to their high contents of lipids [5]. The fifth instar in BSF larvae development was highly advised for Csn extraction, as after this stage, more pigments and melanin are found in covalently binding with chitin, which leads to poor color attributes for the product [4, 7].

The BSF-Csn spectrum (Figure 1-Csn) indicated typical bonds of standard Csn, illustrated in literature. The main designative bands were detected at 3456 cm^{-1} (NH_2), 2311 cm^{-1} (C—H), 1635 cm^{-1} (amide I, C=O), 1402 cm^{-1} (amide III, C—N), 1112 cm^{-1} (C—O), and 896 cm^{-1} (C—H), respectively. Additionally, the C—H stretching vibrations and C—O bending were also observed at 2879 cm^{-1} and 1069 cm^{-1} , respectively. The detected designative bands in BSF-Csn here are in accordance with former modern investigations concerned insect Csn extraction and characterizations from different types included mealworm, cicada slough, beetles “*Calosoma rugosa*,” desert locust “*Schistocerca gregaria*,” honey bee “*Apis mellifera*,” grasshopper, silkworm chrysalis, and BSF [2, 3, 7].

TABLE 1: The obtained yields throughout insect chitosan extraction.

Extraction process	Weight after process*	Yield from previous row material (%)	Yield from initial row material* (%)
Oil press	43.2	8.64	8.64
Defatting	41.6	96.43	8.32
Demineralization	23.8	57.21	4.76
Deproteinization	11.6	48.57	2.32
Deacetylation	7.8	67.42	1.56

* The initial raw material weight was 500 g of complete insects.

FIGURE 1: FTIR spectra of insect chitosan (Csn), propolis extract (Pro), and their composite (Pro/Csn).

For Pro (Figure 1-Pro), 889 cm⁻¹ (C–H bonds of phenolic rings), 1151 and 1282 cm⁻¹ (stretched C–O and C–N bond of amino acids aromatic), 1621 cm⁻¹ (–COO⁻ carboxylate anion), 1654 and 2971 cm⁻¹ (C=O of flavonoids), 2924 cm⁻¹ (stretched CH₂ vibrations of phenolics), and broadband around 3432 cm⁻¹ (Free N–H) [33, 39, 40].

Regarding the Csn/Pro composite spectrum (Figure 1-Pro/Csn), the interactions between Csn and Pro were evidently confirmed from appeared bands in the composite that belong to both constituting agents (indicated with vertical blue lines in figure).

The Ag-NPs were efficaciously synthesized using Pro extract, as evinced from their color changing from bale white to deep brown, after interaction with Pro solution (Figure 2-upper part). The observable color change after mixing of Pro and AgNO₃ solutions advocated the Ag⁺ reduction to NP form that have free electrons and SPR “surface plasmon resonance” excitation absorption [41].

The UV-vis absorption spectrum of Pro-synthesized Ag-NPs revealed a distinctive apparent peak at 422 nm (Figure 2-lower part), which belong to the designative SPR for biogenic synthesized Ag-NPs (within 220–425 nm), performed formerly using varied plant extracts [19, 22, 25]. They reported also that SPR absorption band frequency/width are princi-

FIGURE 2: Visual guise and optical density of propolis extract and propolis-synthesized Ag-NPs.

pally based on NPs size, shape, dielectric constants “the composition of the particles and surrounding medium” [41]. The detection of single SPR band frequently indicates the NPs spherical shape, while $\geq$ two SPR bands are mostly corresponding to anisotropic particles [42]. As the UV-vis spectroscopy is frequently distinguishing size-controlled NPs and their shapes in aqueous solutions, the current pattern could indicate the capability and efficiency of Pro solution to reduce Ag to Ag-NPs and advocate the phytosynthesis of NPs [31].

The structural analysis of Pro-synthesized Ag-NPs revealed that NPs were well-distributed and spherically shaped (Figure 3(a)). The NPs diameter size ranged from 3.89 to 24.12 nm, with a mean diameter of 8.73 nm and median diameter of 8.42 nm. Additionally, the Pro-synthesized Ag-NPs had a ζ value of -21.34 mV.

The XRD analysis of phytosynthesized Ag-NPs disclosed clear peaks' existence at $2\theta = 38.13^\circ$, 44.28° , 64.61° , 77.42° , and 81.84° , which are accredited to (111), (200), (220), (311), and (222) reflections of fcc “face-centered cubic”, respectively, indicating metallic Ag structure (Figure 3(b)). These attributes were formerly indicated for the biogenic synthesized Ag-NPs, especially using plants' extracts and phytochemicals [20, 22, 26, 43].

The surface of Ag-NPs was suggested as frequently carriers for negative charges, when dispersed in medium; these negative values reinforce the NPs repulsion and elevated stability [26].

The XRD pattern (Figure 3(b)) evidently exhibited the Ag-NPs crystalline nature, as expected from these biosynthesized NPs [22]. As the appeared peaks markedly indicate the NPs cubic crystallinity [20], Pro proved its capability as a promising candidate (from waste materials) for efficient Ag-NPs development.

The antimicrobial potentialities of insect Csn, Pro, synthesized Ag-NPs with Pro, and their composite against skin pathogens (*S. aureus* and *C. albicans*), comparing with customary (vancomycin and nystatin), are demonstrated in Table 2. The microbial inhibitory activities were observable for all examined agents; the most forceful treatment was the combined Csn+Pro+Ag-NPs, as it exhibited the least MIC

FIGURE 3: TEM depiction (a) and XRD analysis (b) of phytosynthesized Ag-NPs with propolis extract.

TABLE 2: Antimicrobial potentialities* of insect chitosan, propolis extract, synthesized Ag-NPs with propolis extract, and their composite against skin microbial pathogens.

Agents	<i>Staphylococcus aureus</i>		<i>Candida albicans</i>	
	IZ (mm)**	MIC ($\mu\text{g/mL}$)	IZ (mm)	MIC ($\mu\text{g/mL}$)
Chitosan	14.6 ± 2.1^a	85.0	9.9 ± 1.8^a	92.5
Propolis extract	17.3 ± 3.3^a	75.0	11.7 ± 2.1^a	85.0
Propolis/Ag-NPs	24.6 ± 3.2^b	37.5	19.1 ± 2.5^b	45.0
Chitosan/Propolis/Ag-NPs	26.3 ± 4.8^b	35.0	23.4 ± 2.2^c	45.0
Vancomycin	23.5 ± 3.7^b	40.0	ND	ND
Nystatin	ND	ND	19.8 ± 3.3^b	52.5

*IZ: appeared inhibition zones included diameter of assay disc; MIC: "minimum inhibitory concentrations." ** Within one column, dissimilar letters (superscript) indicate significant differences.

values and the largest ZOI diameters toward both microorganisms. The effect of Ag-NPs was very noticeable for inhibiting skin pathogens; the antibacterial actions (against *S. aureus*) of examined agents were more effectual than their antimycotic actions (against *C. albicans*).

The antimicrobial powers of phytosynthesized Ag-NPs were proved toward numerous pathogens; the assumed mechanisms depended on the NPs ability to attach and penetrate into microbial cells, disturb membrane permeability and interrupt respiration functions, disrupt DNA replication, and ATP production [17, 20, 22, 44, 45].

The observation of SEM micrographs of treated microorganisms with Pro-synthesized Ag-NPs appointed the consequences of NPs treatment on microbial topography and structure (Figure 4). The control cells in the initiation of exposure, i.e., *C. albicans* (Figure 4-C0) and *S. aureus* (Figure 4-S0), appeared with healthy normal structures and utterly smooth intact membranes. After 4 h of exposure (Figure 4-C4 and S4), the interactions between NPs and microbial cells' walls were noticeable, with the appearance of partial cell lysis, especially in *S. aureus* cells. The extension of NPs exposure to 8 h lead to complete cell lysis in *S. aureus*

cells (Figure 4-S8) that released their interior contents and exploded, whereas the *C. albicans* cells were obviously deformed and many lysis signs were appeared in this stage (Figure 4-C0).

The attachments of Ag-NPs to microbial surfaces are mostly due to electrostatic attraction [46]; this nanoparticulate attribute was proposed as the leading basis for NPs bactericidal action [47]. Due to high electrostatic affinity and attractions of Ag^+ ions towards sulfur proteins in cells' surface, the NPs can frequently adhere to cells' cytoplasm and walls, upsurge membranes' permeability and elasticity, which derive microbial casings' disruptions [23, 24]. Once the Ag^+ ions get uptaken inside microbial cells, the deactivations of respiratory enzymes are occurred, leading to elevated ROS production and ATP release interruption [17]; these ROSs are aggressive factors for enforcing cellular membranes' disruption and DNA distortions. Furthermore, via denaturing the ribosomal cytoplasm components, Ag^+ ions could competently hinder cellular protein synthesis [48]. Conversely, the biosynthesized Ag-NPs were reported to have elevated biocompatibility and minor toxicity toward mammalian natural cells, e.g., RBCs "red blood cells," PBMCs "peripheral

FIGURE 4: SEM micrographs of exposed *Candida albicans* (C) and *Staphylococcus aureus* (S) cells to synthesized Ag nanoparticles with propolis extract for 0, 4, and 8 h.

blood mononuclear cells,” and HEK “human embryonic kidney,” which advocated their practical biomedical applications [23, 44, 45].

Ag-NPs themselves can eradicate exposed microbes; the cumulative NPs trigger cell's membranes denaturation and can permeate through these membranes to modify their structural arrangements [24]. The interior cell's organelles could be also ruptured after membranes' denaturation, and this promotes cell lysis/death [49]. The shape of Ag-NPs was demonstrated for influencing their bactericidal power [50], and the spherical shapes with tinier sizes provide more reactive contact facets that strengthen NPs activity [21].

The skin wounds' treatment with Csn/Pro/Ag-NPs composite promoted faster healing of wounded rats' skin during 14 days of local treatment (Figure 5). For the untreated group (Figure 5-control), no complete healing was observed after 14 days, and the mean wound size reduced from 65.4 mm^2 to 59.8 , 41.3 , 28.5 , 22.7 , 18.2 , and 6.84 mm^2 after 4, 6, 8, 10, 12, and 14 days, respectively. The healing signs appeared much faster in the treated group with Csn/Pro/Ag-NPs composite (Figure 5-treated); the wounded parts became mostly healed after 12 days of treatment.

The composite-treated wounds had no signs of microbial contamination, pus formation, or bleeding throughout treatment, whereas untreated wounds exhibited remarkable inflammation and infection signs. By the 4th day onwards, Csn/Pro/Ag-NPs-treated wounds showed notable wound size reduction and distinguished closure, which were further progressed in the subsequent days of treatment.

FIGURE 5: Consequence of treatment rats' skin wounds with chitosan/propolis extract/Ag-NPs composite (treated) on the healing rate of wounds during 14 days, compared to untreated (control) wounds.

The selection of *S. aureus* and *C. albicans* as models for skin pathogens based on their hazardous infections and contamination of epidermal wounds/burns, which resulted in many complications and disorders of infected parts [51]. Additionally, the inclusion of both bacterial and fungal skin pathogens in challenging trials can give more reliability and trustworthiness of the effectiveness and applicability of examined agents. Both *S. aureus* and *C. albicans* can possess the antimicrobial resistance, which require innovative microbicidal agents to overcome their skin wounds' infections [52, 53]. The combined presence of the two pathogens could increase their biofilm formation and antimicrobial resistance [54].

S. aureus is from the extremely dangerous pathogenic microorganism that threatens humans; the bacteria habitually located in skin and mucus membranes and could enter the bloodstreams through wounds and skin scratches [55]. In numerous skin and wound infections' studies, involving colonization and resistance characteristics, *S. aureus* was focused and reported to repeatedly cause severe bloodstream infections [51]. *C. albicans* was categorized among the hazardous fungal infections in diabetic ulcers and skin wounds, which remarkably delayed their healing [56, 57].

The usage of natural derivatives for wound treatments, as alternatives to chemotherapy, attained great concerns to manage skin infections and promote its regeneration [34]. Pro-based formulations were proposed as effectual treatments to accelerate the natural reorganization of injured tissues, through augmenting keratinocyte proliferation [58]. Even diabetic wounds were effectually healed with Pro via decrement the levels of MMPs “matrix metalloproteinases” and proinflammatory cytokines, enhancing the deposition of collagen I and VEGF “vascular endothelial growth factor”

in injured skin [28, 59], thus hastening the healing development. The healthy skin could too benefit from Pro protective properties, e.g., its persuasive anti-inflammatory, antimicrobial, photoprotective, and antioxidants activities [37, 60].

The wound size reduction and their closure are principally resulting from the antimicrobial and anti-inflammatory actions of the composite components, i.e., Csn, Pro, and Ag-NPs. The microbicidal activity of Ag-NPs could prevent wound microbial contamination, which consequently enabled tissue integrity restoring and resulted in adequate repairing of injured sites [22]. The Ag-NP potential role in rapid wound healing was reported to have dose-dependent manner with a healthier cosmetic appearance [61]. Besides, by their microbicidal potentials, Ag-NPs displayed positive consequences for decreasing wound inflammation through diminishing mast cell and lymphocyte infiltration and fibrogenic cytokines amendments [62]. Likewise, the potentiality of Ag-NPs in epidermal reepithelialization and dermal contraction throughout the healing process was the supposed factors for reproduction promotion, keratinocytes migration increment, and fibroblasts differentiation into myofibroblasts [18, 44, 61, 63].

Chitin and Csn were supposed to enhance the healing process in wounded skins. The multiple of mono-subunit (N-acetyl glucosamine), in these polymers' composition, is an imperative constituent in dermal tissue and has a vital necessity for repairing scar tissues [10, 64]. The Csn surface has numerous free amine groups, which can conjoin with blood acidic groups and enhance their coagulation [35, 65, 66]. Csn molecules (by their high positive charge) can effectively promote cells' growth and assist thrombosis/blood coagulation [12, 63, 67, 68], which greatly foster damaged tissue repairing.

4. Conclusion

The biosynthesis of Ag-NPs from Pro and extraction of insect Csn from BSF were promisingly attained in the current work. Csn was extracted from BSF "black soldier fly as an unconventional source for this polymer. The Pro-biosynthesis of Ag-NPs applied simple, direct, and eco-friendly protocol that generated the desired size, shape, and distribution of nanoparticles. The composite of these agents (Pro-synthesized Ag-NPs and Csn) exhibited powerful antimicrobial potentialities against skin pathogens (*C. albicans* and *S. aureus*) and high capability for fast wound healing, without the emergence of infection or inflammation signs, which strongly advocate their applications for skin protection, disinfection, and regeneration.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The author declares that they have no conflicts of interest.

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