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Research Article

EXTRACTION AND EVALUATION OF ANTIMICROBIAL POTENTIAL OF *ANTHERAEAMYLITTA* SILK SERICIN

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ABSTRACT

The cocoons of silkworms consist of two major proteins a hydrophobic fibroin and hydrophilic sericin. The fibroin protein recognized as biomaterial for various biomedical applications as surgical sutures, hydrogels, scaffolds and nanoparticles as a drug delivery vehicle in addition to textile uses. Silkworm produces a large amount of sericinat the end of fifth instar along with the fibroin and form the silk thread used in the construction of the cocoon, which provides the ideal conditions for the larval metamorphosis to adults. The silk textile industries discardsericinas waste after degumming process. But recently research is initiated on biomedical applications of domesticated *Bombyxmori* silk sericin whereas Indian tropical wild tasar silk proteins related research is still in juvenile stage. Hence in present study we extracted the sericin and evaluated its antimicrobial potential from Tasar silkworm *Antheraeamylitta* on pathogenic bacterial strains of *Escherichia coli*, *Staphylococcus aureus* and fungal strains of *Candida albicans*, *Aspergillusflavus.*

INTRODUCTION

Lepidopteran insects belong to the families *Saturnidae* and *Bombycidae* spins silk fibres. The domestic *Bombyxmori* silkworms belongs to Bombycidae and the Indian tropical wild *Antheraeamylitta* silkworms of saturnidae produces a delicate twin thread of silk protein fibroin, which is coated by a glue like hydrophilic sericin protein. Silkworms during pupation spin the cocoons to protect the inactive pupae. The silk proteins are synthesized by silk gland cells and stored in the lumen of the glands. The sericin protein is specifically biosynthesized in the middle silk gland of the mature silkworm larvae, which constitutes 25-30% of silk proteins (Aramwit *et al*., 2012). It is a water soluble globular protein family whose molecular mass ranges from 10 to 310 kDa (Wei*et al*., 2005). Naturally sericin is a hydrophilic, amorphous and glue-like proteinwhich helps tojoin both the fibroin filaments to maintain the structural integrity of the cocoon. During degumming process of silk textile industry sericin is removed as waste from fibroin to make silk fibers more lustrous, soft, smooth, white, and dye able (Gupta *et al*., 2013). The global discarded sericin constitutes approximately 50,000 tons out of the 1 million tons of fresh cocoons annually (Aramwit *et al*., 2012).

Silk sericin of *Bombyxmori* is one of the most researched proteins. It has been shown to be useful as a degradable biopolymer for forming articles, functional membranes, fibers and fabrics. Because of its nature sericin can be used in food, cosmetics and pharmaceutical products as well as for biomaterials manufacture (Wu *et al*., 2006). The sericin proved as antioxidant, anticoagulant and anti-wrinkle agent. It is also reported to suppress tumor growth and to reduce oxidative stress (Khampieng *et al*., 2015). But many studies on sericin of mulberry silkworms and their biomaterials are elucidated for adverse immunological response by sericin mediated macrophage activation, it is dependent on a physical association with the core fibroin fibers responsible for the allergic reaction and was observed in surgical sutures (Santin *et al*., 1999; Mandal *et al*.,2011). To overcome this major immunological issue we explored another major contributor of Indian silk industry the non-mulberry Indian tropical Tasar silk sericin as a potent replaceable source for for biomedical applications.

The wild sericigenous Indian tropical tasar silkworm *Antheraeamylitta* is a polyphagous insect; it has rich genetic resources of forty four races acclimatized to diverse ecological zones. In the course of evolution it has been evolved with many advanced qualities such as silk quality, fecundity, disease

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resistance and tolerance to various environmental conditions. The tasar cocoons are exposed to various biotic and abiotic environmental stresses. Therefore the quality of the silk and silk proteins is differing than of mulberry silk proteins (Ahmad *et al*., 2004). Most of the proteomics and genomics studies on sericin restricted with *Bombyxmori* and quite less information is reported on the non-mulberry silk sericin in perticulartasarsericin.

The domesticated *Bombyxmori* silk sericin contains 18 amino acids including polar amino acids such as 32% serine and 17% aspartic acid gives higher hydrophilic property and processing ability (Zhang *et al*., 2011). In contrast to this Tasarsericin contains 19% serine contents (Dash *et al*., 2008; Aramwit 2010). The Mulberry and Tasarsericins are biochemically distinct due to differences in their amino acid compositions, leading to differences in the immunological responses. Being non-domesticated and wild type, tasar cocoons are more resistant towards biotic and abiotic stresses such as microbial decomposition, heat, drying and the sericin coat may contributed for toughness and resistance properties (Dash *et al*., 2007). Therefore Tasarsericin has been proposed as a promising natural protein source for developing protein based biomaterials and potent antimicrobial agent.

With this insight, in this study we tried to develop effective, economic and simple method of extraction for pure and stable Tasarsericin for biomedical applications. The antimicrobial property of Tasarsericin was screened against pathogenic bacterial strains of *Escherichia coli*, *Staphylococcus aureus* and fungal strains of *Candida albicans*, *Aspergillusflavus*.

MATERIALS AND METHODS

***Collection of wild Anthereamylitta cocoons*:** Tasar cocoons were procured from Regional tasar research station, Warangal, Telangana, India.

***Extraction of TasarSericin*:** 5g of peeled *Antheraeamylitta* cocoons were boiled in 125 ml of 0.02M Sodium carbonate in 100˚c for 1 hour under refluxed condition and the same protocol was followed to extract sericin in Distilled water to compare the degumming ratio.The degumming ratio was calculated according to the formula

$$\text{Degumming ratio (\%)} = \frac{\text{Wo} - \text{Wf}}{\text{Wo}} \text{X100}$$

Where, W_o- initial weight of the cocoons and W_f- final weight of the cocoons after extraction.

***Lyophilization of Sericin*:** Post extracted sericin solution centrifuged at 5000rpm and supernatant was dialysed against distilled water in a dialysis tube (Spectra/Por. USA molecular porous membrane tubing, MWCO 6-8kDa) for 3 days with 2 changes to remove sodium carbonate. The dialysate was rota evaporated in Buchi rota evaporator at 40˚c to concentrate the sericin content. The concentrated solution of sericin was freeze dried in SCANVAC freeze dryer at -55˚c at 0.200 mpa pressure.

***Antimicrobial Assay of Sericin*:** Antimicrobial activity of Tasarsericinwas evaluated with slight modification by Agar well diffusion method on Lag phase bacterial strains of *Escherichia coli*, *Staphylococcus aureus* and antifungal activity on *Candida albicans*, *Aspergillusflavus*(Aneja *et al*., 2011). The bacterial cultures on nutrient agar plates (HimediaLaboratories Pvt Ltd, Mumbai) and the suspension of fungal spores were swab inoculated onto the sterile PDA plates (Himedia Laboratories Pvt Ltd, Mumbai) for four different concentrations of sericin in DMSO (25, 50, 75 and 100 µg/ml) into the respective labeled wells. Bacterial plates incubated at 37˚c for 24 hours and fungal plates were incubated at room temperature for 3 days. The zone of inhibition was measured in millimetres.

RESULTS

Extraction of Tasarsericin

The aqueous extraction and sodium carbonate extraction methods were compared to extract the sericin from Tasar cocoons. The sodium carbonate extraction is effective to remove the sericin content from the cocoons as shown in table 1.

Table 1 Methods of Sericin Extraction and Degumming ratio

Method of Extraction	Degumming ratio
Aqueous extraction Method	17.5%
0.02M Sodium carbonate extraction	26.6%

Antimicrobial activity of Tasarsericin

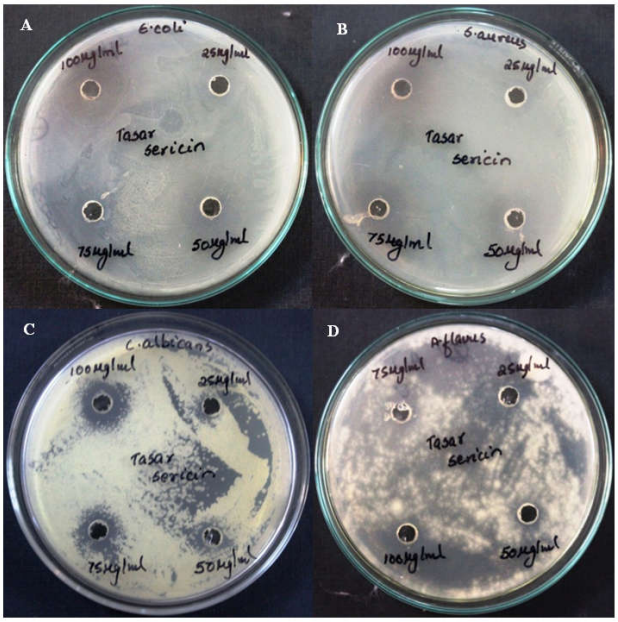

Figure 1 Antimicrobial potential of tasarsericin in different concentrations on A. *Escherichia coli* B. *Staphylococcus aureus*C. *Candida albicans* D. *Aspergillusflavus*

Table 2 Antimicrobial activity of Tasarsericin in different concentrations

Concentration Of Tasarsericin (µg/ml)	Average zone of Inhibition (mm)			
	Bacterial strains		Fungal strains	
	Escherichia coli	*Staphylococcus aureus*	*Candida albicans*	*Aspergillusflavus*
25	10.33±0.57	12.33±0.57	11.66±1.15	10.33±0.57
50	15.33±1.52	17.33±0.57	13.00±1.00	14.16±0.28
75	17.33±0.57	18.66±1.15	15.33±0.57	16.50±0.50
100	22.66±0.57	22.16±0.28	20.50±0.50	17.33±0.57

The results are expressed as Mean ±SD (n=3)

The antimicrobial activity of Tasarsericin at the concentrations of 25, 50, 75 and 100 µg, spontaneously inhibited the growth of bacterial strains of *Escherichia coli*, *Staphylococcus aureus*and fungal strains of *Candida albicans*, *Aspergillusflavus*(Fig.1). The maximum antibacterial activity was observed against *Escherichia coli* (22.66mm) followed by *Staphylococcus aureus*(22.16mm) at 100µg/ml of sericin concentration. At the same concentration of sericin anti-fungal activity on *Candida albicans* (20.50 mm) and *Aspergillusflavus* (17.33 mm) was observed. The antimicrobial potential of tasarsericin was mentioned in table 2.

DISCUSSION

Antimicrobial chemotherapeutic drugs have been universally used to control infectious diseases. But the alternative natural, biocompatible agents are encouraged over antibiotics due to the emergence of drug resistance in pathogenenic microorganisms. Hence interest is increasing in the exploration of antibacterial proteins with different mechanisms of action over conventional drug compounds. Various alternate mechanisms have been established other than for the known action of lysozyme like the human antibacterial psoriasin protein which causes pore formation on bacterial membranes and the antibacterial RNase 7 enzyme (Harder and Schroder, 2002; Michalek *et al*. 2009). The usage of silk protein such as sericin may provide the future world with a new futuristic antibacterial protein.

Recently mulberry sericin has reported for medical applications such as antioxidants, anticancer, anticoagulants and cell culture additive. Similarly Tasarsericin shown antioxidant activity to suppressive chemical-induced and UV radiation-induced skin tumerogenesis (Kundu *et al*., 2008). But antimicrobial potential of the Tasarsericin on pathogenic microorganisms still needs more research. We presume that being wild silkworm, tasar cocoons are highly exposed to several microorganisms hence we standardized the extraction method and long lasting sericin sample preparation by lyophilisation and evaluating the antimicrobial potential of Tasarsericin on selected pathogens. The methods of extraction of Tasarsericin for biomedical applications play an important role in biocompatibility and to avoid the chemical contaminations during extraction processes. The sericin was utilizing for biological applications, so extraction chemicals should not be hazardous or carcinogenic. We selected the non-toxic sodium carbonate and hot water extractions to avoid chemical toxicity and least degradation of sericin during extraction. The earlier studies on extraction conditions and the minimum molecular weight degradation of *Antheraeamylitta* sericin was evaluated by Soap-alkaline method, Aqueous-extraction method, Urea method, Urea-Mercaptoethanol method, NaCl and Na_2CO_3 methods. The Na_2CO_3 extraction method was reported most suitable for the extraction of tasarsericin, based on the yield, least molecular weight degradation and the optimum condition for the extraction was 0.02 M Na_2CO_3 extraction for 60 min was standardized (Yun *et al*., 2013). The Silk sericinafter lyophilisation can preserve and utilize for years(Kundu *et al*., 2014).

The antimicrobial property on bacterial and fungal strains revealed strong microbicidal action against *Escherichia coli*, *Staphylococcus aureus* and fungal strains of *Candida albicans* and *Aspergillusflavus*. Basically tasar cocoons sustain in wild environmental conditions, the antimicrobial potential can be achieved along with the architecture of cocoons and other chemical constituents, the peptides present in sericin may be responsible. Our results are contradictory to earlier report on antimicrobial activity of mulberry and wild silkworm sericin on E.coli (Kaur *et al*., 2014). It may be due to the different extraction conditions and chemicals used for the extraction process, might be denatured the functional antimicrobial peptides from the sericin. The previous studies on *Bombyxmori* cocoon proteins contain number of polypeptides which are amphipathic basic molecules that act as detergent on microbial cell membranes causing death of the microorganism by lysis (Pandiarajan *et al*., 2011). The two major small molecular polypetides seroin-1 and seroin-2 present in *Bombyxmori* sericin are reported as antimicrobial proteins (Zhang *et al*., 2015).Similar polypeptides may be present in Tasarsericin which may be the key factors to inhibit the microbial growth.

CONCLUSIONS

One of the most researched natural protein biopolymer *Bombyxmori* sericin was reported for adverse immunological response. The preparation and applications of biomaterials from it is the matter of concern. To overcome this issue, Indian native tropical *Antheraeamylitta* silk sericin; which is also the waste byproduct from Tasar silk textile industries can be explored as biocompatible natural biopolymer for biomedical applications. The extraction and lyophilization conditions made Tasarsericin powder as potent antibacterial and antifungal agent and confirmed it as a natural biopolymer to be utilized as microbicidal agent. But further proteomic studies, mechanism of action and pharmacological studies are required for medical applications. Ultimately Tasarsericin can be a preferable biopolymer over *Bombyxmori* sericin for biomedical applications.

Conflict of interest

Authors do not have any conflict of interest related to the manuscript.

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References

Ahmad, S.E., Kamra, A., Hasnain, S.E. (2004). Fibroin silk proteins from the nonmulberry silkworm *Philosamiaricini* are biochemically and immuno-chemically distinct from those of mulberry silkworm *Bombyxmori*. *DNA Cell Biol*., 23: 149-154.

Aneja, D.K., Lohan, P., Arora, S., Sharma, C., Aneja, K. R., & Prakash, O. (2011). Synthesis of new pyrazolyl-2, 4-thiazolidinediones as antibacterial and antifungal agents. *Org Med Chem Lett*., 1(1)15: 1-11.

Aramwit, P., Siritientong, T., Srichana, T (2012).Potential applications of silk sericin, a natural protein from textile industry by products.*Waste Manag. Res*. 30: 217-224.

Dash, R., Acharya, C., Bindu, P. C., & Kundu, S. C. (2008). Antioxidant potential of silk protein sericin against hydrogen peroxide-induced oxidative stress in skin

fibroblasts. *BMB Reports*.,41: 236-241.

Dash, R., Ghosh, S. K., Kaplan, D. L., & Kundu, S. C. (2007). Purification and biochemical characterization of a 70 kDa sericin from tropical tasar silkworm, *Antheraea mylitta*. *Comp Biochem Physiol B Biochem Mol Biol*, 147(1): 129-134.

Gupta, D., Agrawal, A., & Rangi, A. (2014). Extraction and characterization of silk sericin. *Indian J Fibre Text Res*, 39(4): 364-372.

Harder, J. and Schroder, J.M. (2002). RNase 7, a novel innate immune defense antimicrobial protein of healthy human skin. *J. Biol. Chem*, 277: 46779- 46784.

Kaur, J., Rajkhowa, R., Afrin, T., Tsuzuki, T., Wang, X., 2014. Facts and myths of antibacterial properties of silk. *Biopolymers*,101: 237-245.

Khampieng, T., Aramwit, P., & Supaphol, P. (2015). Silk sericin loaded alginate nanoparticles: preparation and anti-inflammatory efficacy. *Int. J. Biol. Macromol.*, 80: 636-643.

Kundu, B., Kurland, N.E., Yadavalli, V.K., Kundu, S.C.(2014). Isolation and processing of silk proteins for biomedical applications. *Int. J. Biol. Macromol*.,70: 70-77.

Kundu, S.C., Dash, B.C., Dash, R., Kaplan, D.L., (2008). Natural protective glue protein,sericin bioengineered by silkworms: Potential for biomedical and biotechnological applications. *Prog. Polym. Sci*., 33: 998-1012.

Mandal, B. B., Ghosh, B., & Kundu, S. C. (2011). Non-mulberry silk sericin/poly (vinyl alcohol) hydrogel matrices for potential biotechnological applications. *Int. J. Biol. Macromol*, 49(2): 125-133.

Michalek, M., Gelhaus, C., Hecht, O., Podschun, R., Schröder, J.M., Leippe, M. and Grötzinger J. (2009). The human antimicrobial protein psoriasin acts by permeabiliza- tion of bacterial membranes. Developmental & Comparative Immunology,33: 740-746.

Pandiarajan, J., Cathrin, B. P., Pratheep, T., & Krishnan, M. (2011). Defense role of the cocoon in the silk worm *Bombyx mori* L. *Rapid Commun Mass Spectrom*, 25(21): 3203-3206.

Santin, M., Motta, A., Freddi, G., & Cannas, M. (1999).In vitro evaluation of the inflammatory potential of the silk fibroin. *J Biomed Mater Res B ApplBiomater*, 46(3): 382-389.

Wei, T., Li, M. Z., & Xie, R. J. (2005).Preparation and structure of porous silk sericin materials. *Macromol.Mater. Eng*., 290: 188-194.

Wu, J. H., Wang, Z., & Xu, S. Y. (2007). Preparation and characterization of sericin powder extracted from silk industry wastewater. *Food Chem.*, 103(4): 1255-1262.

Yun, H., Oh, H., Kim, M. K., Kwak, H. W., Lee, J. Y., Um, I. C., ... & Lee, K. H. (2013). Extraction conditions of *Antheraea mylitta* sericin with high yields and minimum molecular weight degradation. *Int. J. Biol. Macromol*, 52: 59-65.

Zhang, H., Deng, L., Yang, M., Min, S., Yang, L., & Zhu, L. (2011). Enhancing effect of glycerol on the tensile properties of *Bombyx mori* cocoon sericin films. *Int. J. Mol. Sci.*, 12(5): 3170-3181.

Zhang, Y., Zhao, P., Dong, Z., Wang, D., Guo, P., Guo, X., … Xia, Q. (2015). Comparative proteome analysis of multi-layer cocoon of the silkworm, *Bombyx mori*. *PLoS One*, *10*(4): 1-14.

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