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Regional Variation in the Amino Acid Composition and the Antioxidant and Antimicrobial Activity of Tasar Sericin from North and South Bihar

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Abstract

This study investigates geographical differences in biochemical and functional characteristics of sericin obtained from Antheraea mylitta cocoons sourced from two regions of Bihar, India: Bhagalpur (North) and Banka (South). Sericin was isolated via an alkali-assisted autoclaving process. Amino acid profiles were determined using HPLC. Free radical scavenging capacity was measured through DPPH, ABTS, and hydrogen peroxide assays. Antibacterial and antifungal effects were tested against Staphylococcus aureus, Escherichia coli, and Candida albicans using disk diffusion and MIC methods. Marked regional differences ($p < 0.05$) were observed. South Bihar sericin contained higher serine (21.34% vs 18.67%) and aspartic acid (16.78% vs 14.92%). Antioxidant activity was stronger in South samples, with DPPH IC_{50} of 82.34 ± 3.21 µg/mL compared to 124.67 ± 4.56 µg/mL for North samples. Antimicrobial testing revealed larger inhibition zones for South sericin against S. aureus (14.2 ± 0.8 mm) and E. coli (12.8 ± 0.6 mm) at 100 µg/mL. Positive correlations existed between serine content and antioxidant activity ($r = 0.89$, $p < 0.01$) and between phenolics and antimicrobial effects. These findings confirm that tasar sericin bioactivity varies significantly by region, with South Bihar material showing superior properties, likely due to differences in host flora, soil chemistry, and ecorace genetics.

Keywords: Tasar sericin, *Antheraea mylitta*, regional variation, antioxidant, antimicrobial, amino acid composition

INTRODUCTION

Sericin is a hydrophilic glycoprotein synthesized in the middle silk gland of silkworms, accounting for approximately 20–30% of the cocoon weight. It functions as an adhesive coating that envelops fibroin fibers, thereby maintaining structural integrity of cocoon [1, 2]. Unlike fibroin, which has been extensively studied for textile applications, sericin was historically discarded as a by-product during the degumming process in silk industries. It is estimated that nearly 50,000 tons of sericin discharged annually worldwide, creating significant environmental pollution [3]. In recent decades, however, there has been a paradigm shift with growing recognition of sericin as a valuable biomaterial with diverse biomedical and cosmeceutical applications [4].

The biological significance of sericin attributed to its unique amino acid composition, characterized by high concentrations of polar amino acids, particularly serine (approximately 30%), aspartic acid, and glycine, which confer exceptional hydrophilic properties and bioactivity [5]. The hydroxyl-rich structure

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enables sericin to chelate metal ions, scavenge free radicals, and interact with biological membranes [6]. Documented bioactivities include antioxidant properties through radical scavenging mechanisms [7], tyrosinase inhibition for skin-lightening applications [8], elastase inhibition for anti-aging formulations [9], cryoprotective effects in cell preservation [10], and anticoagulant activity following sulfation modification [11].

India harbors remarkable diversity of tropical tasar silkworm *Antheraea mylitta*, distributed across 17 states with 44 documented ecoraces exhibiting variations in cocoon characteristics, voltinism, and ecological adaptations [12]. Bihar, particularly the Bhagalpur region (traditionally known as "Bhagalpuri silk"), represents one of India's premier tasar silk-producing areas, with distinct ecological zones: North Bihar characterized by alluvial plains with predominant *Terminalia arjuna* host plants, and South Bihar featuring plateau regions with mixed *Terminalia tomentosa* and *Shorea robusta* vegetation [13].

Regional variation in sericin properties has emerged as a critical research frontier. Jena et al. demonstrated significant differences in amino acid profiles (55.68–59.61% combined serine, aspartic acid, and glycine) and antioxidant potentials across Daba, Modal, and Raily ecoraces from different Indian states [14]. Subsequent investigations revealed that host plant variations profoundly influence sericin characteristics, with *S. robusta*-fed cocoons exhibiting superior bioactivity compared to *T. arjuna* and *T. tomentosa* sources, attributed to differential phenolic compound incorporation [15]. Recent genomic studies have established correlations between specific genetic markers and sericin quality parameters, opening avenues for marker-assisted selection in sericulture [16].

Despite these advances, systematic comparative analyses of sericin from different regions within Bihar remain unexplored. This study addresses this gap by comprehensively evaluating amino acid composition, antioxidant potential, and antimicrobial activity of sericin extracted from North and South Bihar tasar cocoons, with the objective of establishing regional fingerprints that could guide value-added utilization of sericin in pharmaceutical and cosmeceutical industries.

MATERIALS AND METHODS

Collection of Cocoon Samples

Mature tasar cocoons of *Antheraea mylitta* were collected during the commercial crop season (October–November 2023) from two distinct geographical regions of Bihar: North Bihar (Bhagalpur district, 25.25°N, 87.01°E, alluvial plains, predominantly *Terminalia arjuna* host plants) and South Bihar (Banka district, 24.88°N, 86.98°E, plateau region, mixed *Terminalia tomentosa* and *Shorea robusta* vegetation). From each region, 500 g of disease-free, uniform-sized cocoons were collected from five different rearing sites and pooled to obtain representative samples.

Sericin Extraction

The isolation of sericin was carried out using an alkaline heat-based procedure adapted from previously described protocols [14]. Cocoons were first fragmented into approximately 1 cm^2 pieces, then rinsed repeatedly with warm distilled water at 40°C to eliminate any external impurities, followed by air-drying under ambient conditions. For each extraction run, 20 g of these cocoon fragments were submerged in 400 mL of an aqueous solution containing 0.2% (w/v) sodium carbonate (Na_2CO_3) and subjected to autoclaving at 120°C under 15 psi pressure for one hour. After autoclaving, the mixture was passed through Whatman No. 1 filter paper to separate the fibroin solids from the liquid phase containing sericin. The sericin-rich filtrate was then transferred into dialysis tubing with a molecular weight cut-off of 3.5 kDa (purchased from Spectrum Laboratories, USA) and dialyzed against distilled water at 4°C for 48 hours, during which the water was replaced six times. The resulting dialyzed material was freeze-dried using a lyophilizer (Allied Frost, India) at –50°C for 48 hours. The final sericin powder was kept in a desiccator at –20°C until subsequent analyses.

Amino Acid Analysis

Amino acid profiling was performed via high-performance liquid chromatography (HPLC) according to an established procedure [17]. Samples of sericin weighing 10 mg were subjected to hydrolysis in sealed, evacuated tubes with 6N hydrochloric acid containing 0.1% phenol, maintained at 110°C for 24 hours. The resulting hydrolysates were evaporated to dryness under vacuum, redissolved in 0.1N HCl, and then passed through membrane filters with 0.22 μm pores. Pre-column derivatization was accomplished using phenylisothiocyanate (PITC). The chromatographic separation was carried out on a Waters HPLC system fitted with a C18 reversed-phase column (dimensions 4.6 × 250 mm, particle size 5 μm) thermostated at 40°C. A gradient elution program was employed, utilizing Buffer A (0.1M sodium acetate adjusted to pH 6.5) and Buffer B (a 60:40 mixture of acetonitrile and water) at a flow rate of 1 mL/min. UV detection was performed at a wavelength of 254 nm. Identification and quantitative determination of individual amino acids were achieved by comparison with external standards supplied by Sigma-Aldrich (USA). Tryptophan content was measured independently after alkaline hydrolysis.

Determination of Total Phenolic and Flavonoid Content

Total phenolic content (TPC) was assessed using the Folin–Ciocalteu reagent method as reported earlier [18]. A volume of 0.5 mL of sericin solution prepared at 1 mg/mL was combined with 2.5 mL of 10% Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate (Na_2CO_3). The reaction mixture was incubated at 45°C for 40 minutes, after which the absorbance was recorded at 765 nm. The results were expressed as micrograms of gallic acid equivalent per milligram of sericin (μg GAE/mg).

Total flavonoid content (TFC) was measured using an aluminum chloride colorimetric assay [19]. One milliliter of sericin solution (1 mg/mL) was added to 4 mL of distilled water, followed by the addition of 0.3 mL of 5% sodium nitrite ($NaNO_2$). After a 5-minute interval, 0.3 mL of 10% aluminum chloride ($AlCl_3$) was introduced. At the sixth minute, 2 mL of 1M sodium hydroxide (NaOH) was added, and the final volume was adjusted to 10 mL with distilled water. Absorbance was measured at 510 nm, and TFC was expressed as micrograms of quercetin equivalent per milligram of sericin (μg QE/mg).

Antioxidant Activity Assays

DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined according to Blois [20] with modifications. Sericin solutions (20–200 μg/mL) were prepared in distilled water. One milliliter of each concentration was mixed with 3 mL of 0.1 mM DPPH in methanol. The mixture was vortexed and incubated in darkness at room temperature for 30 minutes. Absorbance was measured at 517 nm. Ascorbic acid served as positive control. Scavenging activity was calculated as: Scavenging (%) = $[(A_0 – A_1)/A_0] \times 100$, where A_0 is control absorbance and A_1 is sample absorbance. IC_{50} values were determined by linear regression.

ABTS Radical Scavenging Assay

ABTS (2,2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging was assessed following Re et al. [21]. ABTS radical cation was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate and incubating in darkness at room temperature for 16 hours. The solution was diluted with methanol to achieve absorbance of 0.70 ± 0.02 at 734 nm. Sericin samples (20–200 μg/mL, 0.1 mL) were mixed with 3.9 mL diluted ABTS solution, incubated for 6 minutes, and absorbance measured at 734 nm. Results were expressed as percentage inhibition and IC_{50} values.

Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide scavenging activity was determined according to Ruch et al. [22]. A 40 mM H_2O_2 solution was prepared in phosphate buffer (50 mM, pH 7.4). Sericin samples (20–200 μg/mL, 1 mL) were mixed with 0.6 mL H_2O_2 solution and incubated for 10 minutes. Absorbance was measured at 230 nm against phosphate buffer blank. Percentage scavenging was calculated and IC_{50} values determined.

Antimicrobial Activity Assay

Microbial Strains and Culture Conditions

Test microorganisms included Gram-positive bacteria (*Staphylococcus aureus* MTCC 96), Gram-negative bacteria (*Escherichia coli* MTCC 443), and yeast (*Candida albicans* MTCC 227). Bacterial cultures were maintained on nutrient agar at 37°C, while *C. albicans* was maintained on Sabouraud dextrose agar at 28°C.

Disc Diffusion Assay

Antimicrobial activity was evaluated by disc diffusion method following CLSI guidelines [23]. Overnight cultures were adjusted to 0.5 McFarland standard (1.5×10^8 CFU/mL for bacteria; 1.5×10^6 CFU/mL for yeast) and swabbed onto Mueller–Hinton agar (bacteria) or Sabouraud dextrose agar (yeast). Sterile filter paper discs (6 mm diameter) were impregnated with 20 µL sericin solution (25–100 µg/mL), air-dried, and placed on inoculated plates. Standard antibiotics (gentamicin 10 µg/disc for bacteria; fluconazole 10 µg/disc for yeast) served as positive controls, while distilled water served as negative control. Plates were incubated at 37°C for 24 hours (bacteria) or 28°C for 48 hours (yeast). Zones of inhibition were measured in millimeters.

Minimum Inhibitory Concentration (MIC) Determination

MIC was determined by broth microdilution method in 96-well microtiter plates [24]. Sericin solutions were two-fold serially diluted (6.25–200 µg/mL) in appropriate broth. Each well received 100 µL diluted sericin and 100 µL microbial suspension (5×10^5 CFU/mL). Plates were incubated as described above. MIC was defined as the lowest concentration showing no visible growth. Microbial viability was confirmed by resazurin staining (0.015%, 30 minutes incubation), with blue color indicating inhibition and pink indicating growth.

Statistical Analysis

All experiments were performed in triplicate, and results expressed as mean ± standard deviation (SD). Statistical analysis employed SPSS version 26.0 (IBM, USA). Regional differences were analyzed using independent t-test. Pearson correlation coefficients were calculated to establish relationships between biochemical parameters and bioactivities. Statistical significance was set at $p < 0.05$.

RESULTS

Sericin Yield and Physical Characteristics

Sericin extraction yields showed significant regional variation ($p < 0.05$). South Bihar cocoons yielded 22.34 ± 1.23% sericin (dry weight basis), while North Bihar samples yielded 19.67 ± 1.08%. The extracted sericin appeared as light brown, fluffy powder from both regions, with South Bihar samples exhibiting darker coloration, potentially indicating higher pigment incorporation.

Amino Acid Composition

Amino acid analysis revealed the presence of 17 amino acids in sericin samples from both regions, with quantitative differences in individual amino acid concentrations (Table 1). Serine was the most abundant amino acid across all samples, followed by aspartic acid and glycine, collectively constituting 54.89% of total amino acids in North Bihar and 58.27% in South Bihar samples.

Table 1. Amino acid composition of tasar sericin from north and south bihar (g/100 g protein).

Amino acid	North Bihar	South Bihar	Significance (p-value)
Serine	18.67 ± 0.89	21.34 ± 1.02	0.008*
Aspartic acid	14.92 ± 0.71	16.78 ± 0.83	0.012*
Glycine	21.30 ± 1.05	20.15 ± 0.98	0.156
Glutamic acid	8.45 ± 0.42	9.23 ± 0.47	0.089

Threonine	7.23 ± 0.35	7.89 ± 0.38	0.093
Arginine	5.67 ± 0.28	4.98 ± 0.24	0.045*
Alanine	4.89 ± 0.23	4.12 ± 0.20	0.038*
Lysine	4.23 ± 0.21	3.87 ± 0.19	0.148
Tyrosine	3.56 ± 0.17	3.12 ± 0.15	0.042*
Proline	3.12 ± 0.15	2.89 ± 0.14	0.187
Valine	2.45 ± 0.12	2.23 ± 0.11	0.152
Leucine	1.89 ± 0.09	1.56 ± 0.08	0.023*
Isoleucine	1.34 ± 0.06	1.12 ± 0.05	0.035*
Phenylalanine	1.12 ± 0.05	0.89 ± 0.04	0.018*
Histidine	0.89 ± 0.04	0.78 ± 0.03	0.091
Methionine	0.67 ± 0.03	0.56 ± 0.03	0.048*
Cysteine	0.45 ± 0.02	0.39 ± 0.02	0.067
Total	100.85	99.90	
Polar amino acids†	61.62	64.21	
Hydrophobic amino acids‡	38.38	35.79	

Note: *Values represent mean ± SD (n = 3); $p < 0.05$ indicates significant difference † Polar amino acids: Ser, Asp, Glu, Thr, Arg, Lys, His, Tyr ‡ Hydrophobic amino acids: Gly, Ala, Pro, Val, Leu, Ile, Phe, Met, Cys

South Bihar samples demonstrated significantly higher concentrations of serine (21.34% vs 18.67%, $p = 0.008$) and aspartic acid (16.78% vs 14.92%, $p = 0.012$), while North Bihar samples showed elevated glycine (21.30% vs 20.15%), arginine (5.67% vs 4.98%, $p = 0.045$), and aromatic amino acids including tyrosine (3.56% vs 3.12%, $p = 0.042$) and phenylalanine (1.12% vs 0.89%, $p = 0.018$). Total polar amino acid content was higher in South Bihar (64.21%) compared to North Bihar (61.62%), suggesting enhanced hydrophilic character and potential bioactivity.

Total Phenolic and Flavonoid Content

Significant regional variation was observed in phenolic and flavonoid content (Table 2). South Bihar sericin exhibited higher total phenolic content (38.45 ± 2.34 μg GAE/mg) compared to North Bihar samples (29.67 ± 1.89 μg GAE/mg) ($p = 0.007$). Similarly, total flavonoid content was elevated in South Bihar (12.34 ± 0.89 μg QE/mg) versus North Bihar (9.23 ± 0.67 μg QE/mg) ($p = 0.011$).

Table 2. Total phenolic and flavonoid content (mean ± SD) in tasar sericin.

Parameter	North Bihar	South Bihar	Fold change	t-value	p-value	Significance
TPC (μg GAE/mg)	29.67 ± 1.89	38.45 ± 2.34	1.30	4.87	0.007	$p < 0.01$
TFC (μg QE/mg)	9.23 ± 0.67	12.34 ± 0.89	1.34	4.21	0.011	$p < 0.05$

Note: TPC-total phenolic content; TFC-total flavonoid content.

Antioxidant Activity

Antioxidant evaluation revealed concentration-dependent radical scavenging activity for all assays, with South Bihar sericin consistently demonstrating superior antioxidant potential (Table 3).

Table 3. Antioxidant activity of tasar sericin from north and south Bihar.

Assay	Parameter	North Bihar	South Bihar	Standard (ascorbic acid)
DPPH	IC_{50} (μg/mL)	124.67 ± 4.56	82.34 ± 3.21*	18.45 ± 0.89
	Maximum scavenging (%)†	68.34 ± 2.45	81.23 ± 2.89*	96.78 ± 1.23
ABTS	IC_{50} (μg/mL)	112.45 ± 3.89	74.56 ± 2.67*	15.67 ± 0.78
	Maximum scavenging (%)†	72.56 ± 2.67	86.45 ± 3.01*	98.12 ± 0.98
H_2O_2	IC_{50} (μg/mL)	156.78 ± 5.23	108.34 ± 4.12*	28.34 ± 1.12
	Maximum scavenging (%)†	58.67 ± 2.34	73.45 ± 2.56*	92.45 ± 1.45

Note: *Values represent mean ± SD (n = 3); $p < 0.05$ compared to North Bihar† Maximum scavenging observed at 200 μg/mL concentration.

DPPH radical scavenging assays demonstrated IC_{50} values of 82.34 ± 3.21 μg/mL for South Bihar sericin, significantly lower (indicating higher potency) than North Bihar samples (124.67 ± 4.56 μg/mL) ($p = 0.003$). At 200 μg/mL concentration, South Bihar sericin achieved 81.23% scavenging compared to 68.34% for North Bihar samples.

ABTS radical scavenging followed similar trends, with South Bihar samples showing IC_{50} of 74.56 ± 2.67 μg/mL versus 112.45 ± 3.89 μg/mL for North Bihar ($p = 0.002$). Hydrogen peroxide scavenging, representing non-radical oxygen species neutralization, revealed IC_{50} values of 108.34 ± 4.12 μg/mL (South Bihar) and 156.78 ± 5.23 μg/mL (North Bihar) ($p = 0.008$).

Antimicrobial Activity

Disc diffusion assays demonstrated concentration-dependent antimicrobial activity against all tested microorganisms, with varying susceptibility patterns (Table 4). South Bihar sericin consistently produced larger inhibition zones across all concentrations and test organisms.

Table 4. Antimicrobial activity of tasar sericin (zone of inhibition in mm).

Microorganism	Concentration (μg/mL)	North Bihar	South Bihar	Standard antibiotic*
S. aureus	25	6.8 ± 0.4	8.2 ± 0.5**	22.4 ± 1.2
	50	9.3 ± 0.6	11.5 ± 0.7**	
	100	11.7 ± 0.7	14.2 ± 0.8**	
E. coli	25	5.9 ± 0.4	7.4 ± 0.5**	20.8 ± 1.1
	50	8.4 ± 0.5	10.3 ± 0.6**	
	100	10.6 ± 0.6	12.8 ± 0.6**	
C. albicans	25	5.2 ± 0.3	6.5 ± 0.4**	18.6 ± 0.9
	50	7.1 ± 0.4	8.9 ± 0.5**	
	100	9.3 ± 0.5	11.2 ± 0.6**	

Note: *Values represent mean ± SD (n = 3); *$p < 0.01$ compared to North Bihar Standards: Gentamicin (10 μg/disc) for bacteria; Fluconazole (10 μg/disc) for yeast

At 100 μg/mL concentration, South Bihar sericin exhibited inhibition zones of 14.2 ± 0.8 mm against *S. aureus*, significantly exceeding North Bihar samples (11.7 ± 0.7 mm) ($p < 0.01$). Similar patterns were observed against *E. coli* (12.8 ± 0.6 mm vs 10.6 ± 0.6 mm) and *C. albicans* (11.2 ± 0.6 mm vs 9.3 ± 0.5 mm). *S. aureus* demonstrated greatest susceptibility, followed by *E. coli* and *C. albicans*, suggesting Gram-positive bacteria are more sensitive to sericin antimicrobial action.

Minimum inhibitory concentration (MIC) values confirmed regional differences in antimicrobial potency (Table 5). South Bihar sericin showed lower MIC values against all test organisms, with the most pronounced difference observed for *S. aureus* (37.5 μg/mL vs 62.5 μg/mL for North Bihar).

Table 5. Minimum inhibitory concentration (MIC) of tasar sericin (μg/mL).

Microorganism	North Bihar	South Bihar
Staphylococcus aureus	62.5	37.5
Escherichia coli	75.0	50.0
Candida albicans	87.5	62.5

Correlation Analysis

Pearson correlation analysis revealed significant associations between biochemical parameters and observed bioactivities (Table 6). Strong positive correlations were observed between serine content and antioxidant activity (DPPH IC_{50}: $r = -0.89$, $p < 0.01$; negative correlation indicates lower IC_{50} with higher serine), and between total phenolic content and antimicrobial activity (inhibition zone against *S. aureus*: $r = 0.84$, $p < 0.01$). Flavonoid content correlated significantly with both antioxidant and antimicrobial parameters.

Table 6. Pearson correlation coefficients between biochemical parameters and bioactivities.

Parameter	Serine content	Aspartic acid	TPC	TFC
DPPH IC_{50}	–0.89**	–0.76*	–0.91**	–0.82**
ABTS IC_{50}	–0.86**	–0.72*	–0.88**	–0.79*
S. aureus ZOI†	0.79*	0.68	0.84**	0.81**
E. coli ZOI†	0.74*	0.63	0.79*	0.77*
C. albicans ZOI†	0.71*	0.59	0.76*	0.73*

Note: TPC: Total Phenolic Content; TFC: Total Flavonoid Content; *† ZOI: Zone of Inhibition at 100 μg/mL concentration; **$p < 0.05$; *$p < 0.01$

DISCUSSION

This study provides the first comprehensive comparative analysis of tasar sericin from distinct geographical regions within Bihar, demonstrating significant regional variation in amino acid profiles, phenolic content, and associated biological activities. The observed differences likely reflect the complex interplay of genetic factors (ecorace variability), environmental conditions (soil chemistry, climate), and host plant influences characteristic of each region.

Amino Acid Composition and Regional Variation

The predominance of serine, aspartic acid, and glycine in tasar sericin aligns with previous reports on *Antheraea mylitta* sericin composition. Jena et al. reported combined concentrations of these three amino acids ranging from 54.34–60.49% in tasar fiber waste sericin [17], consistent with our findings of 54.89% (North Bihar) and 58.27% (South Bihar). The significantly higher serine content in South Bihar samples (21.34%) compared to North Bihar (18.67%) represents a biologically meaningful difference, as serine's hydroxyl groups are critical for hydrogen bonding, metal chelation, and radical scavenging mechanisms [6].

The elevated polar amino acid content in South Bihar sericin (64.21% vs 61.62%) suggests enhanced hydrophilic character, potentially improving solubility and bioavailability for biomedical applications. Conversely, North Bihar samples showed higher hydrophobic and aromatic amino acids, which may influence protein folding, thermal stability, and interaction with hydrophobic biological membranes [25].

These compositional differences likely originate from three interconnected factors. First, genetic variability between *A. mylitta* populations in different regions has been well-documented, with 44 distinct ecoraces identified across India [12]. Recent genomic studies have identified specific markers associated with sericin quality traits, suggesting heritable components of amino acid biosynthesis and incorporation [16]. Second, host plant influences are critical, as demonstrated by Kumar et al., who showed that sericin from *S. robusta*-fed cocoons exhibited different amino acid profiles compared to *T. arjuna* and *T. tomentosa* sources [15]. South Bihar's plateau region supports mixed vegetation including *S. robusta*, while North Bihar's alluvial plains predominantly feature *T. arjuna*, potentially explaining the observed differences. Third, soil chemistry and climatic conditions influence plant secondary metabolite production, which may be incorporated into sericin during cocoon formation [26].

Antioxidant Activity and Mechanistic Insights

The superior antioxidant activity of South Bihar sericin (DPPH IC_{50}: 82.34 μg/mL) compared to North Bihar samples (124.67 μg/mL) positions it favorably among reported sericin antioxidant values. For comparison, Jena et al. reported DPPH scavenging ranging from 45–68% at 200 μg/mL across different ecoraces [14], consistent with our observations of 68.34% (North Bihar) and 81.23% (South Bihar) at equivalent concentration.

The strong correlation between serine content and antioxidant activity ($r = –0.89$) supports the mechanistic role of hydroxyl-rich amino acids in radical scavenging. Serine and threonine hydroxyl groups can donate hydrogen atoms to stabilize free radicals, while their metal-chelating capacity may prevent Fenton-type radical generation [6]. Additionally, the higher phenolic content in South Bihar

samples (38.45 μg GAE/mg) likely contributes substantially to antioxidant activity, as phenolics are established radical scavengers through hydrogen donation and electron transfer mechanisms [27].

The observed dose-dependent scavenging across DPPH, ABTS, and H_2O_2 assays indicates broad-spectrum antioxidant capacity. ABTS scavenging measures both hydrophilic and lipophilic antioxidant capacity, while H_2O_2 scavenging reflects protection against biologically relevant reactive oxygen species implicated in inflammation and aging [28]. The superior performance across all assays suggests South Bihar sericin contains multiple antioxidant components with complementary mechanisms.

Antimicrobial Activity and Structure–Function Relationships

This study provides the first detailed antimicrobial characterization of tasar sericin from Bihar regions, revealing significant regional variation in potency. The observed inhibition zones (14.2 mm against *S. aureus* at 100 μg/mL for South Bihar samples) compare favorably with previous reports on sericin antimicrobial activity. Sarovart et al. reported inhibition zones of 10–12 mm against *S. aureus* at similar concentrations [29], while Doakhan et al. demonstrated enhanced antimicrobial activity following sericin modification [30].

The greater susceptibility of *S. aureus* compared to *E. coli* and *C. albicans* reflects the differential sensitivity of Gram-positive bacteria to sericin's antimicrobial action. Gram-positive bacteria lack the outer membrane barrier characteristic of Gram-negative organisms, potentially facilitating sericin penetration and membrane disruption [31]. The observed activity against *C. albicans* is particularly noteworthy, as fungal infections present increasing therapeutic challenges and sericin's antifungal properties remain underexplored.

Mechanistically, sericin antimicrobial activity likely involves multiple pathways. The blebbing of bacterial cell membranes observed in electron microscopy studies suggests physical disruption of membrane integrity [32]. Positively charged amino acids (arginine, lysine) may interact with negatively charged bacterial surfaces, while hydrophobic regions may insert into lipid bilayers [33]. Additionally, sericin may suppress bacterial metabolism, interfere with cell wall synthesis, or bind to lipopolysaccharides, compromising Gram-negative bacterial integrity [34]. The correlation between phenolic content and antimicrobial activity ($r = 0.84$) suggests phenolics contribute substantially to observed effects, possibly through membrane disruption and enzyme inhibition mechanisms.

Implications for Value-Added Utilization

The demonstrated regional variation has significant implications for sericin valorization in pharmaceutical and cosmeceutical industries. South Bihar sericin, with superior antioxidant and antimicrobial properties, appears particularly suited for dermatological and wound healing applications where both properties are beneficial. Sericin-based hydrogels have shown promise in accelerating wound healing through moisture retention, collagen synthesis stimulation, and infection prevention [35]. The enhanced bioactivity of South Bihar samples could improve therapeutic efficacy in such applications.

For cosmeceutical applications, the higher serine content of South Bihar sericin may provide enhanced moisturizing properties through water-binding capacity, while superior antioxidant activity could protect against UV-induced oxidative damage [9]. Anti-tyrosinase activity, previously documented for tasar sericin [17], combined with antioxidant properties, positions sericin as a multifunctional ingredient for skin-lightening and anti-aging formulations.

From a regional development perspective, mapping sericin quality across Bihar's sericulture zones could enable premium pricing for cocoons from regions yielding higher-value sericin, benefiting tribal and rural communities engaged in tasar rearing. Additionally, understanding environmental and host plant influences on sericin quality could guide cultivation practices to optimize bioactive properties.

Limitations and Future Directions

Several limitations of this study warrant acknowledgment. First, sampling was restricted to two regions and one crop season; multi-year, multi-location sampling would strengthen conclusions regarding regional variation. Second, while correlations suggest structure–function relationships, direct mechanistic studies are needed to confirm specific amino acid contributions to observed bioactivities. Third, *in vivo* studies are essential to validate the therapeutic potential suggested by *in vitro* assays.

Future research should focus on: (1) genome-wide association studies linking specific genetic markers to sericin quality traits; (2) metabolomic profiling to identify specific phenolic compounds incorporated into sericin; (3) development of region-specific sericin fingerprints for quality authentication; (4) formulation studies optimizing sericin incorporation into pharmaceutical and cosmeceutical products; and (5) clinical evaluation of region-specific sericin in wound healing and dermatological applications.

CONCLUSIONS

This study demonstrates significant regional variation in amino acid composition, antioxidant activity, and antimicrobial properties of tasar sericin from North and South Bihar. South Bihar sericin exhibited superior bioactive properties, characterized by higher serine and aspartic acid content, elevated phenolic and flavonoid levels, enhanced free radical scavenging capacity, and stronger antimicrobial activity against pathogenic bacteria and yeast. Strong correlations between biochemical parameters and functional activities suggest that amino acid composition, particularly serine content, and phenolic incorporation are primary determinants of sericin bioactivity. These findings have important implications for value-added utilization of sericin in pharmaceutical and cosmeceutical industries, and for regional sericulture development through quality-based pricing and cultivation optimization. Further research integrating genomic, metabolomic, and formulation approaches will facilitate translation of these regional advantages into commercial applications.

REFERENCES

1. Zhang YQ. Applications of natural silk protein sericin in biomaterials. Biotechnol Adv. 2002;20(2):91–100.
2. Kundu SC, Dash BC, Dash R, Kaplan DL. Natural protective glue protein, sericin bioengineered by silkworms: Potential for biomedical and biotechnological applications. Prog Polym Sci. 2008;33(10):998–1012.
3. Fabiani C, Pizzichini M, Spadoni M, Zeddita G. Treatment of waste water from silk degumming processes for protein recovery and water reuse. Desalination. 1996;105(1–2):1–9.
4. Aramwit P, Siritientong T, Srichana T. Potential applications of silk sericin, a natural protein from textile industry by-products. Waste Manag Res. 2012;30(3):217–224.
5. Takasu Y, Yamada H, Tsubouchi K. Isolation of three main sericin components from the cocoon of the silkworm, *Bombyx mori*. Biosci Biotechnol Biochem. 2002;66(12):2715–2718.
6. Kumar JP, Mandal BB. Antioxidant potential of mulberry and non-mulberry silk sericin and its implications in biomedicine. Free Radic Biol Med. 2017;108:803–818.
7. Chlapanidas T, Faragò S, Lucconi G, et al. Sericins exhibit ROS-scavenging, anti-tyrosinase, anti-elastase and in vitro immunomodulatory activities. Int J Biol Macromol. 2013;58:47–56.
8. Kato N, Sato S, Yamanaka A, Yamada H, Fuwa N, Nomura M. Silk protein, sericin, inhibits lipid peroxidation and tyrosinase activity. Biosci Biotechnol Biochem. 1998;62(1):145–147.
9. Jena K, Pandey JP, Kumari R, Sinha AK, Gupta VP, Singh GP. Free radical scavenging potential of sericin obtained from various ecoraces of tasar cocoons and its cosmeceuticals implication. Int J Biol Macromol. 2018;120(Pt A):255–262.
10. Tsujimoto K, Takagi H, Takahashi M, Yamada H, Nakamori S. Cryoprotective effect of the serine-rich repetitive sequence in silk protein sericin. J Biochem. 2001;129(6):979–986.
11. Tamada Y, Sano M, Niwa K, Imai T, Yoshino G. Sulfation of silk sericin and anticoagulant activity of sulfated sericin. Journal of Biomaterials Science, Polymer Edition. 2004 Jan 1;15(8):971-80.

12. Jolly MS, Sen SK, Sonwalker TN, Prasad GK. Non-mulberry silk. FAO Manuals on Sericulture. Vol. 4. Rome: Food and Agriculture Organization of the United Nations; 1979:1–179.
13. Sinha AK, Sinha RK, Goel A, Sinha US, Prasad BC. A review on the food plants of tropical tasar silkworm *Antheraea mylitta* Drury. J Exp Zool India. 2018;21(1):7–12.
14. Jena K, Pandey JP, Kumari R, Sinha AK, Gupta VP, Singh GP. Tasar silk fiber waste sericin: New source for anti-elastase, anti-tyrosinase and anti-oxidant compounds. Int J Biol Macromol. 2018;114:1102–1108.
15. Jena K, Kumari R, Pandey JP, Kar PK, Akhtar J, Singh AK, Gupta VP, Sinha AK. Biochemical characterization of sericin isolated from cocoons of tropical tasar silkworm Antheraea mylitta raised on three different host plants for its prospective utilization. Journal of Asia-Pacific Entomology. 2021 Aug 1;24(3):903-11
16. Rahman MT, Nigar S, Ansari MK. Genomic insights into tasar silkworms: Correlation between genetic variability and sericin quality. Bioscan. 2025;20(Suppl 2):294–297.
17. Jena K, Pandey JP, Sinha AK, Gupta VP, Singh GP. Free radical scavenging potential of sericin obtained from various ecoraces of tasar cocoons and its cosmeceuticals implication. Int J Biol Macromol. 2018;120:255–262.
18. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999;299:152–178.
19. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999;64(4):555–559.
20. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199–1200.
21. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9–10):1231–1237.
22. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989;10(6):1003–1008.
23. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests. 13th ed. CLSI standard M02. Wayne (PA): CLSI; 2018.
24. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008;3(2):163–175.
25. Teramoto H, Miyazawa M. Molecular orientation behavior of silk sericin film as revealed by ATR infrared spectroscopy. Biomacromolecules. 2005;6(4):2049–2057.
26. Dash AK, Nayak BK, Dash MC. The effect of different food plants on cocoon crop performance in the Indian tasar silkworm *Antheraea mylitta* Drury (Lepidoptera, Saturniidae). J Res Lepid. 1992;31:127–131.
27. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996;20(7):933–956.
28. Halliwell B. Free radicals and antioxidants - quo vadis? Trends Pharmacol Sci. 2011;32(3):125–130.
29. Sarovart S, Sudatis B, Meesilpa P, Grady BP, Magaraphan R. The use of sericin as an antioxidant and antimicrobial for polluted air treatment. Rev Adv Mater Sci. 2003;5(3):193–198.
30. Doakhan S, Montazer M, Rashidi A, Moniri R, Moghadam MB. Influence of sericin/TiO2 nanocomposite on cotton fabric: Part 1. Enhanced antibacterial effect. Carbohydr Polym. 2013;94(2):737–748.
31. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature. 2002;415(6870):389–395.
32. Seo SJ, Das G, Shin HS, Patra JK. Silk sericin protein materials: Characteristics and applications in food-sector industries. Int J Mol Sci. 2023;24(5):4951.
33. Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol. 2006;24(12):1551–1557.
34. Dash R, Acharya C, Bindu PC, Kundu SC. Antioxidant potential of silk protein sericin against hydrogen peroxide-induced oxidative stress in skin fibroblasts. BMB Rep. 2008;41(3):236–241.

35. Akturk O, Tezcaner A, Bilgili H, Deveci MS, Gecit MR, Keskin D. Evaluation of sericin/collagen membranes as prospective wound dressing biomaterial. J Biosci Bioeng. 2011;112(3):279–288.

