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FT-IR ANALYSIS AND ANTI BACTERIAL ACTIVITY OF SILK SERICIN

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ABSTRACT

Realization of current trends and innovative uses of insect fiber, silk is required to exploit its compatibility, eco friendly and value addition potential. An adhesive silk protein sericin has high potential for use in biomedical applications. It has important properties such as excellent oxygen permeability, cell protecting and antioxidant action, moisture regulating ability, protection from ultraviolet (UV) radiation and microbes, wound healing, anticancer and anticoagulant properties. Hence this study was taken to find out the bioactive functional groups present in silk extract of Sericin for their antibacterial activity. The characterization and presence of functional groups in sericin was achieved by Fourier Transform Infrared Spectroscopy (FT-IR) analysis. Antibacterial activity of silk extract of sericin was evaluated against two pathogenic bacterial species, namely gram negative bacterial pathogen *Escherichia coli* and gram positive bacterial pathogen *Staphylococcus aureus* by agar well diffusion method. The FT-IR analysis revealed the presence of functional groups such as primary amine, carboxylic acid, alkane, aromatic ring and alcohol. Major peaks were observed at 3425.58 and 1064.71 cm^{-1} which corresponds to N-H and O-H functional groups respectively. Both pathogenic bacterial species were sensitive to silk extract of sericin. For the treatment of diseases associated with these pathogenic bacteria the silk extract of sericin have promising antibacterial potential and could be effective natural medicine.

KEYWORDS: Sericin, FT-IR, Agar well diffusion, *Escherichia coli, Staphylococcus aureus*

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INTRODUCTION

Silk has played an important role in the economic life of man. Even today the *Queen of Textiles* continues to reign supreme as natural silk. The texture, luster, comfort, tensile qualities, adaptability to all climatic conditions and the ability to exhaust dyes are the major qualities of silk. Silk is a product of special insects that belongs to the category of Lepidoptera order. The most extensively characterized silks are from spiders (*Nephila clavipes and Araneus diadematus*) and from the domesticated silkworm *Bombyx mori.* Silks are fibrous proteins, which were spun into fibres by insects. For some vital function these fibers are used by insects like protecting their larvae or eggs, for dragline formation and to form capture nets that are able to trap insects and bear high impacts. An essential constituent of cocoon filament is silk protein. Silk proteins were produce from the silk gland cells which are getting stored in the lumen of the silk glands. Subsequently, these proteins are converted into silk fibres. When the silkworms secrete the liquid silk, it passes via the anterior gland and at the time of spinning it comes out through the spinneret opening[8]. Silk derived from silkworm *Bombyx mori* is a natural protein that is mainly made of fibroin and sericin proteins.[1]

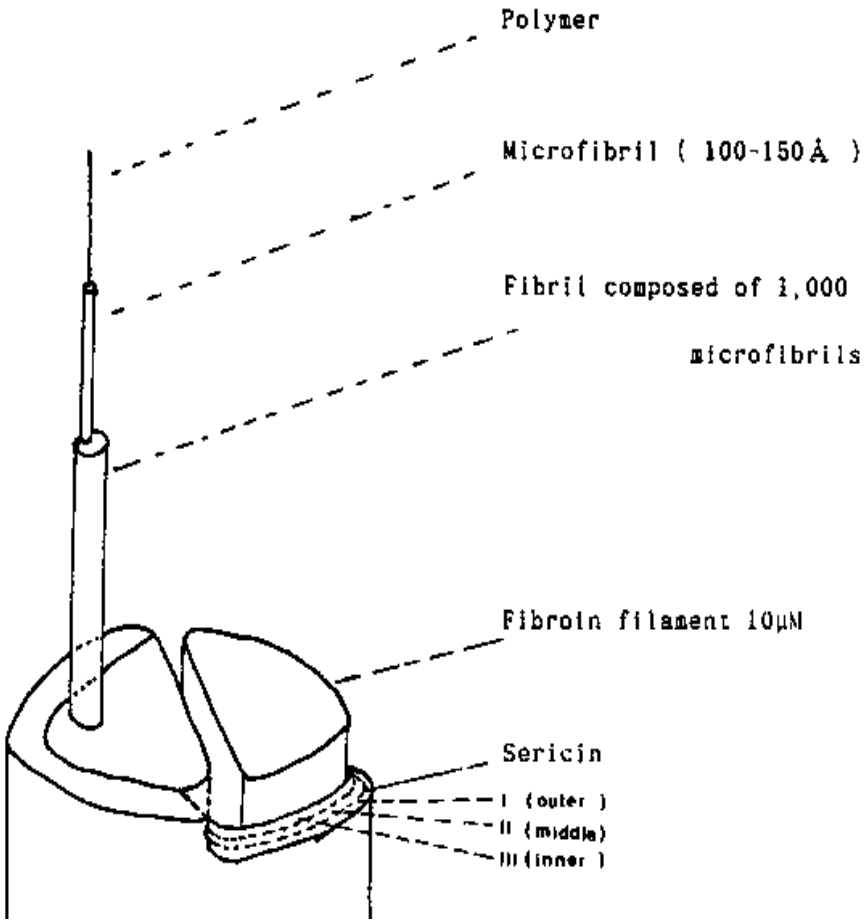


Figure 1
Texture of silk

Natural fibrous protein spun from the *Bombyx mori* is a Silk fibroin (SF). The silk fibroin consists of a light chain (*M*w ~26 kDa) and a heavy chain (*M*w ~390 kDa) linked by a disulfide bonds. Silk fibroin is a block copolymer which is rich in hydrophobic – sheet - forming blocks linked by small hydrophilic linker segments or spacers.[14] Due to higher concentration of hydrophobic amino acids it is insoluble in water.[9]

Table 1
Aminoacid composition of fibroin

Name of Amino acids	Silk Fibroin
Glycine	42.8
Leucine	0.7
Glutamic acid	1.7
Threonine	1.2
Alanine	32.4
Isoleucine	0.9
Serine	14.7
Valine	3.0
Phenylalanine	1.2
Histidine	0.3
Tyrosine	11.8
Lysine	0.5
Aspartic acid	1.9
Proline	0.6
Methionine	0.2
Tryptophan	0.5
Cystine	0.1

High content of the amino acids in the fibroin is glycine (42.8 g) and alanine (32.4 g) respectively as shown in (table 1).[10] Silk I and Silk II are the two types of molecular conformation of the secondary structure of Silk fibroin. Metastable form of silk fibroin is silk I and it is soluble in water and non-crystalline. In addition, the helix conformations are called silk I. Highly stable, organized structure and sheet conformation is silk II. It is insoluble in water.[13] Silk fibroin has highly oriented crystalline domain and the fibroin content of naturally spun silk fibers can be separated from sericin by degumming process.[11] Their specific functional properties like biocompatibility, good oxygen and water vapor permeability and the bio degradability made them to widely usage in tissue engineering field.[12] Silk sericin is the adhesive protein and it is a group of soluble glycoproteins, which is biosynthesized exclusively in the middle silk gland. These proteins cover the surface of fibroin, which is the silk filament core protein in the cocoon filament to fill the gaps and to enhance the toughness of the cocoon fiber.[14] It constitutes 25-30% of silk fiber mass. Sericin protein ensures the cohesion of the cocoon by gluing silk threads together. Sericin must be removed at the reeling mill and the other stages of silk processing, during silk production. At present, in silk processing wastewater sericin is mostly discarded. About 50,000 tons of sericin is produced while processing the raw silk. If this sericin protein is recovered and recycled, it can give a significant economic and social benefit. Sericin is a molecular protein and its molecular weight ranges from 10 – 300 kDa.[1] It is made of 18 amino acids, most of which have strong polar side chains like hydroxyl, carboxyl and amino groups. In addition, its high hydrophilicity arises from the high content of aspartic acid (16.7%) and serine (33.4%) of sericin.[2]

Table 2
Amio acid composition of hot water soluble sericin protein

Amino acids	Mol%
Ser	28.004
Arg	3.516
Phe	0
Asp	17.970
Thr	7.777
Lys	3.722
His	1.316
Ile	0.785
Met	0
Leu	1.211
Val	3.767
Glu	6.249
Ala	5.200
Tyr	2.870
Gly	16.289
Pro	0
Cys	0.691

Among these amino acids approximately 72.11% are polar amino acids. These strong polar side groups of amino acids were able to cross-link covalently with other enzymes by using bifunctional reagent.[5] Sericin is a type of water soluble protein, when it is hydrolyzed in alkaline or acid solutions, or dissolved in a polar solvent, or degraded by a protease, then the size of the resulting sericin molecules depends on following factors such as

pH, temperature and processing time. If sericin peptides have a high molecular weight it can be used as compound polymers, functional bio membranes, hydrogels, functional fibres and fabrics, medical and degradable biomaterials. Low molecular weight of sericin peptides can use in cosmetics.[3] Sericin is insoluble in cold water, but it is easily hydrolyzed, where long protein molecules breakes down to give smaller fractions, which are easily solubilised or dispersed in hot water. Various scientific knowledge classified the sericin of cocoon shell into two major classes: α-sericn and β-sericin .The inner layer of cocoon shell is made of β-sericin while outer layer is made of α-sericin. The α-sericin contains more N and O and less C and H than the β-sericin. Hence α-sericin have higher solubility than β-sericin in hot water. Sericin contains different amino acids in different amount but not as same as fibroin.[4] It is useful because of its special properties like resists oxidation, UV resistant, absorbs and release moisture easily, anti bacterial, inhibitory activity of kinase and tyrosine.[3] Hence sericin can be coated on some textile fabrics like wool, cotton and polyester in the powder form by the different processing method, to enhance the properties of those textile fabrics.[15] A variety of sophisticated techniques are available for the identification of physical and chemical structures amino acid composition in sericin. However spectroscopic and chromatography techniques are the most popular tools used for that purpose. Fourier Transform Infrared Spectrometry (FT-IR) is a physio-chemical analytical technique which is employed to determine the structure of unknown constituents. In addition, it will measure the intensity of the absorption spectra associated with the molecular composition of the chemical group. Moreover, FT-IR Spectroscopy is an established time saving tool to characterize and identifies functional groups in the unknown constituents.[6] Silk fibre is being used as surgical sutures for a long while, as it does not cause inflammatory reactions and is absorbed after wounds heal. Sericin makes silk fibre a good entrant for biomedical joining and sealing applications because of its tenacity and gum like quality. The sericin was reported to suppress tumor promotion, exert other health supporting effects like cell proliferation to provide a basic matrix for wound healing and provide protection against ulcers. It is also used in clinical diagnostic techniques, lowering blood glucose levels and production of medically important enzymes.[7] Though many reports are available on the biological activities of sericin protein but pharmacological knowledge of silk extracts of sericin protein is scanty. Therefore, in the present study, silk extracts of sericin protein powder were considered for its antibacterial activity and FTIR analysis.

MATERIALS AND METHODS

Material

The Silk degumming waste was collected from Central Silk board industry of Hosur, Tamilnadu.

Extraction procedure

Hot water extraction method was used for the extraction of sericin in powder form.[16] 20 litres of degumming waste was boiled and evaporated at 120^0c in the room temperature using vessel for 2hrs. The residue would deposit at the bottom of the vessel which is then dried under direct sunlight to obtain the required sericin in the powder form. At the end of the extraction process, the sericin powder is weighed about 20g. The sericin powder will be stable than in the solution form to avoid contamination.

Yield of extract

Yield of silk extracts of sericin powder was about 20g from 20litres of degumming waste (aqueous extract).

FT-IR analysis

The presence of functional groups in the silk extracts of sericin was analyzed by FT-IR Spectrophotometer. The molecular functional vibration of chemical groups present in the sample was recorded with Perkin-Elmer FT-IR Spectrophotometer, ranging from 4000-500 cm^{-1}. The spectral data obtained were compared with the reference chart to identify the functional groups present in the sample.

Bacterial species

Two pathogenic bacterial species namely gram negative pathogen *Escherichia coli* (MTCC443), gram positive pathogen *Staphylococcus aureus* (MTCC737) were used for testing the antibacterial activity. The bacteria were cultured on nutrient agar slants and subcultured periodically.

Antibacterial bio assay

Antibacterial activity of Silk extract was evaluated by agar well diffusion method.[18-20,23,24]. Muller Hinton Agar and Muller Hinton Broth were swabbed (sterile cotton swabs) with 18hrs old – broth culture using respective bacteria. In these petri dish, agar wells were made using sterile cork borer of 9mm diameter and about 2cm apart. Stock solution of silk extract of sericin was prepared at concentration of 10-100 µg/ml in Dimethyl sulphoxide (DMSO) as a solvent. About 100µl of different concentrations of silk extract was added into the wells and allowed to diffuse at room temperature for 2hrs. Control experiments comprising inoculum without silk extract was prepared. The petri dish was incubated at 37^o C for 18 – 24 Hours for bacterial pathogens. The diameter of zone of inhibition (mm) was measured and the activity index was also calculated.

Statistical analysis

Results obtained in the present study were analyzed for one way analysis of variance (ANOVA) to find out significant differences between sample means, with significant level being considered at $p<0.05$. All data are expressed as Mean ± standard deviation of ten values (n=10) obtained from ten separate runs.

RESULTS AND DISCUSSION

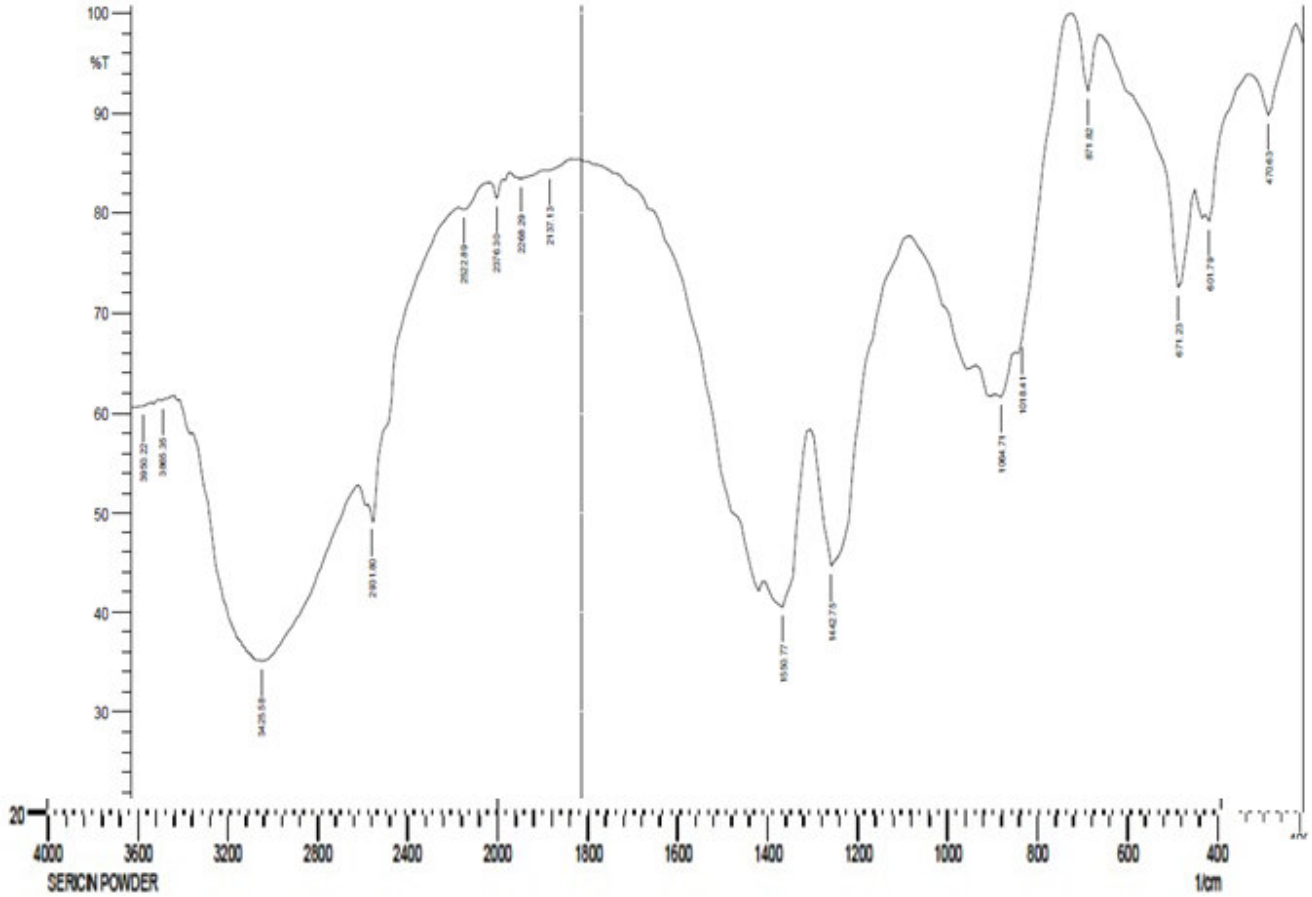
FT-IR spectroscopy analysis of silk sericin powder

The FT-IR spectrum of aqueous silk extracts of sericin is shown in (Fig.2). Results regarding FTIR analysis of silk extracts are in accordance with other findings tool[17,21,22]. Our FT-IR analysis revealed the presence of amine, carboxylic acid, hydroxyl, nitriles, alkane, alkyne, aromatic, nitro, alcohol, ether and ester. Major peaks were observed at 2000 - 3000 cm^{-1} which correspond to C≡C, C≡N, O-H, C-H functional groups, respectively which shown in (Table 3)

Table 3
FT-IR profile of silk extract of sericin

S.No	Absorption frequency/ Wavenumber(cm^{-1})	Functional groups
1.	3425.58	Primary Amine (N-H bond)
2.	2931.80	Carboxylic acid(C-H Stretch)
3.	1442.75	C=C aromatic ring
4.	1064.71	Alcohol(O-H stretch)
5.	871.82	Alkane (C-H bond)

Figure 2
FT-IR spectrum of silk extract of sericin



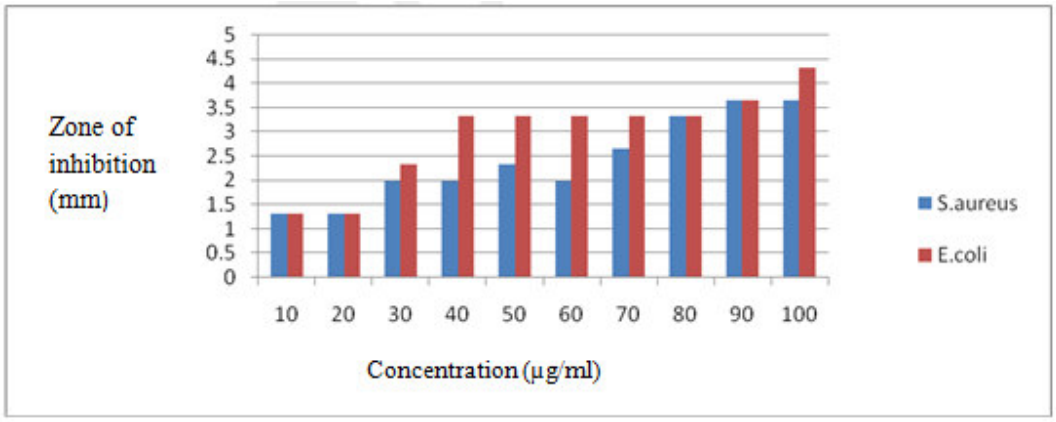
Antibacterial activity of silk extract of sericin

Silk extract of sericin is evaluated for antibacterial activity against different pathogenic bacteria species (*Escherichia coli, Staphylococcus aureus*). The antibacterial activity of silk extract of sericin was analysed by agar well diffusion method at different concentrations (10- 100 µg/ml) and inhibitory efficacy towards the tested pathogenic species were showed by silk extract of sericin. The maximum activity was observed against *Staphylococcus aureus* (3.66 ± 0.57 mm) and *Escherichia coli* (4.33 ± 0.57mm) as shown in the (table 4).

Table 4
Zone of inhibition of silk extract of sericin against different bacteria

Pathogenic Bacteria	Zone of inhibition (mm)									
	Concentration(µg/ml)									
	10	20	30	40	50	60	70	80	90	100
Staphylococcus.aureus	1.33±0.57	1.33±0.57	2±0	2±0	2.33±0.57	2±0	2.66±0.57	3.33±0.57	3.66±0.57	3.66±0.57
Escherichia.coli	1.33±0.57	1.33±0.57	2.33±0.57	1.33±0.57	3.33±0.57	3.33±0.57	3.33±0.57	3.33±0.57	3.66±0.57	4.33±0.57

Graph 1
Inhibitory concentration values of silk extract of sericin against different bacteria species



From the test result it was observed that, silk extracts works on a dose dependent manner that is when the concentration of sericin increases the activity also increases. Presence of amine, alkanes, hydroxyl, nitriles, alkyne, aromatic, alcohol, and ester in the silk extracts of sericin which is indicated by FT-IR analysis might be responsible for the present antibacterial activity. These medicinally bioactive components exert antimicrobial action through distinct mechanisms. Results regarding the antibacterial activity of the silk extracts are in accordance with other findings that the sericin had strong antibacterial activity against a wide panel of tested pathogenic bacteria. The results of our study are promising in the aspect of a drug discovery for both *Staphylococcus aureus* and *Escherichia coli* which are troublesome pathogens. These pathogens are found to be sensitive to the crude extracts of silk extract of sericin.

CONCLUSION

In the present study, silk sericin is a rich source of antibacterial compounds, with the extracts exhibiting strong antibacterial activity. The extract showed N-H, C-H, C=C, C-O, C-H are the major groups, which indicate the presence of primary amine, carboxylic acid, aromatic ring, alcohol and alkane compounds with regard to the functional group of compounds. The results were used to develop safe and effective drugs for the treatment of infectious diseases and may serve as a scientific basis. However, especially with regard to in vivo toxicity, should be conducted, as additional studies.

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Conflict of interest
Author declared no conflict of interest.

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