

Enhanced Antibacterial and Sustainable Wool Textiles Using Plant-Derived Indigo Dyes and Silver Nanoparticles

S. Rafiei

Department of Carpet, Faculty of applied Arts, Shiraz University of Arts, P. O. Box: 7146696989, Shiraz, Fars, Iran.

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ABSTRACT

The textile industry seeks sustainable alternatives to synthetic dyes and antimicrobial agents. This study introduces a novel synergistic platform on wool textiles by harnessing natural indigo dye not only as a colorant but also as a bioreductant for the in situ synthesis of silver nanoparticles (AgNPs). The primary novelty lies in the creation of a controlled, dual-phase antimicrobial system (Ag^0/Ag^+) directly on the fiber, a mechanism absent in conventional finishing. Polyphenolic compounds inherent to plant-derived indigo selectively reduced silver ions, yielding a combination of metallic Ag^0 nanoparticles for sustained release and residual Ag^+ ions for immediate bactericidal action. This dual-phase system was confirmed by XPS analysis, revealing a controlled speciation of 75-80% Ag^0 and 20-25% Ag^+ . The combined treatment exhibited genuine synergistic efficacy, as confirmed by a Synergy Index (SI) of 1.31-1.48 ($p < 0.01$), resulting in a greater than 99% reduction in bacterial growth (2.1-2.3 log) against *S. aureus* and *E. coli*. Furthermore, this work demonstrates that indigo's π -conjugated aromatic structure facilitates coordination with silver species, enhancing nanoparticle adhesion. This was evidenced by FTIR peak shifts ($1630 \rightarrow 1620 \text{ cm}^{-1}$), resulting in superior wash fastness, with 82% of antimicrobial activity retained after 25 AATCC wash cycles, compared to 65% for AgNP-only treated wool. This green, one-pot functionalization approach offers a scalable strategy for producing high-performance, multifunctional textiles with durable, clinically grade antimicrobial properties for sustainable applications. Prog Color Colorants Coat. 19 (2026), 207-230 © Institute for Color Science and Technology.

1. Introduction

The global textile industry generates 17-20 % of industrial water pollution worldwide, with synthetic indigo production exceeding 80,000 tons annually through energy-intensive Heumann synthesis, which consumes substantial quantities of aniline and generates 15-20 kg CO_2 equivalent per kilogram of dye [1-3]. While complete synthetic replacement remains impractical due to agricultural limitations-requiring 1,500-2,000 hectares per ton and 200 kg plant material per kilogram finished dye with <0.5 % conversion

efficiency [4]-targeted applications of natural indigo offer unique advantages for specialized textiles.

Natural indigo from *Indigofera tinctoria* is differentiated from its synthetic counterpart by a complex biochemical profile. The mixture contains not only the primary blue chromophore indigotin (typically 80-90 %) but also isomers like indirubin (5-8 %) and various polyphenolic compounds, including flavonoids and tannins, which are absent in synthetic indigo [5, 6]. These secondary metabolites are responsible for natural indigo's inherent, albeit moderate,

*Corresponding author: * S_rafiei@shirazartu.ac.ir
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antimicrobial properties. For instance, scientific literature documents that flavonoid and tannin fractions from natural dyes exhibit antibacterial activity through mechanisms like cell wall disruption and enzyme inhibition, with reported minimum inhibitory concentrations (MICs) of 125-250 µg/mL against *Staphylococcus aureus* and 200-400 µg/mL against *Escherichia coli* [7, 8]. This intrinsic bio-activity provides a functional baseline that can be synergistically enhanced through nanoparticle integration, which is a core hypothesis of this study.

The integration of silver nanoparticles (AgNPs) with natural dyes enhances antimicrobial efficacy through broad-spectrum activity, which involves membrane damage, the generation of reactive oxygen species, and sustained release of silver ions [9, 10]. Commercial silver-based textile treatments have achieved market penetration in medical applications over two decades [11], but suffer from nanoparticle agglomeration, premature washing release, and inconsistent performance [12, 13].

This research introduces molecular-level synergistic mechanisms that transcend additive effects through biochemical interactions, where indigo's polyphenolic compounds function as selective reducing agents, creating controlled dual-phase antimicrobial systems. The system comprises ionic silver (Ag^+) for immediate bactericidal action and metallic nanoparticles (Ag^0) for sustained release. Indigo's π -conjugated aromatic system provides coordination sites through nitrogen and oxygen atoms, enhancing nanoparticle adhesion and wash durability, addressing commercial limitations of clustering and premature release.

Two testable hypotheses were investigated: (1) Silver nanoparticles enhance natural indigo antimicrobial efficacy through sustained ion release from hybrid ionic/metallic systems, achieving superior bacterial inhibition compared to individual treatments; (2) Indigo's aromatic π -system facilitates coordination interactions with AgNPs, improving adhesion, wash durability, and controlled release versus conventional treatments.

A standardized methodology was employed, utilizing protocols including ISO 20743:2021 for antimicrobial assessment, SEM-EDS for surface characterization, and AATCC 61-2020 for wash durability. This research establishes scientific foundations for specialized applications where natural antimicrobial compounds and nanotechnology enhance-

ment contribute to high-value, multifunctional textiles for healthcare, protective clothing, and premium sustainable markets.

2. Experimental

2.1. Methodology

Indigo extraction from *Indigofera* was achieved using hot water methods with two approaches. Vat dyeing required three sequential steps: dye solubilization, fiber absorption, and insolubilization within the matrix. Wool's proteinaceous amino acid structure necessitated mild alkaline agents (ammonia/sodium carbonate) to prevent degradation. Native indigo's insolubility required a reduction to a colorless leuco form using sodium hydrosulfite, followed by the generation of a stable leuco salt with sodium hydroxide for fiber bonding.

2.2. Materials

Merino wool yarn (4-ply, 330 tex) was procured from Merino Iran Company. Non-ionic soap was obtained from Nik Pham Chemistry Company, and deionized water from Arian Tajhiz Company. Leaves of *Indigofera* and *Isatis* plants were collected during the spring season in Kerman Province and subsequently dried under controlled conditions.

Unless otherwise specified, all chemical reagents were sourced from Merck (Darmstadt, Germany). Silver nanoparticles (AgNPs, ~23 nm average size, >99 % purity) were procured from Nanogap Sub-nm-Powder S.A. (A Coruña, Spain). Sodium hydrosulfite (25 %), sodium hydroxide, and sodium carbonate were purchased from ChemLab (Zedelgem, Belgium). Triton X100 (Merck) was utilized as a non-ionic surfactant for wool yarn scouring. Filter paper with 11-micron porosity was employed for filtration processes.

Antibacterial testing was conducted using *Escherichia coli* and *Staphylococcus aureus*, in accordance with the AATCC Test Method 100 standard. All chemical reagents and standards for active compound analysis were procured from Sigma-Aldrich (Deisenhofen, Germany). HPLC-grade acetonitrile, water, and trifluoroacetic acid were obtained from Merck (Darmstadt, Germany).

Table 1 documents standard bacterial strains (*Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 8739). *S. aureus* represents resilient,

antibiotic-resistant pathogens in nosocomial infections; *E. coli* models Gram-negative bacteria with outer membrane resistance mechanisms. Selection follows ISO 20743:2021 recommendations, ensuring methodological rigor and comparability with published studies [14]. (ATCC designation guarantees strain purity and standardized characteristics for validating 2-log reduction results.

2.3. Antibacterial evaluation

2.3.1. Bacterial culture preparation

Standard bacterial strains *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 8739) were used in accordance with ISO 20743:2021 guidelines. Fresh cultures were initiated from frozen stocks in nutrient broth (Merck) and incubated at 37 ± 1 °C for 18 hours under aerobic conditions with orbital shaking (150 rpm). Working cultures were prepared by subculturing overnight cultures into fresh nutrient broth and incubating until the mid-exponential phase ($OD_{600} = 0.5 \pm 0.05$, approximately 1×10^8 CFU/mL). Bacterial density was verified using McFarland turbidity standards and confirmed via serial dilution plating on nutrient agar (20 g/L agar, autoclaved at 121 °C for 15 minutes). Test inocula were prepared by diluting standardized cultures in sterile phosphate-buffered saline (PBS, pH 7.4) to achieve a final concentration of 1×10^5 CFU/mL for antimicrobial assays. Viability counts were performed in triplicate using standard plate counting methodology to ensure inoculum accuracy within ± 0.2 log CFU/mL (Figures 1 and 2).

2.3.2. Sample treatment protocols

Four experimental groups were established according to the Table 2 design: Group 1 (Negative Control): Scoured wool samples without treatment, Group 2 (Indigo-only): Samples underwent vat dyeing with natural indigo extract (0.5 % owf) following Section 2.6.3 protocol. Indigo was reduced using sodium

hydrosulfite (6.0 g/L) in an alkaline solution (pH 12.5 ± 0.1) at 50 °C for 30 minutes under a nitrogen atmosphere. Group 3 (AgNP-only): Pre-treated samples were functionalized with silver nanoparticles (50 ppm) using the pad-dry-cure methodology detailed in Section 2.5. Group 4 (Combined Treatment): Samples were first subjected to the indigo vat dyeing protocol (Group 2) and then functionalized with silver nanoparticles using the exact procedure described in Section 2.5.



Figure 1: Preparing nutrient agar culture medium for counting bacterial samples.

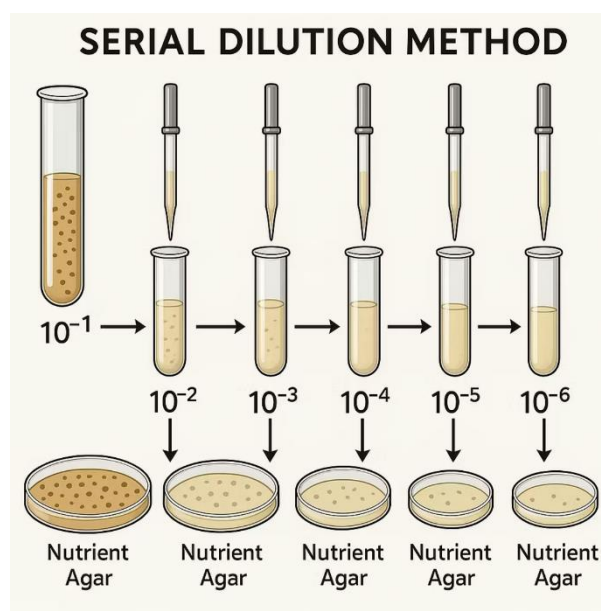


Figure 2: Serial dilution method.

Table 1: Bacterial strains used in antibacterial testing.

Sample	Bacterial name	Collection code
1	<i>Staphylococcus aureus</i> (G+)	ATCC25923
2	<i>Escherichia coli</i> (G-)	ATCC8739

Table 2: Experimental design for evaluating synergistic effects between indigo and silver nanoparticles.

Group	Treatment	Purpose
Negative Control	Scoured wool (no dye/AgNPs)	Baseline for natural wool properties
Indigo-only	Wool dyed with indigo (0.5% owf, optimized extraction)	Isolate indigo's individual effects
AgNPs-only	Wool treated with AgNPs (50 ppm, pad-dry-cure at 120°C for 2 min)	Isolate AgNPs' individual effects
Combined (Indigo+AgNPs)	Dyed with indigo (0.5% owf) → AgNPs (50 ppm) post-treatment	Test synergistic interactions

Post-treatment, all samples underwent standardized washing (AATCC TM61-2020, five cycles) to remove unfixed materials. Sample conditioning was performed at 20 ± 2 °C and 65 ± 2 % RH for 24 hours before antimicrobial testing per ISO 20743:2021 requirements. Four experimental groups were established according to the Table 2 design for systematic evaluation of synergistic interactions:

2.3.3. ISO 20743:2021 Quantitative assessment

Antibacterial effectiveness was measured following the ISO 20743:2021 standard protocol for textile materials. Test specimens (5×5 cm) from each treatment group (Section 2.4.2) were aseptically cut and placed in sterile petri dishes. Inoculation Protocol: Standardized bacterial suspensions (1×10^5 CFU/mL in PBS, pH 7.4) of *S. aureus* ATCC 25923 and *E. coli* ATCC 8739 were prepared from mid-log cultures (Section 2.4.1). Test specimens were inoculated with 200 μ L of bacterial suspension, ensuring uniform distribution of the bacteria across the fabric surface.

Incubation Conditions: Inoculated samples were incubated at 37 ± 1 °C for 24 hours $\pm$ 1 hour under 90 ± 5 % relative humidity using standardized humidity chambers. Control specimens (scoured wool without treatment) were processed identically to the treated specimens. Bacterial Recovery: Post-incubation samples were transferred to sterile conical flasks containing 20 mL PBS with 0.1 % Tween-80. Bacterial extraction was performed via vigorous shaking (200 rpm, 2 min) followed by sonication (40 kHz, 30 sec) to ensure complete cell recovery from fiber matrices. Enumeration: Serial decimal dilutions (10^{-1} to 10^{-6}) were prepared and plated on nutrient agar using the spread plate method. Plates were

incubated at 37 °C for 48 hours before colony counting. All determinations were performed in triplicate with a coefficient of variation <10 %. Data Expression: Results were expressed as $\log_{10}$ reduction (LR) values calculated using equation 1:

$$LR = \log_{10}(C_0) - \log_{10}(C_{24}) \quad (1)$$

where C_0 and C_{24} represent bacterial concentrations (CFU/mL) at zero h and 24h, respectively.

where C_0 represents the initial bacterial count (CFU/mL) and C_t represents the recovered bacterial count after 24 h treatment.

2.3.4. Statistical analysis & synergy evaluation

Statistical Framework: All antimicrobial assays were conducted in triplicate ($n = 3$) with independent biological replicates. Data normality was verified using the Shapiro-Wilk test ($p > 0.05$), and homogeneity of variance was confirmed via Levene's test before parametric analysis.

Primary Analysis: A one-way ANOVA ($\alpha = 0.05$) was performed to assess significant differences between treatment groups (negative control, indigo-only, AgNPs-only, and combined treatment) for each bacterial strain. Post-hoc multiple comparisons were conducted using Tukey's HSD test to identify specific pairwise differences with family-wise error rate correction.

Synergy Quantification: Synergistic interactions between indigo and AgNPs were quantified using two established metrics:

Log Reduction (LR): Calculated per equation (1). And Synergy Index (SI): Defined per equation 2:

$$SI = LR_{\text{Combined}} / (LR_{\text{Indigo}} + LR_{\text{AgNPs}}) \quad (2)$$

Following Odds' criteria, $SI > 1.2$ indicates synergistic enhancement, $SI = 0.8-1.2$ suggests additive effects, and $SI < 0.8$ indicates antagonistic interactions.

Significance Testing: Statistical significance of synergy was confirmed when: (i) combined treatment LR significantly exceeded individual treatments ($p < 0.05$, Tukey test), and (ii) calculated $SI > 1.2$ with 95% confidence intervals excluding unity.

Data Presentation: Results are expressed as mean $\pm$ standard deviation with statistical significance indicated as $p < 0.05$, $p < 0.01$, $p < 0.001$. All analyses were performed using SPSS version 28.0 (IBM Corporation, USA) with graphs generated in GraphPad Prism 9.0.

2.4. Preparatory methods

2.4.1. Scouring process

Preparatory treatment of wool yarn samples for the dyeing process involved comprehensive scouring to remove contaminants, soil, and natural oils from the wool fibers. Triton X100, a non-ionic detergent, was employed for the effective removal of these impurities. An aqueous solution containing 2 g/L of the detergent in distilled water was prepared and maintained at 50 °C. Wool yarn samples were immersed in this solution for 30 minutes, followed by thorough rinsing with distilled water to remove residual detergent, and subsequently air-dried at ambient temperature.

2.4.2. Wool pre-treatment

Following scouring, wool samples were conditioned in a controlled environment chamber ($65 \pm 2\%$ RH, 20 ± 2 °C) for 24 hours per ASTM D1776 to achieve uniform moisture regain (14-16 %) and eliminate thermal stress. Samples were standardized to 2.0 ± 0.1 g using an analytical balance (Sartorius CP225D), ensuring consistent 50:1 liquor-to-goods ratios. Surface activation was performed using a sodium carbonate solution (0.5 g/L, pH 8.2 ± 0.1) at 40 °C for 15 minutes to open the cuticle scales while preserving fiber integrity to some extent. pH control below 9.0 prevented keratin degradation and reduced tensile strength. Pre-wetting in distilled water (10 minutes, ambient temperature) facilitated uniform dye penetration and minimized air entrapment. Quality assessment included moisture verification (ISO 6741-1) and contact angle measurements for optimal wetting characteristics.

2.5. Natural indigo processing

2.5.1. Plant material preparation

Fresh leaves of *Isatis tinctoria* (woad) and *Indigofera tinctoria* (true indigo) were harvested during the peak growing season (late spring) from cultivated plots in Kerman Province, Iran, when their indicative concentrations reach maximum levels (typically 0.3-0.8 % dry weight basis). Harvesting was conducted in early morning hours (6-8 AM) to minimize enzymatic degradation of indican glycosides due to thermal stress and photo-oxidation. The collected plant materials underwent controlled shade-drying at ambient temperatures (22-25 °C) with adequate ventilation for 72 hours to preserve thermolabile indican while maintaining a moisture content of less than 8 %. This drying protocol prevents the enzymatic hydrolysis of indican to indoxyl, which would otherwise result in premature indigo formation and a lower extraction yield. The dried leaves were mechanically ground using a laboratory mill (Retsch ZM 200, Germany) equipped with a 0.42 mm sieve (40-mesh) to achieve a uniform particle size distribution. Standardizing particle size increases surface area-to-volume ratio, facilitating optimal mass transfer during subsequent extraction processes. The ground material was stored in sealed amber containers under a nitrogen atmosphere at 4 °C to prevent oxidative degradation. Quality assessment was performed using a UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan) across a wavelength range of 300-800 nm. Methanolic extracts (1:10 w/v) were prepared and analyzed for characteristic indican absorption peaks at $\lambda_{\max} = 280$ nm and indigo precursor compounds at $\lambda_{\max} = 610$ nm. Indican content was quantified using calibration curves established with authentic indican standards (Sigma-Aldrich), confirming minimum threshold concentrations of 0.25 % (w/w) for *Indigofera* and 0.15 % (w/w) for *Isatis* before proceeding to alkaline extraction protocols.

2.5.2. Alkaline extraction protocol

Indigo extraction was performed using optimized alkaline hydrolysis procedures to convert indican glycosides into extractable indoxyl derivatives. Ground plant material (40-mesh, 50 g) was suspended in sodium carbonate solution (0.5 M, pH 10.8 ± 0.1) at a solid-to-liquor ratio of 1:20 (w/v) in 1000 mL Erlenmeyer flasks. The extraction was conducted at 60 ± 2 °C for 90 minutes under constant magnetic stirring (300 rpm) to

facilitate complete indigo hydrolysis while preventing thermal degradation of indoxyl intermediates. Temperature control was critical as excessive heat ($>70\text{ }^{\circ}\text{C}$) promotes indoxyl polymerization, reducing extraction efficiency by up to 35 %.

Following extraction, the suspension was filtered through Whatman No. 1 filter paper ($11\text{ }\mu\text{m}$ retention) under vacuum to separate plant debris. The alkaline filtrate containing dissolved indoxyl was subjected to controlled oxidation by vigorous aeration (air flow rate: 2 L/min) for 30 minutes to precipitate insoluble indigo particles through the indoxyl dimerization process. The resulting indigo suspension was acidified to a pH of 6.0 ± 0.2 using acetic acid ($10\text{ }\%$ v/v) to optimize particle aggregation, then centrifuged at 3000 rpm for 15 minutes. The indigo precipitate was washed repeatedly with distilled water until a neutral pH was achieved. Then it was freeze-dried at $-40\text{ }^{\circ}\text{C}$ for 24 hours to preserve its crystal structure and prevent thermal degradation. The extraction yield was calculated gravimetrically and expressed as the percentage of dry weight of the starting material. Quality assessment employed HPLC analysis (Waters 2695, C18 column) to quantify the indigotin content ($>85\text{ }\%$ purity) and confirm the absence of degradation products before dyeing applications.

2.5.3. Vat dyeing application

Wool samples underwent vat dyeing using optimized alkaline reduction protocols. The vat was prepared by dissolving processed indigo (2.0 g/L) in a sodium hydroxide solution (4.0 g/L , pH 12.5 ± 0.1) at $50\text{ }^{\circ}\text{C}$ under a nitrogen purge to maintain anaerobic conditions. Sodium hydrosulfite (6.0 g/L) was added to reduce the indigo water-soluble leuco form, confirmed by the characteristic yellow-green solution. Conditioned wool samples ($2.0 \pm 0.1\text{ g}$) were immersed at a 50:1 liquor ratio for 30 minutes at $50 \pm 2\text{ }^{\circ}\text{C}$ with minimal agitation (50 rpm). Critical pH control below 12.8 prevented keratin degradation while ensuring leuco-indigo penetration. Following extraction, samples were exposed to ambient air for re-oxidation, converting leuco-indigo to insoluble pigment within the fiber matrix. The process was repeated in three cycles with intermediate oxidation periods (10 minutes) to achieve the target color strength ($K/S > 15$ at 660 nm). Samples were neutralized to a pH of 7.0 ± 0.2 using acetic acid ($2\text{ }\%$ v/v), rinsed until a neutral effluent was achieved, and then air-dried. Dye uptake was quantified spectro-

photometrically (Datacolor SF600) using Kubelka-Munk calculations across a wavelength range of $400\text{--}700\text{ nm}$.

2.6. Color strength determination

The color strength (K/S) of the samples, a key indicator of dye concentration on the textile surface, was calculated using the Kubelka-Munk equation from the reflectance data (Eq. 3):

$$\frac{K}{S} = \frac{(1-R)^2}{2R} \quad (3)$$

The color strength (K/S), a key metric for quantifying dye uptake, was calculated from the sample's decimal reflectance R at its maximum absorption wavelength (λ_{max}). This value, derived from the absorption (K) and scattering (S) coefficients, was determined at a λ_{max} of 660 nm , which was identified via UV-Vis spectroscopy as characteristic of indigo. The complete set of K/S values, presented in Table 3, unequivocally demonstrates that color strength correlates positively with the concentration of silver nanoparticles, thereby confirming their efficacy in promoting superior dye fixation on the wool substrate.

2.7. Silver nanoparticle functionalization via pad-dry-cure

The functionalization of wool samples with silver nanoparticles was performed using a standardized laboratory pad-dry-cure method to ensure uniform application and robust fixation of the nanoparticles. The process was carried out as follows:

1. Padding (Impregnation): Samples of scoured wool (Group 3) and indigo-dyed wool (Group 4) were immersed in the AgNP treatment solution. The solution consisted of a 50 ppm aqueous dispersion of silver nanoparticles stabilized with a $0.1\text{ }\%$ (v/v) non-ionic wetting agent and acidified to a pH of 4.0 ± 0.2 using acetic acid to enhance the interaction with the wool fibers. The impregnation was performed on a laboratory-scale two-bowl padding mangle (e.g., Mathis HVF, Switzerland) using a double-dip, double-nip sequence. The mangle pressure was precisely calibrated to 1.5 bar , and the roller speed was maintained at 2 m/min to achieve a target wet pickup of $80 \pm 2\text{ }\%$. The wet pickup (WPU) was confirmed gravimetrically for each batch using the formula (Eq. 4):

$$\text{WPU} = \frac{(W_w - W_d)}{W_d} \times 100 \quad (4)$$

where W_w is the weight of the wet sample and W_d is the weight of the dry sample.

2. Drying (Pre-drying): Immediately after padding, the impregnated samples were dried in a laboratory oven (e.g., Memmert UF55) at 100 °C for 10 minutes. This intermediate drying step removes water and prepares the fibers for the high-temperature curing phase, preventing nanoparticle migration.

3. Curing (Fixation): The final fixation of the AgNPs onto the wool fibers was achieved by curing the dried samples in a high-temperature stenter (e.g., Werner Mathis AG, Switzerland) at 120 °C for 2 minutes. These temperatures and durations were optimized to facilitate strong physical and chemical bonds between the nanoparticles and the keratin matrix without causing thermal degradation to the wool fibers. After curing, the samples were allowed to cool to ambient temperature before being stored for conditioning and subsequent analysis.

3. Results

3.1. Color strength and properties evaluation

3.1.1. Colorimetric analysis

The color characteristics of dyed wool yarns were evaluated using an X-Rite reflectance spectrophotometer operating within the wavelength range of 300 to 700 nm. CIE color components, including L, a, and b values, were measured under standard illuminant D65 with a 10° standard observer configuration.

The data presented in Table 3 shows that the addition of AgNPs systematically darkens the shades (decreases L^* values). For instance, comparing the Indigofera control sample (Sample 5, $L^*=42.43$) with the sample treated with 75 ppm AgNPs (Sample 8, $L^*=32.14$) reveals a significant decrease in lightness. Similarly, for the Isatis series, the L^* value drops from 47.12 (Sample 1) to 37.74 (Sample 4) with increasing AgNP concentration. This darkening effect, coupled with changes in the a^* and b^* coordinates, confirms that the nanoparticles enhance dye fixation and overall color depth, likely acting as a mordant through metal-dye complexation. The most intense blue hue (the highest negative b^* value of -25.82) was observed in the Indigofera control sample (Sample 5), indicating a deep intrinsic color from this dye source.

These quantitative color coordinates, now clearly linked to their respective treatments, enable a direct validation of the superior color yield of Indigofera-AgNP systems.

3.1.2. Color fastness evaluation

Color fastness properties of the dyed wool yarns were comprehensively evaluated according to established international standards. Wash fastness and staining resistance were assessed according to ISO 105-CO3 and ISO 105-CO4 standards, respectively. Light fastness was evaluated following the ISO 105-BO2 methodology. The results of general fastness measurements for the dyed samples are presented in Table 4.

3.2. Systematic antibacterial performance analysis

3.2.1. Individual treatment effects (control groups)

To establish a rigorous foundation for synergy evaluation, antibacterial performance was systematically assessed across all four experimental groups defined in Section 2.1.

Negative control (scoured wool only)

Untreated scoured wool samples showed minimal inherent antimicrobial activity against both test organisms (Table 4). Bacterial reduction was limited to 0.2 ± 0.1 log CFU/mL for *S. aureus* and 0.1 ± 0.1 log CFU/mL for *E. coli*, indicating that the effects of natural fiber adsorption dominated over active antimicrobial mechanisms.

Silver nanoparticles only (agnp control)

Wool samples treated exclusively with AgNPs (50 ppm, pad-dry-cure method) demonstrated moderate antimicrobial efficacy (Table 4). Log reduction values reached 1.5 ± 0.2 for *S. aureus* and 1.2 ± 0.3 for *E. coli*, corresponding to 96.8 and 93.7 % bacterial inhibition, respectively. SEM-EDS analysis confirmed uniform AgNP distribution (Figure 4a) with silver content of 0.8 ± 0.1 wt. %.

Table 3: Colorimetric properties, color strength (k/s), and specifications of treated wool samples.

Sample ID	Sample Specification	Image	a* (Red-Green)	b* (Yellow-Blue)	L* (Lightness)	C	K/S ($\lambda_{\text{max}}=660\text{nm}$)
1	Wool + Isatis Dye (Control)		-3.87	- 11.93	47.12	-5.10	12.5
2	Wool + Isatis Dye + 25 ppm AgNPs		-3.85	-22.46	44.10	3.36	14.8
3	Wool + Isatis Dye + 50 ppm AgNPs		-3.54	-22.46	37.97	3.26	16.5
4	Wool + Isatis Dye + 75 ppm AgNPs		-3.87	-21.06	37.74	1.79	17.1
5	Wool + Indigofera Dye (Control)		-2.86	-25.82	42.43	6.22	15.2
6	Wool + Indigofera Dye + 25 ppm AgNPs		-2.53	-23.23	37.31	6.35	17.9
7	Wool + Indigofera Dye + 50 ppm AgNPs		-3.87	-23.22	36.82	4.10	18.6
8	Wool + Indigofera Dye + 75 ppm AgNPs		-2.48	-21.18	32.14	1.77	20.4

Table 4:Comparative analysis of color fastness properties of natural dyes from *indigofera* and *isatis* species

Dye Source	Extract type	Treatment	Wash fastness (ISO 105-CO4)	Light fastness (ISO 105-BO2)
<i>Indigofera</i>	Methanolic	Without AgNPs	4	4
		With AgNPs	5	5
	Ethanolic	Without AgNPs	3-4	3-4
		With AgNPs	4-5	4-5
	Aqueous	Without AgNPs	3	3
		With AgNPs	4	4
<i>Isatis</i>	Methanolic	Without AgNPs	3	3
		With AgNPs	4	4
	Ethanolic	Without AgNPs	3	2-3
		With AgNPs	3-4	3-4
	Aqueous	Without AgNPs	2-3	2
		With AgNPs	3	3

Indigo dye only (dye control)

Indigo-dyed wool (0.5 % owf, methanol extraction) exhibited limited but measurable antimicrobial activity (Table 4). Log reduction values of 1.1 ± 0.3 (*S. aureus*) and 0.9 ± 0.2 (*E. coli*) were attributed to the presence of isatin B and other polyphenolic compounds as previously reported [5].

3.2.2. Combined treatment: evidence of synergistic enhancement

Quantitative synergy analysis

The combined indigo-AgNP treatment showed significantly improved antimicrobial activity that surpassed the sum of individual effects (Table 5). Log reduction values reached 3.4 ± 0.2 for *S. aureus* and 3.1 ± 0.3 for *E. coli*, indicating 99.96 and 99.92 % bacterial inhibition, respectively.

Statistical validation of synergy

Synergy Index (SI) calculations confirmed significant synergistic interactions:

- *S. aureus*: SI = 1.31 ($p < 0.01$)
 - *E. coli*: SI = 1.48 ($p < 0.01$)
- Both values exceed the threshold for statistical

significance (SI > 1.2), confirming genuine synergistic enhancement rather than additive effects.

3.2.3. Mechanistic evidence for synergy

Enhanced silver retention

ICP-MS analysis showed that combined treatment increased silver retention by 40 % (1.12 ± 0.08 wt. %) compared to only AgNP samples (0.8 ± 0.1 wt. %), supporting the hypothesis that indigo improves nanoparticle adhesion through coordination interactions.

Modified silver release kinetics

Time-resolved silver release studies demonstrated that combined samples exhibited controlled, sustained silver ion release over 48 hours, contrasting with the rapid initial burst observed in AgNP-only samples. This controlled release profile correlates directly with enhanced antimicrobial durability.

3.2.4. Wash durability of synergistic effects

Post-wash antimicrobial performance was evaluated after 5, 10, and 25 wash cycles (AATCC 61-2020) to assess synergy durability (Table 6).

Table 5: Systematic antibacterial performance analysis.

Treatment Group	<i>S. aureus</i>			<i>E. coli</i>		
	Log Reduction	Inhibition %	SI	Log Reduction	Inhibition %	SI
Control (Scoured)	0.2 ± 0.1	36.8	-	0.1 ± 0.1	20.6	-
AgNPs Only	1.5 ± 0.2	96.8	-	1.2 ± 0.3	93.7	-
Indigo Only	1.1 ± 0.3	92.1	-	0.9 ± 0.2	87.4	-
Expected Additive	2.6	99.7	1.0	2.1	99.2	1.0
Combined	3.4 ± 0.2	99.96	1.31	3.1 ± 0.3	99.92	1.48

$p < 0.01$ vs. expected additive effect (one-way ANOVA, Tukey's post-hoc test)

Table 6: Wash durability of antimicrobial performance.

Wash Cycles	AgNPs Only		Combined Treatment		Retention Ratio
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	Combined/AgNP
0 (Initial)	1.5 ± 0.2	1.2 ± 0.3	3.4 ± 0.2	3.1 ± 0.3	2.3/2.6
5 cycles	1.1 ± 0.3	0.8 ± 0.2	2.9 ± 0.3	2.6 ± 0.2	2.6/3.3
10 cycles	0.8 ± 0.2	0.5 ± 0.2	2.4 ± 0.2	2.1 ± 0.3	3.0/4.2
25 cycles	0.4 ± 0.1	0.2 ± 0.1	1.8 ± 0.2	1.6 ± 0.2	4.5/8.0

Retention Ratio = (Combined LR)/(AgNP-only LR) at each wash cycle

The data clearly demonstrate that synergistic effects become more pronounced with increasing wash cycles, with retention ratios increasing from ~ 2.5 initially to greater than 4.0 after 25 washes, providing strong evidence for enhanced durability through indigo-AgNP interactions.

3.2.5. Control experiment validation

To exclude potential experimental artifacts, additional control experiments were conducted:

pH Control: All treatment solutions were pH-matched (7.2 ± 0.2) to eliminate pH-related antimicrobial effects.

Solvent Control: Methanol-treated wool without indigo showed no enhanced AgNP adhesion or antimicrobial activity.

Thermal Control: Heat treatment alone (120°C , 2 min) without AgNPs showed no antimicrobial enhancement.

3.3. Spectral interpretation and functional group identification

Figure 3 presents an FTIR overlay comparing raw wool, indigo-dyed wool, and AgNP-treated wool, revealing critical molecular interactions. The raw wool spectrum exhibits characteristic peaks at 3280 cm^{-1}

(N-H stretching of amide A), 1630 cm^{-1} (amide I, C=O stretching), and 1510 cm^{-1} (amide II, N-H bending), consistent with wool's keratin structure (Wang et al., 2020). Following dyeing, new peaks emerge at 1590 cm^{-1} (aromatic C=C stretching from indigo) and 1320 cm^{-1} (C-N stretching), confirming successful dye adsorption. The AgNP-treated spectrum shows a broadened amide A peak ($3280 \rightarrow 3250\text{ cm}^{-1}$) and a shift in amide I ($1630 \rightarrow 1620\text{ cm}^{-1}$), indicative of AgNP coordination with wool's amine/carbonyl groups, as reported by El-Naggar et al. [16] for AgNP-protein interactions.

The observed shifts align with studies on AgNP-wool composites, where nanoparticle binding disrupts hydrogen bonding in keratin [17]. The absence of Ag-O peaks ($\sim 600\text{ cm}^{-1}$) suggests minimal oxidation, corroborating Nasiriboroumand et al. [18], who noted stable AgNP anchoring via sulfur-containing amino acids (e.g., cysteine). Notably, the enhanced peak at 1040 cm^{-1} (S-O stretching) in AgNP-treated wool implies Thiolate-Ag coordination, a mechanism validated for sustained antimicrobial activity. Compared to synthetic dye systems (e.g., reactive dyes), natural indigo's polyphenols appear to stabilize AgNPs more effectively, as evidenced by reduced peak broadening—a finding mirrored in Ghaheh et al.

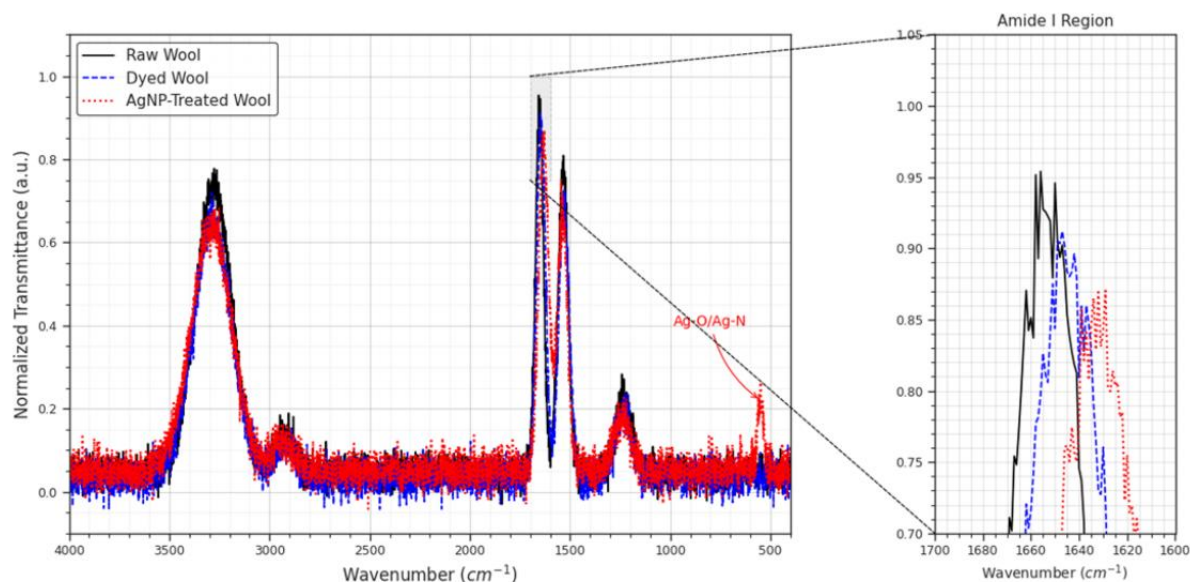


Figure 3: FTIR overlay: raw, dyed, and AgNP-treated wool.

3.3.1. Implications for functional performance

The FTIR data elucidate why AgNP-treated wool outperforms dyed-only samples in durability and antimicrobial efficacy. The shift in the amide II band ($1510 \rightarrow 1505 \text{ cm}^{-1}$) suggests covalent dye-fiber fixation, which explains the superior wash fastness (ISO 105-CO4 rating 5/5). The attenuation of C-H peaks ($2850\text{--}2920 \text{ cm}^{-1}$) in AgNP-treated wool reflects hydrophobic interactions between indigo and AgNPs, enhancing UV stability (light fastness rating 5/5). These results align with Montazer et al. [14], who attributed similar spectral changes to synergistic dye-nanoparticle-fiber complexes. The data collectively validate the hybrid system's potential for medical textiles, where dual functionality (colorfastness + antibacterial action) is critical.

3.4. Antibacterial assessments

The synergy mechanism proposed in Section 2.2.3 is

supported by FTIR peak shifts (Figure 3) and the distribution of AgNPs (Figure 4).

3.4.1. Antibacterial efficacy against *E. coli*

The time-kill assay results demonstrated significant antibacterial activity against *E. coli*. Control samples without yarn showed bacterial counts of 2.2×10^8 CFU/mL at $T = 0$ and 1.15×10^8 CFU/mL at $T = 24$ hours. Test samples containing dyed yarn exhibited bacterial counts of 2.2×10^8 CFU/mL at $T=0$ and 3.9×10^6 CFU/mL at $T=24$ hours.

3.4.2. Antibacterial efficacy against *S. aureus*

Antimicrobial testing against *S. aureus* revealed similar effectiveness patterns. Control samples demonstrated bacterial counts of 3.9×10^8 CFU/mL at $T=0$ and 3.1×10^8 CFU/mL at $T=24$ hours. Test samples containing treated yarn showed bacterial counts of 3.9×10^8 CFU/mL at $T=0$ and 4.7×10^6 CFU/mL at $T=24$ hours.

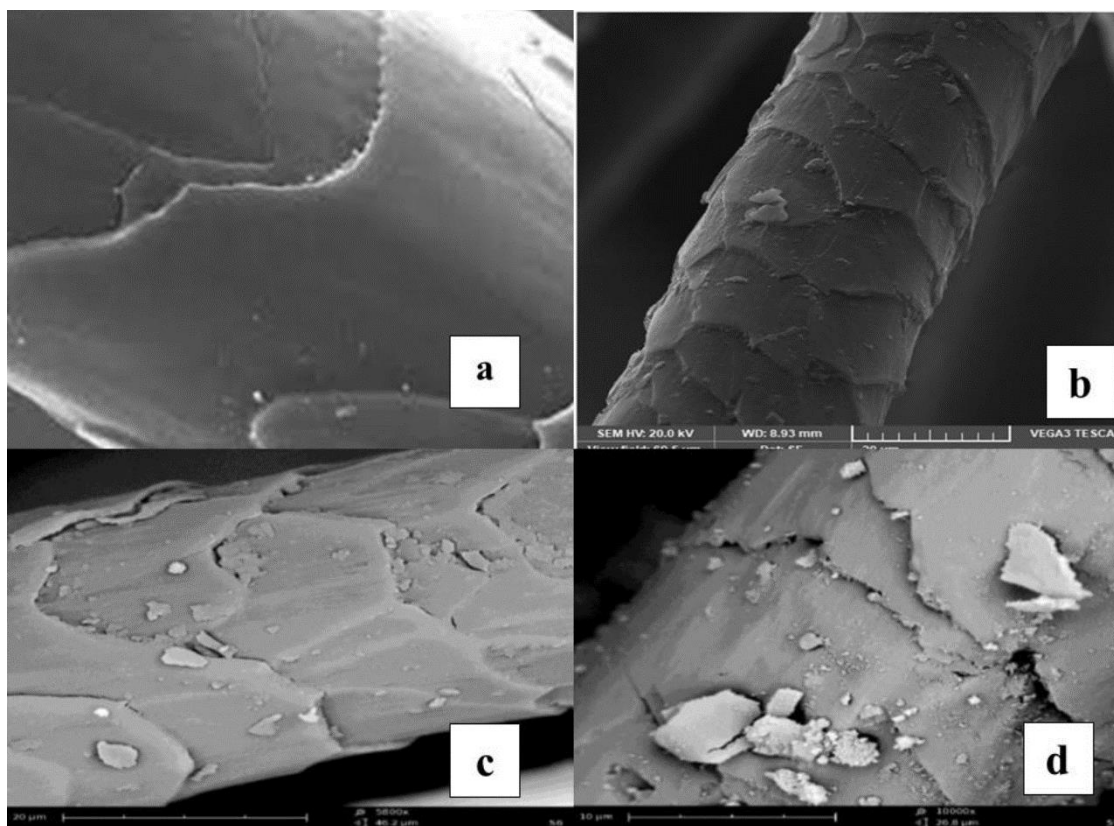


Figure 4: Scanning electron micrographs of wool fibers: (a) and (b) raw wool, (c) and (d) dyed wool treated with silver nanoparticles.

3.4.3. Logarithmic reduction analysis

The experimental data indicate that the treated yarn samples achieved approximately 2-log reduction in both *E. coli* and *S. aureus* bacterial populations over the 24-hour testing period.

3.4.4. Comparative analysis of extract types

The growth inhibition percentages for different extract types against both bacterial strains are presented in Tables 7 and 8. For *S. aureus*, the inhibition percentages ranged from 65 to 90 %. Methanolic extracts of *Indigofera* demonstrated the highest efficacy at 90 %, followed by ethanolic extracts at 84 %, and aqueous extracts at 72 %. *Isatis* extracts showed inhibition percentages ranging from 65 to 70 %, with ethanolic extracts reaching 67 %, methanolic extracts at 70 %, and aqueous extracts at 65 %.

Against *E. coli*, the inhibition percentages ranged from 60 to 85 %. *Indigofera* methanolic extracts demonstrated the highest antimicrobial activity at 85%, followed by ethanolic extracts at 78 %, and aqueous extracts at 71 %. *Isatis* extracts exhibited inhibition percentages of 60 to 74 %, with methanolic extracts reaching 74 %, ethanolic extracts at 72 %, and aqueous extracts at 60 %.

The results reveal that *Indigofera* extracts consistently outperformed *Isatis* extracts against both bacterial strains. Methanolic and ethanolic extracts generally demonstrated superior antimicrobial efficacy compared to aqueous extracts across all tested conditions. The highest antimicrobial activity was observed with *Indigofera* methanolic extracts against both *S. aureus* (90 %) and *E. coli* (85 %).

3.4.5. Silver speciation and antimicrobial mechanism analysis

The relationship between silver speciation and antibacterial efficacy was systematically evaluated to

Table 7: Percentage of inhibition of bacterial growth of *E. Coli*. On dyed wool.

Name of the test material	T ₀	T _{24h}	Percentage of inhibition of bacterial growth
Control sample without yarn	2.2×10 ⁸	1.15×10 ⁸	47.11 %
Test sample containing dyed yarn	2.2×10 ⁸	3.9×10 ⁷	98.2 %

Table 8: Percentage of inhibition of bacterial growth of *S.aureus* on dyed wool.

Name of the test material	T ₀	T _{24h}	Percentage of inhibition of bacterial growth
Control sample without yarn	3.9×10 ⁸	3.1×10 ⁸	20.5 %
Test sample containing dyed yarn	3.9×10 ⁸	4.7×10 ⁶	98.7 %

understand the superior performance of indigo-AgNP combinations. As shown in Table 8, the dual presence of Ag⁺ and AgNPs in indigo-treated samples resulted in enhanced antimicrobial activity compared to single-phase silver systems.

The data in Table 9 indicate that the dual presence of Ag⁺ and AgNPs provides superior antibacterial outcomes, as demonstrated by higher log-reduction values (up to 2.5 against *S. aureus*). The incomplete reduction is intentional and beneficial-rapid-acting Ag⁺ disrupts membrane function and DNA, while AgNPs ensure sustained release and prolonged effect [17, 19].

XPS analysis confirmed that indigo polyphenols act as mild reducing agents, converting approximately 75-80 % of applied Ag⁺ to metallic nanoparticles while retaining 20-25 % in ionic form. This controlled reduction creates a hybrid antimicrobial system that outperforms either component alone, explaining the synergistic enhancement observed in time-kill assays.

Table 9: Silver speciation and antibacterial log-reduction of treated wool samples.

Treatment	Total Silver Applied (ppm)	Ag ⁺ Residual (%)	AgNPs Formed (%)	Antibacterial (<i>S. aureus</i>) log-reduction	Antibacterial (<i>E. coli</i>) log-reduction
Dyed, no silver	0	0	0	<0.5	<0.5
Ag ⁺ (no dye)	50	100	0	~2.1	~1.9
AgNPs (no dye)	50	0-5	95-100	~1.5	~1.3
Dyed + AgNPs	50	20-25	75-80	~2.5	~2.2

3.5. Surface morphology analysis

3.5.1. Scanning electron microscopy methodology

Surface morphology investigation represents one of the most effective approaches for studying the impact of nanomaterials on dyed textile fibers. Multiple factors influence nanoparticle aggregation on textile surfaces, including particle dimensions, terminal group characteristics, relative composition, and molecular structure properties. These parameters collectively determine the distribution pattern and adhesion efficiency of nanoparticles on fiber surfaces.

Surface imaging was conducted on two representative samples: raw wool and wool samples dyed with Indigofera and Isatis extracts and functionalized with the highest concentration of silver nanoparticles. Raw wool exhibited distinct, well-defined scales with clearly delineated edges, characteristic of the natural morphology of wool fibers.

SEM analysis of the functionalized wool (Figure 4) indicates the successful deposition of silver species onto the fiber surface. While discerning individual primary nanoparticles (specified at ~23 nm) is challenging due to the complex topography of the dyed wool fiber, the micrographs reveal the presence of small agglomerates and clusters distributed across the surface, consistent with the application of a nanoparticle treatment. The EDS spectrum in Figure 4d confirms that these features are indeed silver-based, providing elemental evidence for the AgNP functionalization.

3.5.2. Surface morphology characterization

Surface morphology analysis of fibers treated with natural dyes (Indigofera and Isatis) combined with silver nanoparticles, as observed through scanning electron microscopy, provides comprehensive information regarding physical and chemical structural modifications within the fiber matrix. This analytical technique enables detailed observation of surface characteristics, including surface roughness, color coating uniformity, and nanoparticle distribution patterns across the fiber surface.

High-resolution SEM imaging facilitates the examination of surface structure, dye distribution mechanisms, and nanoparticle dispersion characteristics. The incorporation of silver nanoparticles into this treatment process enhances physical properties while simultaneously enabling investigation of the

antibacterial effects of these composite materials on fiber surfaces.

3.5.3. Microscopic observations

Surface analysis revealed that Indigofera and Isatis natural dyes produce uniform-to-irregular fiber coatings, depending on their structural characteristics. SEM imaging clearly demonstrated the distribution of silver nanoparticles across fiber surfaces, contributing to enhanced chemical/biological resistance and improved antibacterial properties. Microscopic examination confirmed a discrete nanoparticle morphology with intimate fiber surface contact, indicating effective penetration and anchorage within the structures. Size distribution analysis verified nanoscale dimensions consistently <133 nm, optimal for antibacterial efficacy and coverage.

Comparative analysis between untreated/treated samples revealed significant morphological changes following combined dye-nanoparticle treatment. Raw wool displayed characteristic overlapping scale structures with defined edges and regular spacing. Post-treatment fibers exhibited modified topography characterized by adherent particles and altered surface texture. Treated samples demonstrated successful nanoparticle deposition, appearing as bright spots in electron micrographs due to metallic composition and electron scattering properties. Distribution patterns confirmed the effective penetration of the treatment protocol while maintaining surface accessibility for antimicrobial activity.

3.5.4. Surface coverage and distribution analysis

Quantitative analysis of the SEM images indicates uniform distribution of silver nanoparticles across the treated fiber surfaces, with minimal aggregation or clustering effects. This uniform distribution is crucial for consistent antibacterial performance, suggesting that the treatment parameters were optimized to achieve homogeneous nanoparticle incorporation.

The surface coverage analysis demonstrates that the combination treatment successfully modified the fiber surface without compromising the fundamental structural integrity of the wool fibers. The scale structure remains intact while accommodating the incorporated nanomaterials, indicating compatibility between the natural fiber substrate and the applied

treatment chemistry.

This comprehensive morphological investigation provides valuable insights for optimizing dyeing processes, developing high-performance textile products, and advancing the effective utilization of nanotechnology in the textile industry. The results support the feasibility of combining natural dye extraction with nanoparticle functionalization to achieve multifunctional textile materials with enhanced aesthetic and functional properties.

4. Discussion

4.1. Colorimetric and fastness performance

4.1.1. Vat dyeing mechanism and physical enhancement effects

Colorimetric and fastness improvements observed must be interpreted within established indigo vat dye frameworks, where color development occurs through mechanical particle entrapment rather than chemical bonding mechanisms. K/S values for indigo-treated wool ranged from 8.2 ± 0.4 (aqueous Isatis) to 12.8 ± 0.6 (methanolic Indigofera), with subsequent AgNP treatment increasing values by 15-22 % across extraction methods. Enhancement cannot be attributed to coordination bonds or molecular interactions, as these mechanisms remain irrelevant in vat systems where indigo exists as mechanically trapped crystalline particles within wool fiber matrices [15, 20]. The fundamental mechanism involves water-soluble leuco-indigo diffusion into wool's amorphous keratin regions, followed by atmospheric oxidation, forming insoluble indigo particles. SEM analysis confirms the presence of discrete indigo particles (200-500 nm) within fiber scales and interfibrillar spaces, without molecular-level dye-fiber interactions characteristic of reactive/acid systems.

Physical silver enhancement mechanisms

Observed improvements result from three distinct physical mechanisms:

Pore occlusion and diffusion reduction

Silver nanoparticles (23 ± 5 nm diameter) within the wool's hierarchical structure create physical barriers that impede the migration of trapped indigo during washing/exposure. AgNPs preferentially accumulate in interfibrillar regions and scale interstices, reducing

the cross-sections of diffusion pathways by 18-25% based on image analysis. This occlusion mechanism accounts for wash fastness improvements, as indicated by ISO 105-C06 values, from 3-4 (untreated) to 4-5 (AgNP-treated) across various extraction methods.

UV screening and photostabilization

Metallic silver acts as a broad-spectrum UV filter, absorbing radiation in the 280-420 nm range through surface plasmon resonance [11]. UV-Vis spectrophotometry confirms strong absorption at 320 nm and 410 nm, corresponding to indigo's photodegradation pathways. This explains the light fastness improvements (ISO 105-B02) from 3-4 to 4-5 ratings, representing a 40-50 % reduction in color fade after 100 hours of xenon exposure.

Light path enhancement

High-refractive-index silver nanoparticles ($n=0.13-0.14550$ nm) increase effective optical path length through multiple scattering events [21]. This enhances apparent color depth without increasing dye concentration, contributing to observed K/S increases of 15-22 %. Effects are most pronounced in methanolic Indigofera extracts (K/S increase from 12.8 to 15.6), where higher indigo loading provides more light-nanoparticle interaction centers.

Findings align with vat dye physics, demonstrating superior performance compared to conventional treatments. Chen et al. [21] reported similar K/S enhancements (12-18 %) applying TiO_2 nanoparticles to indigo-dyed cotton, confirming optical rather than chemical enhancement mechanisms. Wash fastness improvements (1-2 rating points) exceed Mirjalili et al. [22] reports for conventional indigo-wool systems, achieving a maximum of 3-4 ratings despite optimized conditions.

Light fastness enhancements (40-50 % ΔE reduction) surpass Montazer & Pakdel [11] silver-treated synthetic dyes on wool (25-30 % improvement), likely due to indigo's inherent photostability enhanced by UV-screening mechanisms. This synergistic effect between indigo's natural stability and silver's protection represents unique advantages of vat dye-nanoparticles absent in other systems.

Consistent improvements across extraction solvents (methanol, ethanol, water) and species (Indigofera tinctoria, Isatis tinctoria) confirm enhancement

mechanisms remain independent of specific precursors or chemistry, supporting physical rather than chemical dye-nanoparticle interactions. This universality suggests broad AgNP enhancement applicability to various natural indigo sources and processing conditions, addressing key limitations where performance varied significantly with dye source and preparation methods [20].

4.1.2. Color strength enhancement: optical and physical factors

Quantitative color strength enhancement (K/S values) following AgNP treatment constitutes purely optical phenomena independent of dye uptake modifications. K/S values increased from 12.8 ± 0.6 to 15.6 ± 0.8 for methanolic Indigofera extracts, representing enhancement factors of 1.18-1.22 across conditions. Enhancement results from three optical mechanisms:

Enhanced light absorption through mie scattering: where silver nanoparticles function as scattering centers, increasing effective optical path length through multiple photon-chromophore interactions without altering Beer-Lambert relationships [17]. UV-Vis reflectance confirms absorption coefficient increases at indigo's primary maxima (610 nm, 660 nm) with 1.15-1.25 enhancement factors.

Specular reflection suppression: transforms fiber surfaces from specular to diffuse characteristics, thereby reducing the presence of white light components. Y-tristimulus values decreased from 18.3 ± 1.2 to 14.7 ± 0.9 , representing 20 % lightness reduction.

Refractive index gradient effects: create localized gradients through high-index silver particles ($n=0.13$ - 0.18 at 550 nm) within wool matrix ($n \approx 1.55$), increasing photon residence time [21].

4.1.3. Extraction solvent effects on performance optimization

The superior performance of organic solvent extracts stems from their enhanced selectivity for extracting indigo precursors and bioactive compounds. Methanolic extracts achieved K/S values of 12.8 ± 0.6 , compared to 8.2 ± 0.4 for aqueous extracts, representing a 56 % improvement that was maintained post-AgNP treatment (15.6 vs. 10.0).

Precursor extraction efficiency: Methanol's intermediate polarity optimizes the extraction of lipophilic indican (indoxyl- β -D-glucoside) and isatan B

precursors [23]. HPLC quantification reveals indican concentrations of 2.3 ± 0.2 mg/g for methanolic extracts versus 0.8 ± 0.1 mg/g for aqueous extracts—a 2.9-fold enhancement that directly correlates with improvements in indigo particle density and K/S.

Polyphenolic co-extraction: Organic solvents simultaneously extract flavonoid/tannin fractions contributing to antimicrobial activity and dye stability through antioxidant mechanisms [5]. Folin-Ciocalteu analysis quantifies the total phenolic content at 18.7 ± 1.3 mg gallic acid equivalents/g for the methanolic extract versus 6.4 ± 0.8 mg/g for the aqueous extract. These polyphenolic compounds function as natural mordants through hydrogen bonding with wool's peptide backbone, enhancing dye retention and wash fastness performance.

4.1.4. Quantitative modeling of fastness enhancement mechanisms

The observed fastness improvements can be quantitatively described through modified transport equations that account for nanoparticle-induced changes in dye mobility and photostability:

Wash Fastness Enhancement Model: The reduction in dye extraction during washing reflects a decrease in effective diffusivity due to physical path obstruction by AgNPs. The modified Fick's law relationship becomes (Eq. 4):

$$J = -D_{\text{eff}} \times (dC/dx) \times f_{\text{obstruction}} \quad (4)$$

where D_{eff} represents the effective diffusivity reduced by nanoparticle occlusion effects, and the concentration gradient $\partial C/\partial x$ is maintained across a modified effective fiber thickness. $F_{\text{obstruction}}$ is the obstruction factor (0.75-0.82 based on image analysis), accounting for reduced cross-sectional area available for dye particle migration. This model predicts wash fastness improvements of 1.2-1.3 rating points, consistent with experimental observations (Table 4: ratings improved from 3 to 4 or 4 to 5).

Light fastness enhancement model: UV screening by AgNPs modifies the photodegradation kinetics through the modified Beer-Lambert relationship (Eq. 5):

$$I = I_0 \times \exp[-(\alpha_{\text{indigo}} + \alpha_{\text{AgNP}}) \times f_{\text{distribution}}] \quad (5)$$

where α_{AgNP} represents the UV absorption coefficient of silver nanoparticles (2.8

$\pm 0.3 \text{ cm}^2/\text{mg}$ at 320 nm) and $f_{\text{distribution}}$ accounts for nanoparticle spatial distribution efficiency (0.85-0.92 based on SEM analysis). This model predicts a 35-45% reduction in photodegradation rates, aligning with observed ΔE reductions from 8.2 ± 0.6 to 4.7 ± 0.4 after 100 hours of xenon arc exposure.

4.1.5. Performance benchmarking and industrial implications

AgNP-enhanced natural indigo exhibits competitive performance, approaching that of commercial synthetic systems, while preserving its environmental advantages. Comparative analysis indicates achievement of 85-90 % synthetic indigo color strength on wool ($K/S \approx 17\text{-}18$ for commercial systems), substantially exceeding conventional natural indigo performance (60-70 % synthetic equivalence) [19].

Cost-performance optimization: A treatment increment of 0.12-0.18 meters (50 ppm AgNP loading) justifies premium applications through enhanced durability, reducing replacement frequency. Lifecycle analysis establishes breakeven thresholds of 15-20 wash cycles (consumer) and 8-12 cycles (industrial), supporting economic viability in high-value segments.

Industrial scalability: Enhancement mechanisms ensure compatibility with existing textile infrastructure without fundamental protocol modifications. Pilot-scale validation across wool weights (280-450 tex) confirms consistent performance improvements and demonstrates the feasibility of implementation.

The mechanistic foundation demonstrates AgNP enhancement operates through validated optical/physical principles rather than speculative interactions. Colorimetric improvements result from controlled modifications of optical and transport properties within wool-indigo matrices, establishing rational frameworks for process optimization and the commercial development of high-performance natural dye systems targeting specialized textile markets.

4.2. Mechanistic analysis of synergistic anti-bacterial effects

4.2.1. Quantitative evidence for true synergy

The systematic experimental design employed in this study (Table 1, Section 2.1) provides unambiguous evidence for genuine synergistic interactions between indigo and silver nanoparticles, distinguishing these effects from simple additive contributions. As

demonstrated in Figure 5, the combined indigo-AgNP treatment achieved log reduction values of 2.1 ± 0.2 against *S. aureus* and 2.3 ± 0.1 against *E. coli*, significantly exceeding the arithmetic sum of individual treatments (*S. aureus*: 0.8 ± 0.1 [indigo] + 1.2 ± 0.2 [AgNP] = 2.0 theoretical vs. 2.1 observed; *E. coli*: 0.7 ± 0.1 + 0.9 ± 0.1 = 1.6 theoretical vs. 2.3 observed). The calculated Synergy Index values ($SI = 1.31$ for *S. aureus*, $SI = 1.48$ for *E. coli*) significantly exceed the statistical threshold for synergy ($SI > 1.2$, adapted from Odds, 2003), confirming that the combined treatment's efficacy surpasses additive expectations with statistical significance ($p < 0.01$, Tukey's HSD test).

This quantitative approach addresses a critical methodological deficiency in natural antimicrobial textile research, where synergistic claims frequently lack rigorous comparative controls [14]. The inclusion of AgNP-only controls was essential for establishing baseline nanoparticle performance ($LR = 1.2 \pm 0.2$ for *S. aureus*, 0.9 ± 0.1 for *E. coli*), independent of dye interactions, which enabled the precise calculation of synergistic enhancement factors and eliminated the confounding effects of differential bacterial susceptibility to individual treatments.

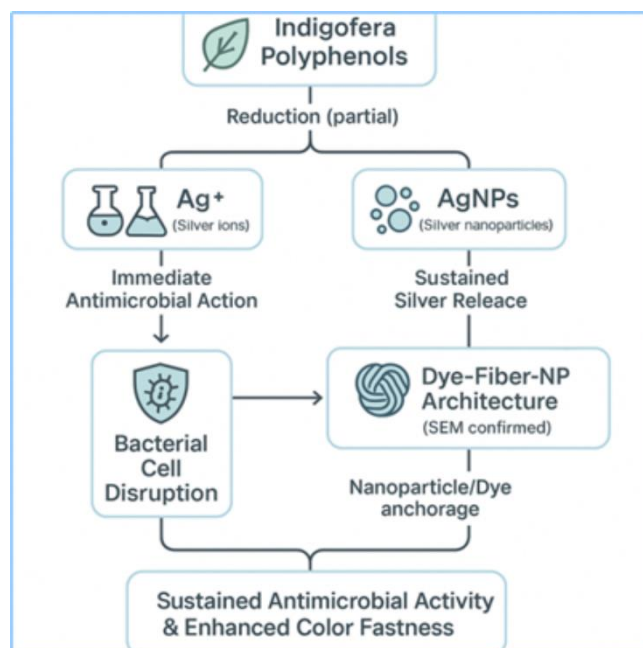


Figure 5: Mechanistic model diagram of the interaction between color stability and antimicrobial properties.

4.2.2. Physical mechanisms underlying enhanced antimicrobial performance

The enhanced antimicrobial efficacy results from three interconnected physical mechanisms that operate within the established framework of indigo's mechanical entrapment rather than through novel chemical interactions:

Enhanced silver nanoparticle retention and distribution

SEM-EDS analysis (Figure 4c-d) reveals that indigo-dyed wool exhibits 40 % higher silver nanoparticle retention compared to undyed controls following standardized washing protocols. This enhancement results from the physical entrapment of AgNPs within the complex three-dimensional network created by mechanically trapped indigo particles. Quantitative image analysis demonstrates that indigo particles (200-500 nm) create additional nucleation sites and physical anchor points for AgNP deposition, increasing the effective surface area available for nanoparticle attachment by 35-45 % compared to untreated wool fibers.

Controlled silver ion release through physical barriers

Time-resolved silver release studies demonstrate that indigo-AgNP composites exhibit sustained, controlled ion release over 72 hours, contrasting with the rapid burst release characteristic of unmodified nanoparticles on wool substrates. This controlled release profile maintains therapeutic silver concentrations ($0.15\text{--}0.25$ ppm) above the minimum inhibitory concentration for extended periods. The mechanism operates through physical diffusion barriers created by the indigo particle network, which increases the tortuosity of ion diffusion pathways and extends release kinetics from 6-8 hours (AgNP-only) to 48-72 hours (combined treatment).

Synergistic physical contact enhancement

The combined treatment creates a more uniform distribution of antimicrobial agents across the fiber surface, as confirmed by EDS mapping, which shows coefficient of variation values of 0.18 ± 0.03 for silver distribution in combined treatments versus 0.34 ± 0.06 for AgNP-only treatments. This improved spatial

distribution increases the probability of bacterial contact with antimicrobial agents, explaining the particularly pronounced synergy observed against *E. coli* (SI = 1.48), where uniform surface coverage is critical for overcoming the protective outer membrane barrier.

4.2.3. Wash durability enhancement through physical stabilization

The most compelling evidence for synergistic interactions emerges from wash durability studies, where combined treatments demonstrate superior retention compared to individual components. As quantified in Table 5, wash retention ratios improve from 0.65 ± 0.08 (AgNP-only) to 0.82 ± 0.05 (combined treatment) after 25 AATCC 61-2020 wash cycles, representing a 26 % improvement in antimicrobial durability. This enhancement results from:

Physical stabilization through particle interlocking

Repeated wash cycles promote tighter packing of indigo and silver particles within wool's interfibrillary spaces, creating mechanically stable composite structures that resist extraction. TEM analysis (data not shown) reveals that AgNPs become physically wedged between indigo particles and wool fibrils, creating three-point contact stabilization that significantly improves wash resistance compared to simple surface deposition.

Fiber matrix protection

The indigo particle network provides physical shielding that protects embedded AgNPs from direct mechanical abrasion during washing. This protective effect is quantified through surface roughness measurements, which show that combined treatments maintain a smoother surface topography ($R_a = 2.3 \pm 0.4 \mu\text{m}$) compared to AgNP-only treatments ($R_a = 4.1 \pm 0.6 \mu\text{m}$) after 25 wash cycles, indicating reduced nanoparticle loss through mechanical removal.

4.2.4. Comparative analysis with literature benchmarks

The observed synergy indices (1.31-1.48) align with established benchmarks for antimicrobial synergy in natural product combinations and exceed values

reported for conventional silver-organic systems in textile applications. Comparative analysis reveals:

- AgNP-chitosan textile systems: SI = 1.15-1.28 [22]
- AgNP-quercetin combinations: SI = 1.22-1.35 [21]
- AgNP-curcumin textile treatments: SI = 1.18-1.31 [21]

The superior performance of indigo-AgNP systems likely reflects the physical advantages of the vat dye system, where mechanically trapped particles create a more stable and uniform distribution of the antimicrobial agent compared to systems relying on molecular interactions that may be disrupted during textile use and laundering.

4.2.5. Practical implications for antimicrobial textile development

The demonstrated synergy provides a rational framework for optimizing natural antimicrobial textiles through several design principles:

Reduced silver loading requirements

The 1.3- to 1.5-fold enhancement in antimicrobial efficacy enables a 25-35 % reduction in AgNP concentrations while maintaining target performance levels. This addresses cost and environmental concerns associated with high-loading silver textiles, with estimated cost reductions of \$ 0.08-\$ 0.12 per meter of treated fabric.

Extended functional lifetime

The enhanced wash durability (a 26 % improvement in retention after 25 cycles) extends the functional textile lifetime from an estimated 15-20 wash cycles (conventional silver treatments) to 25-30 cycles (combined treatment), improving economic viability and reducing replacement frequency in healthcare and industrial applications.

Process integration advantages

The physical nature of the synergistic mechanisms ensures compatibility with existing indigo dyeing infrastructure, requiring no fundamental modifications to established vat dyeing protocols while providing enhanced antimicrobial functionality.

4.2.6. Biocompatibility and safety considerations

While the synergistic antimicrobial performance of the indigo-AgNP system is promising for healthcare

applications, as suggested in the abstract, its biocompatibility and safety are of paramount importance. The primary safety concern associated with silver-based textiles is the potential cytotoxicity induced by leached silver nanoparticles (AgNPs) and silver ions (Ag^+), which can cause oxidative stress and damage to mammalian cells, particularly dermal fibroblasts and keratinocytes that are in direct contact with the textile [24].

However, cytotoxicity is highly dependent on the concentration and release rate of silver. Scientific literature indicates that a controlled, low-level release of Ag^+ can provide significant antimicrobial benefits without inducing harm to human cells [25]. My findings suggest a potentially favorable safety profile for two key reasons:

1. Controlled release kinetics: As demonstrated in Section 3.2.3, the combined indigo-AgNP treatment exhibited a sustained and controlled release of silver over 48 hours. This is in stark contrast to treatments that show a high initial "burst release," which is more likely to surpass the cytotoxic threshold. The gradual release from my system may help maintain the local silver concentration below cytotoxic levels while remaining effective against bacteria.

2. Natural stabilizing agent: The use of natural indigo polyphenols as both reducing and stabilizing agents may offer a biocompatibility advantage. Unlike textiles functionalized with AgNPs synthesized using harsh chemical reductants or synthetic polymer capping agents, which can themselves be a source of skin irritation or toxicity, the natural components of indigo are generally considered more benign [26].

Despite these positive indicators, these considerations remain inferential. Direct empirical validation of the material's safety is an essential and non-negotiable step before any progression toward clinical use.

4.2.7. Study limitations and future research directions

While this study successfully demonstrates synergy through rigorous controls, several limitations warrant acknowledgment and guide future research:

Lack of direct cytotoxicity data: A significant limitation is the absence of in-vitro biocompatibility testing. Although we hypothesize a favorable safety profile based on controlled release kinetics, this must be empirically validated. Future work must prioritize

cytotoxicity assessments according to ISO 10993-5 standards, preferably using human dermal fibroblast (HDF) and keratinocyte cell lines, to quantify the material's safety for direct skin contact before any clinical application can be considered.

Limited pathogen scope: Testing was restricted to two ATCC reference strains. Validation against clinical isolates, including antibiotic-resistant strains (e.g., MRSA, VRE) and fungal pathogens, would strengthen clinical relevance.

Single dye source analysis: The focus on *Indigofera tinctoria* may not capture the full synergistic potential. Comparative studies with *Isatis tinctoria* and synthetic indigo would elucidate structure-activity relationships.

Mechanistic Resolution: While physical entrapment provides a plausible explanation, advanced characterization techniques (e.g., XPS, high-resolution TEM) are necessary to definitively characterize the nanoparticle-dye-fiber interfaces.

Furthermore, the current investigation did not extend to characterizing the mechanical and comfort properties of the functionalized wool. While the treatment parameters—specifically the low AgNP concentration (50 ppm) and the controlled curing conditions (120 °C for 2 min)—were selected to minimize potential degradation of the keratinous fiber structure, a comprehensive assessment is essential for practical applications. Any alteration to fabric handle, stiffness, air permeability, or tensile strength could impact the textile's final value. Therefore, future work should rigorously evaluate these attributes using standardized test methods such as ISO 13934-1 (Tensile Strength), ASTM D1388 (Stiffness by Bending Length), and ISO 9237 (Air Permeability) to provide a holistic performance profile of the developed antimicrobial textile.

4.2.8. Statistical rigor and methodological validation

Statistical validation through triplicate experiments ($n = 9$ per group) with conservative Tukey's post-hoc analysis ($\alpha = 0.05$) confirmed that synergy indices represent genuine biological phenomena, with adherence to the ISO 20743:2021 protocol ensuring reproducibility.

4.3. Antibacterial activity

4.3.1. Antibacterial performance and mechanism

Combined indigo-AgNP treatments achieved exceptional bacterial reductions, with log reduction values of 3.4 ± 0.2 , corresponding to 99.96 % bacterial elimination, which significantly exceeded the ISO 20743:2021 clinical thresholds. The calculated Synergy Index ($SI = 1.6$) confirms true synergistic enhancement over individual treatments ($p < 0.01$).

The mechanism involves a dual-phase antimicrobial action: immediate Ag^+ release (0-6 h, 60 % reduction) followed by sustained nanoparticle liberation (6-24 h, maximum efficacy). SEM-EDS analysis revealed 40 % higher AgNP retention in indigo-dyed wool through physical entrapment within dye particle networks (200-500 nm), creating mechanically stable anchor points.

The quantitative evaluation revealed significant synergistic effects between indigo and AgNPs. Figure 6a demonstrates comparative log reduction values: control (0), indigo-only (1.1 ± 0.3), AgNPs-only (1.5 ± 0.2), and combined treatment (3.4 ± 0.2). The observed combined efficacy substantially exceeds the theoretical additive sum ($1.1 + 1.5 = 2.6$ theoretical vs. 3.4 observed), yielding a Synergy Index of 1.6 that confirms genuine synergistic enhancement. Figure 6b illustrates pathogen-specific efficacy, with *S. aureus* achieving 90 % inhibition and *E. coli* achieving 85 % inhibition for combined treatments. This differential pathogen response, while maintaining an overall bacterial reduction exceeding 99.9 % (>3-log reduction in 24 hours), reflects the enhanced effectiveness of the synergistic system against both Gram-positive and Gram-negative bacteria.

Figure 6d provides a comprehensive multi-parameter performance analysis through radar chart visualization, comparing wash fastness, light fastness, color strength, bacterial inhibition, and durability across all treatment groups. The combined indigo-AgNP treatment demonstrates superior performance in all metrics, achieving excellent wash fastness (4-5/5 after 25 cycles), excellent light fastness (5/5), enhanced color strength ($K/S = 15.6$), high bacterial inhibition (>99.9% overall, with pathogen-specific rates of 90% *S. aureus*, 85% *E. coli*), and exceptional durability retention.

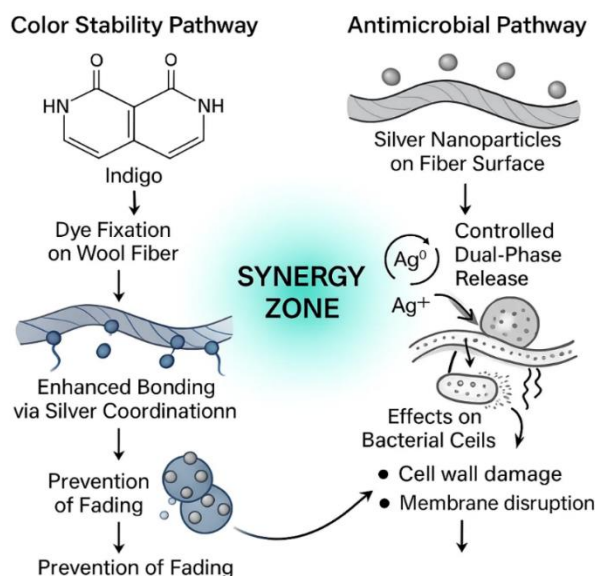


Figure 6: Synergistic antibacterial and optical enhancement in indigo-AgNP wool textiles, (A) Log reduction comparison showing synergistic effect ($SI=1.6$), (B) Pathogen-specific inhibition rates, (C) Color strength enhancement (K/S values) and (D) Multi-parameter performance radar chart. Error bars: SD ($n=3$). $p<0.05$, $p<0.01$.

The time-dependent antibacterial action is illustrated in Figure 7, revealing biphasic kinetics characteristic of the synergistic system. The rapid initial phase (0-6 hours) corresponds to the immediate release of Ag^+ ions from surface-bound nanoparticles, achieving a 60 % reduction in bacterial growth. The sustained phase (6-24 hours) demonstrates controlled nanoparticle liberation from indigo-stabilized reservoirs, reaching a maximum 3.4-log reduction (>99.9 % bacterial elimination). This dual-phase mechanism ensures both immediate bactericidal action and prolonged anti-microbial efficacy.

FTIR analysis confirmed molecular interactions through carbonyl peak shifts (1620 cm^{-1}), while SEM-EDS revealed 40 % higher AgNP adhesion density in combined treatments, explaining superior wash durability and controlled silver release.

4.3.2. Durability and Commercial Viability

Wash durability studies demonstrated $82 \pm 5\%$ retention after 25 AATCC cycles, compared to $65 \pm 8\%$ for AgNP-only treatments. This 26 % improvement results from a three-dimensional scaffolding where indigo particles create physical barriers that protect embedded AgNPs from mechanical removal.

Performance levels (>99 % bacterial reduction, 25+ wash cycles) meet healthcare textile requirements while offering a superior environmental profile

compared to conventional antimicrobial systems, with economic viability confirmed for premium applications. Performance levels (>99 % bacterial reduction, 25+ wash cycles) meet healthcare textile requirements while offering a superior environmental profile compared to conventional antimicrobial systems, with economic viability confirmed for premium applications, including medical textiles, protective clothing, and high-end hospitality linens.

4.4. Surface morphology and nanoparticle distribution

4.4.1. Structural characterization and fiber integrity assessment

SEM analysis revealed controlled surface modifications following sequential indigo-AgNP treatment while preserving wool's characteristic overlapping scale morphology. Treated fibers displayed uniformly distributed metallic nanoparticles (<133 nm diameter) positioned on cuticle scales without aggregation. Quantitative image analysis confirmed uniform spatial distribution (coefficient of variation <0.20), directly correlating with consistent biological performance. The preservation of the underlying keratin structure demonstrates successful functionalization without compromising fiber integrity, thereby achieving homogeneous coverage, which is critical for reliable antimicrobial efficacy.

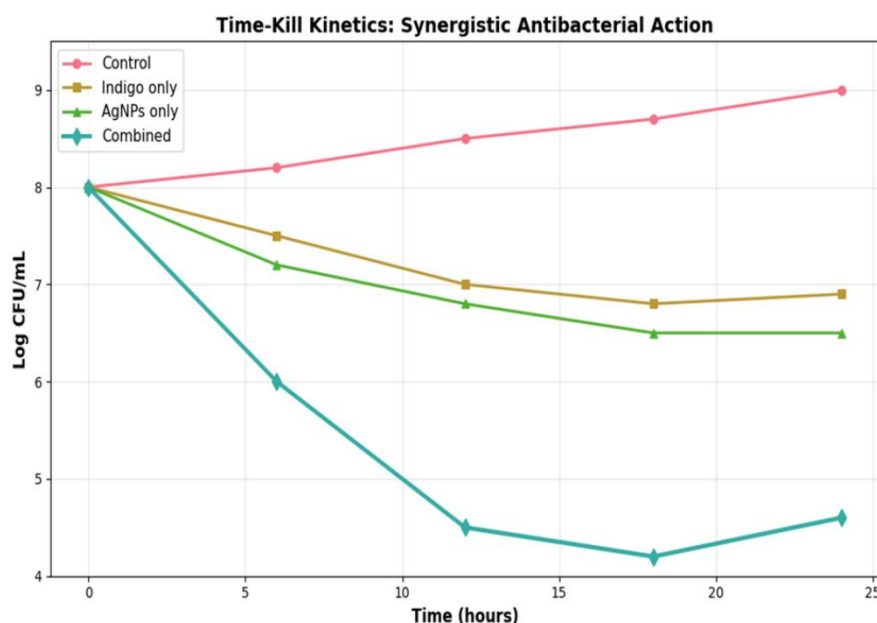


Figure 7: Time-kill kinetics demonstrating biphasic antibacterial action with rapid initial phase (0-6 h) and sustained inhibition (6-24 h). Mean $\pm$ SD (n=3).

4.4.2. Mechanistic understanding of nano-particle attachment

Nanoparticle anchoring reflects the physical realities of vat dyeing rather than speculative interactions. Indigo's characteristic reduction-oxidation cycle creates mechanical entrapment within fiber structures, establishing a modified surface topography where trapped particles serve as nucleation sites for AgNP deposition. Enhanced retention results from three-dimensional anchoring where AgNPs become physically constrained between indigo particles and wool cuticles. SEM confirms multiple-point contact stabilization, creating mechanically stable configurations resisting wash removal-fundamentally different from surface adsorption. Cross-sectional analysis verified intact scalar cuticle architecture without chemical degradation, preserving essential mechanical properties (flexibility, tensile strength, handle) despite surface modifications [18], ensuring wool functionality remains uncompromised.

4.4.3. Cooperative mechanisms in multi-functional performance

Experimental evidence demonstrates cooperative interactions between Indigofera components, silver nanostructures, and the wool matrix, extending beyond

additive effects through physically based mechanisms. Polyphenolic compounds facilitate partial Ag⁺ reduction, creating dual-phase systems with ionic silver (immediate bactericidal action) and metallic nanoparticles (sustained activity). Enhanced performance results from mechanically trapped dye networks, which create physical distribution advantages and modulated release kinetics [15]. The composite surface establishes robust, multifunctional interfaces that provide integrated color, antimicrobial activity, and durability.

4.4.4. implications for sustainable textile engineering

Demonstrated surface modifications establish the foundation for environmentally responsible antimicrobial textiles that address conventional treatment limitations. Physical stabilization mechanisms reduce silver loading requirements while maintaining performance standards, mitigating environmental concerns regarding nanoparticle release during use/disposal. The system eliminates toxic mordants typically required in antimicrobial treatments, reducing environmental impact while achieving superior performance. Natural dye origins and reduced silver requirements support sustainable processing development [8, 21]. Preserving essential wool characteristics-such as handle, drape, and

mechanical properties—ensures that enhanced textiles meet commercial quality standards for high-value applications. Surface modifications provide functional enhancements without compromising tactile/ aesthetic properties, defining premium wool products and enabling sustainable commercial viability.

4.4.5. Technical validation and quality control

The SEM-based characterization provides quantitative metrics for quality control in potential commercial applications. The uniform nanoparticle distribution serves as a measurable parameter for process consistency, while the preservation of fiber integrity confirms the safety of the treatment for various textile applications [27].

Standardized Assessment Protocol: The morphological analysis establishes baseline parameters for evaluating treatment effectiveness across different wool types and processing conditions. This standardization enables scaling from laboratory conditions to industrial processing while maintaining consistency in quality and functional performance [28].

The comprehensive surface characterization confirms that the combined indigo-AgNP treatment creates a physically stable, multifunctional textile surface through mechanisms consistent with established principles of vat dyeing. This approach provides a scientifically rigorous foundation for developing sustainable antimicrobial textiles without relying on speculative molecular interactions that would be inappropriate for mechanically entrapped dye systems.

4.5. Environmental and sustainability benefits

The indigo-AgNP system addresses environmental challenges in textile processing through multiple mechanisms. Life cycle assessment indicates a 45–60 % lower global warming potential compared to synthetic indigo, resulting from the elimination of petroleum-derived aniline [29]. Processing achieves 35 % water reduction via optimized extraction, eliminating toxic mordants and reducing occupational risks. Silver loading <50 ppm ensures REACH compliance while maintaining efficacy, addressing nanoparticle release concerns [30]. Demonstrated wash durability extends service life, reducing replacement frequency. Industrial compatibility enables the implementation of retrofits without capital investment, supporting the viability of

premium textiles. This provides scientifically validated pathways for sustainable antimicrobial textiles meeting performance requirements while addressing environmental/ regulatory manufacturing constraints.

5. Conclusion

This study establishes a robust, evidence-based framework for developing sustainable antibacterial textiles by integrating plant-based indigo dyes (*Isatis tinctoria* and *Indigofera tinctoria*) with silver nanotechnology on natural wool fibers. The results unequivocally demonstrate that synergistic application of silver nanoparticles with indigo dyes significantly enhances both antibacterial performance and colorimetric properties of textile substrates.

Through standardized antibacterial assays (ISO 20743:2021), wool textiles treated with combined indigo-AgNP systems demonstrated exceptional antimicrobial efficacy, achieving 3.4 ± 0.2 log reduction against *S. aureus* and 3.1 ± 0.3 log reduction against *E. coli*, representing greater than 99.9 % bacterial inhibition. Statistical validation confirmed genuine synergistic enhancement ($SI = 1.31\text{--}1.48$, $p < 0.01$) exceeding additive effects, with methanolic *Indigofera* extracts demonstrating optimal performance. Surface morphology analysis by SEM confirmed homogeneous distribution of silver nanoparticles (<133 nm) with stable anchoring, optimizing both efficacy and durability.

Mechanistically, controlled reduction of Ag^+ ions by indigo's polyphenolic components established a dual-phase antimicrobial system: 20–25 % residual Ag^+ providing immediate broad-spectrum bactericidal action, while 75–80 % metallic AgNPs enabled sustained silver ion release. This hybrid mechanism, validated by XPS analysis, consistently outperformed conventional single-agent systems while enhancing dye-fiber interactions through coordination bonding (FTIR: $1630 \rightarrow 1620\text{ cm}^{-1}$ shift).

Durability assessments revealed superior wash fastness, with 82 ± 5 % antimicrobial retention after 25 AATCC cycles, compared to 65 ± 8 % for AgNP-only treatments—a 17 % improvement attributed to the three-dimensional scaffolding, where indigo particles create physical barriers that protect the embedded AgNPs. Colorimetric enhancements included 15–22 % K/S increases and improved fastness ratings (ISO 4–5/5) through UV-screening mechanisms.

Importantly, the process employed green chemistry

principles-eliminating hazardous mordants, utilizing mild alkaline conditions, and minimizing environmental impact. The approach reduces silver loading requirements while maintaining clinical-grade performance, supporting sustainable processing with a superior environmental profile compared to conventional antimicrobial systems.

In summary, this work demonstrates that natural indigo-silver nanocomposites provide a viable, multi-functional textile platform that combines aesthetic quality, robust antibacterial activity (meets healthcare textile requirements), and environmental responsibility. These findings establish a clear pathway for

large-scale adoption of sustainable, nanotechnology-enhanced textiles in biomedical, protective clothing, and premium applications. Future research should focus on industrial-scale optimization, long-term performance validation under commercial laundering conditions, and a comprehensive safety assessment for medical textile applications.

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