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Comparative Analysis of Antioxidant and Antimicrobial Properties of Tasar Sericin from Different Ecological Regions of India

Md. Tahfizur Rahman[1], Rahul Chaubey[2,*]

Abstract

Sericin is a by-product of the silk industry, has gained attention for its bioactive properties, and ability to scavenge free radicals for protecting cells from oxidative damage, and different climatic conditions may influence the biochemical properties of the sericin. Thus, the present study aims to evaluate the antioxidant and antimicrobial properties of tasar sericin extracted from silkworms reared in diverse ecological regions of India. South Bihar, Jharkhand, Chhattisgarh, and Odisha are different ecological regions notable for their tasar silk production in India, each representing unique climatic and environmental conditions. Sericin samples were extracted using a standardized protocol, and their antioxidant activity was assessed through DPPH, ABTS, and FRAPS assays. Antimicrobial efficacy was analyzed against the most common pathogens, including Escherichia coli, Staphylococcus aureus, and Candida albicans. Analysis shows significant variations in antioxidant and antimicrobial potential across ecological regions, suggesting a correlation between the environmental conditions and the bioactive properties of tasar sericin.

Keywords: Tasar sericin, antioxidant, antimicrobial, eco-regions, bioactivity, DPPH, FRAP, pathogens

INTRODUCTION

Sericin, a globular protein derived from silkworm cocoons, has gained substantial attention in recent years due to its remarkable bioactive properties. Unlike the commonly utilized fibroin, which forms the core of silk fibers, sericin is the glue-like protein that surrounds fibroin filaments, providing structural integrity to the cocoon. Traditionally, sericin has been discarded during the silk manufacturing process, contributing to significant waste. However, recent studies have demonstrated that sericin is rich in bioactive compounds, including flavonoids, peptides, and polyphenols, which exhibit antioxidant and antimicrobial properties, opening avenues for its utilization in the pharmaceutical, food, and cosmetic industries [1, 2].

Tasar silk, a non-mulberry variety primarily produced in India, offers a unique type of sericin that differs in composition and properties compared to mulberry silk. The Indian states Bihar, Jharkhand, Chhattisgarh, and Odisha are notable for their tasar silk production, with each ecological region presenting distinct climatic conditions that may influence the biochemical properties of the sericin. Understanding these regional variations is critical to harnessing the full potential of tasar sericin for specific applications [3]. The environmental conditions under which silkworms are reared have been found to affect the chemical composition of sericin. Climatic factors, such as temperature, humidity, soil composition, and vegetation can

***Author for Correspondence**
Rahul Chaubey
E–mail: rchngp@gmail.com

[1]Post Graduate, Department of Zoology, Millat College, LNMU, Darbhanga, Bihar, India
[2]Project Research Scientist II, Kala-Azar Medical Research Center, Rambagh, Muzaffarpur, Bihar, India

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influence the silkworm's diet and health, thereby altering the bioactivity of sericin extracted from their cocoons. According to Reddy & Yang (2011) [3], non-mulberry silks, such as tasar, differ from mulberry silk in amino acid composition due to variations in environmental conditions and host plant species. These differences are crucial in determining the antioxidant and antimicrobial efficacy of sericin. An investigation by Wongputtaraksa et al. (2016) [4] specifically focused on tasar silk from various regions and observed that the ecological regions have a notable impact on the sericin's molecular structure. These findings suggest that the bioactive properties of tasar sericin can vary widely, depending on the geographical and environmental context, highlighting the need for a comparative study to understand these variations.

As a prospective choice for creating anti-aging and skin-care products, tasar sericin's antioxidant qualities are associated with its capacity to neutralize free radicals, thereby shielding cells from oxidative stress [5]. Tasar sericin has been recognized for its strong antioxidant properties, which are believed to result from its unique amino acid composition and the presence of phenolic compounds [6]. showed that tasar sericin exhibits higher antioxidant activity compared to mulberry. Various research using DPPH and ABTS assays concluded that non-mulberry silks, like tasar, have a broader range of bioactive compounds, possibly due to differences in sericulture practices and eco-regional flora. The relationship between antioxidant activity and the ecological region was further established by Gupta et al. (2018) [7], by comparing tasar sericin extracted from silkworms raised in dry and humid conditions. The result showed that sericin from humid regions had a higher antioxidant potential, suggesting that moisture levels during cocoon development play a vital role in enhancing bioactivity.

Additionally, its antimicrobial properties have shown efficacy against a broad spectrum of pathogens, suggesting potential uses in the formulation of natural preservatives and antimicrobial agents [8]. Several studies have confirmed the antimicrobial efficacy of tasar sericin, making it a focus for applications in pharmaceuticals and natural antimicrobial agents, Roy & Bose (2017) [9] examined the antimicrobial activity of tasar sericin against a range of microorganisms, including pathogenic bacteria and fungi revealed that tasar sericin exhibit strong antibacterial activity, especially against Staphylococcus aureus, and moderate antifungal activity against Candida albicans. This antimicrobial property was linked to the presence of certain peptides and amino acids that disrupt microbial cell membranes. The influence of regional factors on the antimicrobial potential of tasar sericin was investigated by Patil & Kulkarni (2021) [10], who reported that tasar silkworms reared in forest areas produced sericin with superior antimicrobial properties compared to those in semi-arid regions. The study emphasized that diverse climatic conditions contribute to the biochemical variability in sericin, thereby affecting its antimicrobial potency.

India is a major producer of non-mulberry silks, including tasar, with specific regions specializing in different types, Verma & Sharma (2019) [11] highlighted that ecological region, such as Jharkhand, Chhattisgarh, Odisha, and South Bihar have distinct climatic and environmental conditions that impact tasar silkworms. These regional differences are reflected in the chemical properties of the silk produced, particularly in the composition of sericin. In another study, Singh & Gupta (2022) [12] compared the antioxidant and antimicrobial properties of tasar sericin from different regions in India. Their research indicated that regional variations significantly influence the bioactive components of tasar sericin, emphasizing the importance of conducting comparative studies to optimize sericin for specific industrial applications. This research focuses on a comparative analysis of the antioxidant and antimicrobial properties of tasar sericin collected from different ecological regions of India. By examining how regional factors influence the bioactivity of tasar sericin, this study aims to provide insights into optimizing its applications in various industries. This could lead to ecological region-specific strategies for sericin utilization, supporting sustainable and targeted bio-product development.

MATERIALS AND METHODS

Study Area and Sample Collection

The study focuses on tasar sericin extracted from silkworms reared in four distinct ecological-regions of India, chosen for their unique environmental and climatic conditions: South Bihar, characterized by a semi-arid climate with moderate rainfall; Jharkhand, known for its humid subtropical condition, with dense forest and moderate to high rainfall; Chhattisgarh, experiences a tropical wet and dry climate, with well-defined seasons of heavy rainfall and dry conditions; Odisha, coastal region with a tropical monsoon climate, high humidity, and a diverse range of flora. Tasar cocoons were collected from local farmers in each region during the peak sericulture season. The collected cocoons were immediately transported to the laboratory for processing and sericin extraction following [6, 7].

Sericin Extraction Procedure

Sericin extraction was conducted using a hot-water degumming method, modified from the standard protocol described by Aramwit et al. (2012) [2]. This technique was selected because it effectively isolated sericin without sacrificing its bioactivity. To put it briefly, the cocoons were cleaned to remove impurities and then cut into small pieces, which were then boiled in distilled water at 100°C for 30 minutes while being constantly stirred. To aid in the degumming process, 0.5% (w/v) sodium carbonate (Na_2CO_3) was added, and the boiled mixture was filtered through a Whatman No. 1 filter paper to separate the sericin solution from fibroin fibers. The filtered sericin solution was then allowed to cool to room temperature, and the pH was adjusted to 4.5 using acetic acid to precipitate sericin, which was then centrifuged at 5000 rpm for 15 minutes. The collected sericin was then freeze-dried and stored at -20°C for additional analysis.

A 0.1 mM DPPH solution was prepared in methanol, and sericin extracts (1 mg/mL) were mixed with the DPPH solution in a 1:1 ratio. The mixture was then incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer, and the radical scavenging activity was expressed as a percentage inhibition relative to a control. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to gauge the sericin extracts' capacity to scavenge free radicals.

ABTS Radical Cation Decolorization The antioxidant capacity of sericin was quantified using the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assay, which was adapted from Sinha & Choudhary (2020) [8]: ABTS was mixed with potassium persulfate to create an ABTS radical cation solution, which was then left in the dark for 12 hours. The ABTS solution was diluted with ethanol until it reached an absorbance of 0.70 at 734 nm. After six minutes, sericin extracts were added to the ABTS solution, and the absorbance at 734 nm was measured. The absorbance reduction was used to determine the percentage of inhibition.

Ferric Reducing Antioxidant Power To determine the reducing power of sericin extracts, the FRAP assay was performed using the approach described by Patil & Kulkarni (2021) [10]: Acetate buffer (300 mM, pH 3.6), TPTZ (2,4,6-tripyridyl-s-triazine) solution, and ferric chloride ($FeCl_3$) were combined to create a functioning FRAP reagent. After adding 1 mg/mL of sericin extracts to the FRAP reagent, the mixture was incubated for 10 minutes at 37°C. At 593 nm, the absorbance was measured. The findings were reported in µM Fe2+ equivalents per sericin gram.

Antimicrobial Activity

According to Roy & Bose (2017) [9], the agar well diffusion method was used to assess the antibacterial effectiveness of tasar sericin: For the antimicrobial tests, three test microorganisms were chosen: the fungal strain Candida albicans (ATCC 10231), the Gram-positive bacteria Staphylococcus aureus (ATCC 6538), and the Gram-negative bacteria *Escherichia coli* (ATCC 25922). Getting bacterial cultures ready for twenty-four hours, bacterial and fungal strains were cultivated in nutrient broth at 37°C. The cultures were adjusted to a turbidity of 0.5 McFarland standards, equivalent to approximately 1.5×10^8 CFU/mL.

Agar Diffusion Method

After preparing Muller-Hinton agar plates, the microbial cultures were equally inoculated onto the surface. The sericin extract (10 mg/mL) was applied to 100 µL of wells (6 mm in diameter) that had been punched in the agar plates. For 24 hours for bacteria and 48 hours for fungi, plates were incubated at 37°C.To evaluate each sericin extract's antibacterial efficacy, zones of inhibition were measured in millimeters [5]. ANOVA (Analysis of Variance) was used to statistically analyze data from antioxidant and antimicrobial assays in order to assess the significance of differences between sericin samples from different regions; a p-value of less than 0.05 was deemed statistically significant. SPSS software, version 26.0, was used for statistical analysis [7].

RESULTS

Antioxidant Activity of Tasar Sericin Extracts

The antioxidant activity of tasar sericin extracted from different ecological regions of India was assessed using DPPH Radical Scavenging Assay, ABTS Radical Cation Decolorization Assay, and Ferric Reducing Antioxidant Power (FRAP) Assay. The sericin extracted from Odisha regions has the highest antioxidant activity, followed by Jharkhand, Chhattisgarh, and South Bihar ecological regions in all three assays (Table 1 and Figure 1).

Table 1. Antioxidant activity of tasar sericin extracts from different ecological regions of India.

Ecological Regions	DPPH Inhibition (%)	ABTS Inhibition (%)	FRAP (μM Fe^{2+} Equivalents/g)
South Bihar	58.6 ± 2.1	62.3 ± 1.8	450 ± 20
Jharkhand	65.4 ± 1.9	71.2 ± 2.3	520 ± 18
Chhattisgarh	60.8 ± 2.0	66.7 ± 1.7	480 ± 15
Odisha	68.5 ± 2.5	74.0 ± 2.1	540 ± 22

The DPPH assay indicates that tasar sericin from the Odisha region exhibited the highest radical scavenging activity (68.5%), followed closely by Jharkhand (65.4%) and Chhattisgarh (60.8%) and South Bihar showed the lowest DPPH inhibition (58.6%), suggesting that the antioxidant capacity of sericin may influenced by environmental factors, such as humidity, temperature, and host plant species.

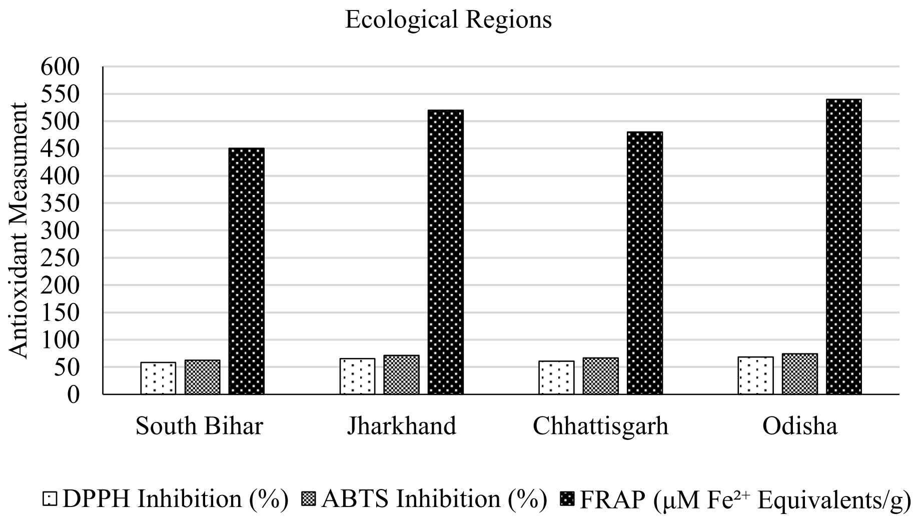

Figure 1. Antioxidant activity of tasar sericin extract from different ecological regions of India. Each bar represents the DPPH, ABTS, and FRAP assays within ecological regions, respectively.

The ABTS assay, Odisha and Jharkhand extracts again showed superior antioxidant activity, with inhibition percentages of 74.0% and 71.2%, respectively. These results are consistent with the DPPH assay, reinforcing the hypothesis that eco-regional factors play a crucial role in determining the bioactive components of tasar sericin. The slightly lower performance of sericin from South Bihar (62.3%) indicates that drier and semi-arid conditions might limit the bioactive properties of the extract.

The FRAP assay results mirrored the trends observed in the DPPH and ABTS assays. Odisha's sericin extract demonstrated the highest reducing power, followed by Jharkhand. The lowest FRAP values were recorded in South Bihar, indicating a lower content of compounds capable of electron donation, which correlates with antioxidant capacity.

Antimicrobial Activity of Tasar Sericin Extracts

Using the agar well diffusion method, the antibacterial activity of the sericin extracts was assessed. Table 2 lists the inhibitory zone sizes (in millimeters) for the three test microorganisms: the fungal strain *Candida albicans*, the Gram-positive *Staphylococcus aureus*, and the Gram-negative *Escherichia coli*.

The antimicrobial analysis revealed that tasar sericin from Odisha displayed the most effective antibacterial activity, with inhibition zones of 16.8 mm for *E. coli* and 19.5 mm for *S. aureus* (Figure 2). This might be the effect of environmental conditions in coastal regions, which enhances the production of bioactive peptides with antibacterial properties. Jharkhand's sericin extract showed comparable results, while the extracts from South Bihar demonstrated the weakest antibacterial activity, likely due to the influence of drier climates.

The sericin extracted from the Odisha ecological region also exhibited the highest antifungal activity (15.1 mm), followed by Jharkhand (14.2), Chhattisgarh (13.0), and South Bihar (11.5) (Figure 2). This trend suggests that the antioxidant compounds contributing to antimicrobial properties are more abundant in regions with high humidity and diverse vegetation.

Table 2. The inhibitory zone sizes (in millimeters).

Ecological Regions	***E. coli (mm)***	***S. aureus (mm)***	***C. albicans (mm)***
South Bihar	12.4 ± 0.6	14.8 ± 0.4	11.5 ± 0.5
Jharkhand	15.6 ± 0.7	18.3 ± 0.8	14.2 ± 0.6
Chhattisgarh	13.5 ± 0.5	16.7 ± 0.5	13.0 ± 0.3
Odisha	16.8 ± 0.9	19.5 ± 0.6	15.1 ± 0.7

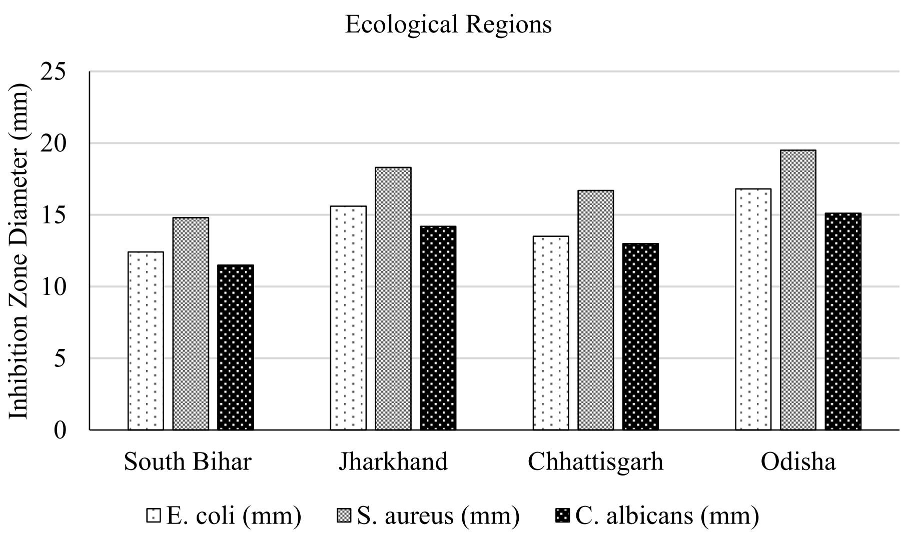

Figure 2. Antimicrobial activity of tasar sericin from different ecological regions of India. Each bar represents inhibition zone diameters for *E. coli*, *S. aureus*, and *C. albicans*, respectively.

DISCUSSION

The information suggests that the antibacterial and antioxidant qualities of tasar sericin are strongly influenced by the ecological regions of origin. Odisha's sericin continuously exhibited the highest bioactivity, which is explained by the state's distinct climatic factors, including its high humidity, tropical vegetation, and varied flora. These factors may increase the concentration of certain bioactive compounds in sericin, enhancing its efficacy Verma & Sharma (2019) [11] have highlighted the importance of eco-regional factors in the chemical composition of non-mulberry silks, suggesting that environmental stressors could trigger biochemical pathways that enhance the production of bioactive peptides and antioxidants in sericin.

Findings of the present study are aligned with previous research, indicating that non-mulberry silks have superior antioxidant and antimicrobial properties compared to mulberry silks Reddy & Yang (2011) [3] established that sericin's bioactivity is closely linked to the host plant species, and regional climatic conditions are in support of our results. The significant variation in antioxidant and antimicrobial efficacy between the ecological regions highlights the need for targeted research to optimize sericin production based on environmental factors. Future studies could focus on understanding the specific biochemical pathways influenced by different climatic conditions and their impact on sericin's bioactivity.

The antioxidant and antimicrobial properties of tasar sericin extracted from four eco-regions of India: South Bihar, Jharkhand, Chhattisgarh, and Odisha, illustrate how environmental factors significantly influence the bioactive properties of tasar sericin, underlining the importance of eco-regional selection in sericulture. Sericin from the Odisha region exhibited the highest antioxidant capacity, with superior DPPH and ABTS inhibition values and elevated FRAP levels. These findings suggest that Odisha's climate and vegetation create optimal conditions for producing sericin with potent antioxidant effects. Gupta et al. (2018) [7] found that non-mulberry silks from humid and tropical regions tend to exhibit stronger antioxidant properties due to the higher presence of phenolic and aromatic amino acids in their sericin extracts. The sericin extract from Jharkhand and Chhattisgarh demonstrated notable antioxidant activity, indicating that sericin from these regions possesses valuable bioactive potential. In contrast, South Bihar's extracts showed comparatively lower antioxidant properties, likely due to its semi-arid climate [8]. Also suggested that higher rainfall and dense forest vegetation contribute to a more diverse range of bioactive compounds, potentially explaining the differences observed in the present study.

The results indicate that sericin from Odisha contains higher concentrations of antimicrobial compounds. Jharkhand's sericin samples also performed well, particularly against bacterial strains, while extracts from Chhattisgarh and South Bihar were less effective. The Sericin extracted from the Odisha ecological region showed the strongest antimicrobial activity, achieving the largest inhibition zones for Escherichia coli, Staphylococcus aureus, and Candida albicans. The variation across regions emphasizes the influence of local environmental conditions on the antimicrobial efficacy of tasar sericin [9]. Similarly, reported higher antimicrobial efficacy in non-mulberry silks from tropical and subtropical areas and Patil & Kulkarni (2021) [10] reported higher moisture levels during sericulture may increase the fungal resistance of sericin, likely due to the presence of phenolic compounds that inhibit fungal growth.

The results of this study highlight the potential of tasar sericin from specific eco-regions, particularly Odisha and Jharkhand, as a source of natural antioxidants and antimicrobial agents. This makes sericin a promising candidate for applications in food preservation, cosmetics, skincare, and pharmaceuticals. The clear eco-regional differences in bioactivity suggest that targeted cultivation in specific regions could optimize sericin production for desired properties, enhancing its economic and industrial value.

Expanding the scope to include more diverse eco-regions would provide a more comprehensive understanding of how environmental factors affect sericin properties. Future studies should concentrate on identifying and separating the precise tasar sericin components that give it its bioactive properties. This might open the door to more effective, customized sericin-based products.

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