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Extraction and characterization of sericin obtained from unreelable cocoons of *Bombyx mori L.*

Aabid Ahmad Bhat[1]; Aabid Khaliq Tantray[1]; Phalisteen Sultan[2]; Mir Shahid Maqbool[3]; Sadaqat Farooq[4]; Lubna Altaf[1]; Khursheed Ahmad Sahaf[1] and Irfan Latief Khan[1]

Address:

[1]College of Temperate Sericulture, Mirgund, Sher-e-Kashmir University of Agricultural Science and Technology of Kashmir (SKUAST-K), Shalimar, J&K, India

[2]Division of Plant Biotechnology, CSIR - Indian Institute of Integrative Medicine, Sanatnagar, Srinagar - 190005, J&K, India.

[3]Cancer Pharmacology Division, CSIR - Indian Institute of Integrative Medicine, Sanatnagar, Srinagar - 190005, J&K, India.

[4]Fermentation and Microbiology Division, CSIR - Indian Institute of Integrative Medicine, Sanatnagar, Srinagar - 190005, J&K, India.

*Corresponding author: ***Aabid Ahmad Bhat*** e-mail*: aabidbhat905@gamil.com*

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ABSTRACT

Silkworm, *Bombyx mori* L. cocoon is a protective covering consisting of two important proteins, sericin and fibroin. Sericin, a water-soluble protein that represents about 30% of the cocoon weight is perse dissolved by cooking to unwind the silk filament from the cocoon during reeling. In the present study, the sericin was extracted from cut cocoons. Extraction was carried out by two methods i.e., open boiling and autoclave method at different time regimes ,30, 60 and 90 min. The recovery of crude sericin powder was done by hot air drying and freeze drying. Maximum recovery (14.12 ± 0.05 %) of crude sericin powder was obtained when extraction was carried out by autoclave method and drying by lyophilization. The sericin samples were studied for qualitative, quantitative, and biological criteria. The autoclave extraction showed maximum protein content and protein purity as compared to open boiling method. The maximum gelation formation in the extracted samples was observed for 60 minutes followed by a time of 30 minutes. Differential molecular weight protein bands were observed at three-time intervals. A molecular weight of 15-25 KDa was obtained in the sample extracted for 30 min and 60 min. While reduced molecular weight ranges of 10-15 KDa were obtained when extraction was carried for 90 min. The sericin showed concentration dependent antioxidant activity to the tone of 64 ± 7 % at 40 mg/mL concentration. Sericin did not show any antibacterial and antifungal activity. Results of this study suggests sericin protein can be used in cosmetic, pharmaceutical and food industry.

Keywords: *Bombyx mori* L., Sericin Extraction, SDS - PAGE electrophoresis, antibacterial and antifungal

INTRODUCTION

Silkworm is a symbolic monophagous insect, feeding on mulberry leaves as its sole food. After completing the larval stage, the silkworm surrounds itself in a hard protein shell called a cocoon, where the transformation of the larva into the pupa takes place. Silkworm cocoon is a complex structure consisting of two complementary essential proteins., fibroin and sericin. About 20-30% of the cocoon's total weight consists of sericin (Abhilasha and Lalit., 2015). Sericin encapsulates and protects the fibroin fiber with sequentially layers but sericin is usually discarded and is considered as a waste by-product (Mondal *et al.,* 2007). The foremost amino acid compositions in sericin are 32% of serine, 16% glycine and 18% aspartic acid (Kwang *et al*., 2003). The total amount of hydroxyl amino acids in sericin is 45.8 percent. There are 62.93 percent of polar amino acid and 37.07 percent of non-polar amino acid residues in sericin (Padamwar and Pawar., 2004). The occurrence of polar groups in its amino acid side chains effect their solubility which led to its classification into 3 fractions A, B and C (Dong *et al*., 2019). Mainly sericin occurs in an unstructured random coil and to a slighter extent in a β-sheet organized structure (Gulrajani *al*., 2009). Efforts to recover and salvage it as a natural biopolymer have been ongoing for many applications. Sericin shows several important useful properties such as anti-tyrosinase (Sarovart *et al*., 2003), antioxidant **(**Takechi *et al*., 2014**)** moisture engrossing properties and UV absorbing (Aramwit *et al*., 2010). The sericin is used in cosmetics industry in various products of skin care due to its hydrophilicity that makes it 50 times more water soluble than glycerine

(Sothornvit *et al*., 2010). In food industry sericin can also be a valued natural ingredient (Puangphet *et al.,* 2015). Sericin has a high content of bioactive peptides that are specific fragments of proteins, their amino acid sequence is directly related to the beneficial effects on corporal functions, especially on systems such as cardiovascular, nervous system, gastrointestinal and immune system (Gabriela *et al.,* 2020). Sericin protein has been confirmed to have anticancer activity against few types of cancer cells, in addition to its antioxidant effects (Fakharany *et al.,* 2020). Various studies have shown the anticancer effects in such models as breast, colon, colorectal, lung, cervical, and prostate cancer cells (Kumar *et al.,* 2019). Cancer cells treated with sericin show decreasing viability with increasing concentrations of sericin. Sericin based formulations are a great example of nanotechnological tools applied to the design of an economically viable, biocompatible, and biodegradable compound, as well as its use as nanomedicine. There is a variety of sericin-based nanomaterials usually as composites, for instance, nanoparticles, flms, used as drug haulers taking benefit of its biological activities to improve them (Salunkhe and Jadhav., 2018). Sericin hydrogel possesses high porosity and degradation. In addition, the sericin hydrogel system is appropriate as a transporter of cells or bioactive molecules contribute from its exceptional cell-adhesive capability, efficiently promoting cell attachment, proliferation, and a continual manner for drug release. Sericin-based edible coating material containing chitosan, aloe vera, and glycerol has the potential to prolong the storage life of tomatoes under storage at 25 ◦C and relative humidity of 70% (Tarangini *et al.,* 2022). Extraction parameters such as temperature and time of treatment are recognized to impact sericin quality and extraction efficiency (Vaithanomsat and Kitpreechavanich, 2008). The production of sericin is essential because it determines the monetary value of the method of separating sericin. In this regard, sericin powder retrieval is critical for the efficient end utilization of this protein. In the textile industry, the cocoon is processed and sericin is mostly removed in a course known as degumming. The unscientific disposal of sericin into the open creates a high chemical and biological oxygen demand, as well as water adulteration (PadmaShree D., 2022). On the other hand, the elimination and reutilization of sericin could have significant economic, social and environmental consequences especially in countries where sericulture is mostly practiced, such as China and India. Cooking and reeling of 8 kg of dry cocoon yields approximately 01 kg of sericin. Sericin and its derivatives have been included by the FDA (2001) in the "Generally recognized as safe – GRAS" list (GRAS notice GRN 1026). Sasaki *et al*. 2000 stated sericin increases the bioavailability of Zn, Fe, Mg and Ca in rats. Therefore, several researchers have been considered to extract pure sericin from cocoon and degummed solution (Wu *et al.* 2007). Moreover, its role as a biodegradable, biocompatible functional biomaterial begins to get explored nowadays. Over the 2017-2021 antique period, the sericin global market recorded a CAGR (Compound annual growth rate) of 3.1%, and the market of sericin is expected to reach an appraisal of US$ 2.76 million in China (Fact MR., 2022). The market of sericin is anticipated to offer huge progress opportunities. In literature, there exist very few studies on the extraction and characterization of sericin from cut open unreelable cocoons. In this study we focused on extraction and characterization of crude sericin powder and investigate its biological properties.

MATERIALS AND METHODS

Cut cocoons of FC1 (CSR6×CSR26) was obtained from the Sericulture Development Department, Jammu and Kashmir. For the extraction of sericin, deionized water was used, and all other chemicals were obtained commercially of reagent grade.

Extraction of sericin:

Extraction of sericin was done by boiling water method (Remi *et al*., 2022) and autoclave method (Rocha *et al*., 2017) with slight modifications. In the former method the 40 gram of cocoons after cutting them into small pieces were subjected to boiling water at 100 ºC for different time regimes viz., 30, 60 and 90 minutes. Whereas in the later method, the 40 gram of cocoons after chopping into very small pieces were autoclaved for different time regimes viz., 30, 60 and 90 minutes at 121 ºC temperature and 15 psi pressure.

Recovery of Sericin: The recovery of crude sericin powder was done by the following methods:

Hot air-drying method:

The protocol of Bharathi, *et al.* (2020) was employed with slight modifications for the hot air drying of sericin. The sericin solution obtained by different methods was placed onto the Petri plates and those Petri plates were placed in the hot air dryer at 70ºC till the solution was dried. After complete drying, the plates were scraped to get the crude sericin powder.

Freeze drying method:

The protocol followed by the (Lorella *et al*., 2021) was employed for the freeze drying sericin samples. The sericin samples were completely frozen and placed under deep vacuum, well below the triple point of water. The added thermal energy caused the ice to sublime and sericin powder was recovered from freeze dryer.

Electrophoretic profiling of sericin:

The MW (molecular weight) of the sericin samples was determined by SDS-PAGE Laemmli (1970) with slight modifications. The agglomeration and casting gels were 12.5% and 4%, respectively, while the resolving gel was 7.5%. The system was powered by a Dual Mini Slab Kit at 100 V (Bio-Rad, Mini-PROTEAN 3 Cell). Silver stain was used for staining the gels. Molecular weight markers included the standard proteins phosphorylase B (97 KDa), bovine serum albumin (66 kDa), ovalbumin (43 kDa), carbonic anhydrase (31 kDa), soy trypsin inhibitor (22 kDa), and lysozyme (14 kDa) were used.

Measurement of radical scavenging activity (DPPH assay):

Using the 2.2-diphenyl-1-picryl-hydrazil (DPPH) reagent and slightly altered Amarowicz (2004) approach, the radical scavenging activity was determined. Freshly generated 3.5 mL of DPPH radical in a methanol solution (1.0 $\times 10^{-4}$ mol L^{-1}) was added and vortexed with 0.5 mL of sericin that had been dissolved in distilled water at gradually increasing concentrations of 40 mg/mL, 20 mg/mL, and 10 mg/mL. The reaction mixtures were centrifuged at 6400 rpm for 5 minutes after the reaction for 25 minutes at room temperature (25 °C) in the dark. The following equation was used to measure the free radical scavenging capacity.

$$\text{Radical scavenging activity (RSA)} = \frac{\text{1- sample absorbance}}{\text{control absorbance}} \times 100$$

Where sample absorbance is 3 mL of DPPH methanol solution combined with 1 mL of solution of sericin and control absorbance is DPPH methanol solution.

Protein quantification:

The total amount of sericin in the samples extracted by open boiling and autoclave method was determined by the method followed by Nitin *et al*., (2018) with slight modification. 1 mL of NaOH solution was transferred into the test tube and heated up to 100 ^{o}C. The test tube was added with 1 mL of sample and was suspended into the above solution for 5 minutes and 5 mL of alkaline copper reagent was mixed properly and the mixture was left at room temperature for 10 min. 0.5 mL of the Folin-Ciocalteau reagent (FCR) was quickly added with immediate mixing, and the absorbance at 750 nm was measured after 30 minutes at room temperature. The protein concentration in the sample was calculated using the BSA standard graph

Qualitative estimation: - Qualitative estimation of sericin was done first on UV-Vis (Hitachi U-1800) spectrophotometer determining the A-ratio. A-ratio, signifying the purity of proteins, was determined by calculating the ratio of absorbance of the sample at 280 nm and 260 nm. Since nucleic acids absorb UV- radiation at 260 nm and proteins absorb UV - radiation at 280 nm, A-ratio above 1.8 typically corresponds to a sample that is free of contamination.

The gelation property:

The gelation property of the sericin solution extracted by open boiling and autoclave method at different time regimes was determined after incubating sericin solution at room temperature.

Anti-fungal activity:

Sericin powder was used to test the antifungal activity against *Aspergillus flavus* using the agar well diffusion assay method. To create pure isolates, a fungus was sub cultured in potato dextrose media at 27 °C for 7 to 15 days. The agar wells were made by cautiously cutting 6 mm diameter wells from the agar plates, and then 50 µL of the sericin solutions was poured into the wells, DMSO was used as negative control and Nystatin as positive control. The agar plates were then incubated for 5-7 days at 27 °C while being monitored every day. Using a micrometer, the zones of inhibition surrounding the agar wells were noted. The diameter of the zone of inhibition, including the well size, was measured in order to calculate the amount of fungal growth.

Antibacterial activity tests:

The microtiter plate assay was performed to test the crude sericin powder antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus*. In the first column of a 96-well microtiter plate, 200 µl and 100 µl, respectively, of Mueller Hinton Broth were added to each well. 100 µl of Mueller-Hinton broth with the substance added and serially diluted at various concentrations, including 50, 25,12.5 and 6.25 µg/mL. Each well received 10^4 cells of the

appropriate test bacteria as an inoculum, and it was then incubated for 24 hours at 37 °C. Each plate had a set of controls, a column without the test extract, one without the relevant test organism, and one with a broad-spectrum antibiotic (ciprofloxacin) as positive controls. At 620 nm absorbance was measured spectrophotometrically.

Statistical analysis:

All data are shown as mean ± standard deviation and One- way ANOVA (Kruskal- Walli's test) Statistical analysis was carried out in a statistical software SPSS (version 20) and MS excel were used for the analysis purpose.

RESULTS

Recovery of sericin by hot air drying:

The results of the recovery of sericin from cut cocoons by hot air drying are presented in Table 1. Maximum recovery of crude sericin powder (Fig, 1). was obtained when extraction was carried out by autoclave method as compared to open boiling method. Among the three-time regimes tested extraction for 90 mins yielded maximum crude sericin powder. Pursual of Table 1 it is evident that recovery of the sericin powder increased with increase in extraction time from 30-90 mins to the tone of 2.740% to 9.700 % in open boiling method and 3.660 % to 11.130 % in autoclave method.

Table 1. Effect of Different Methods of Extraction and Recovery of Sericin by Hot Air Drying

Method / **Treatment time (min)**	**Open boiling (recovery %)** **Mean ± SD**	**Autoclave (recovery %)** **Mean ± SD**
30	2.740 ± 0.06	3.660 ± 0.03
60	5.430± 0.03	7.230 ± 0.1
90	9.700± 0.20	11.130± 0.03
LSD (P value)	0.248 (0.027)	0.128 (0.025)

Recovery of sericin by freeze drying:

Table 2 summarizes the data on the recovery of sericin protein using a Freeze dryer. In general, maximum recovery was observed when drying was carried out using freeze drying compared to that of hot air drying. Although the recovery of sericin followed the same increasing trend as that of hot air drying in both extraction methods viz., open boiling and autoclave method but the total content of crude sericin powder recovered was more comparatively. Sericin recovery showed increasing trend from 3.467 % to 11.133 % in open boiling and 4.557 % to 14.12% in autoclave method with increase in extraction time from 30 min to 90 mins.

Table 2. Effect of Different Methods of Extraction and Recovery of Sericin by freeze drying method

Method / **Treatment time (min)**	**Open boiling (recovery %)** **Mean ± SD**	**Autoclave (recovery %)** **Mean ± SD**
30	3. 467 ± 0.02	4.557 ± 0.03
60	6.267 ± 0.06	9.03 ± 0.07
90	11.133 ± 0.02	14.12 ± 0.05
LSD (P value)	0.166 (0.022)	0.014 (0.024)

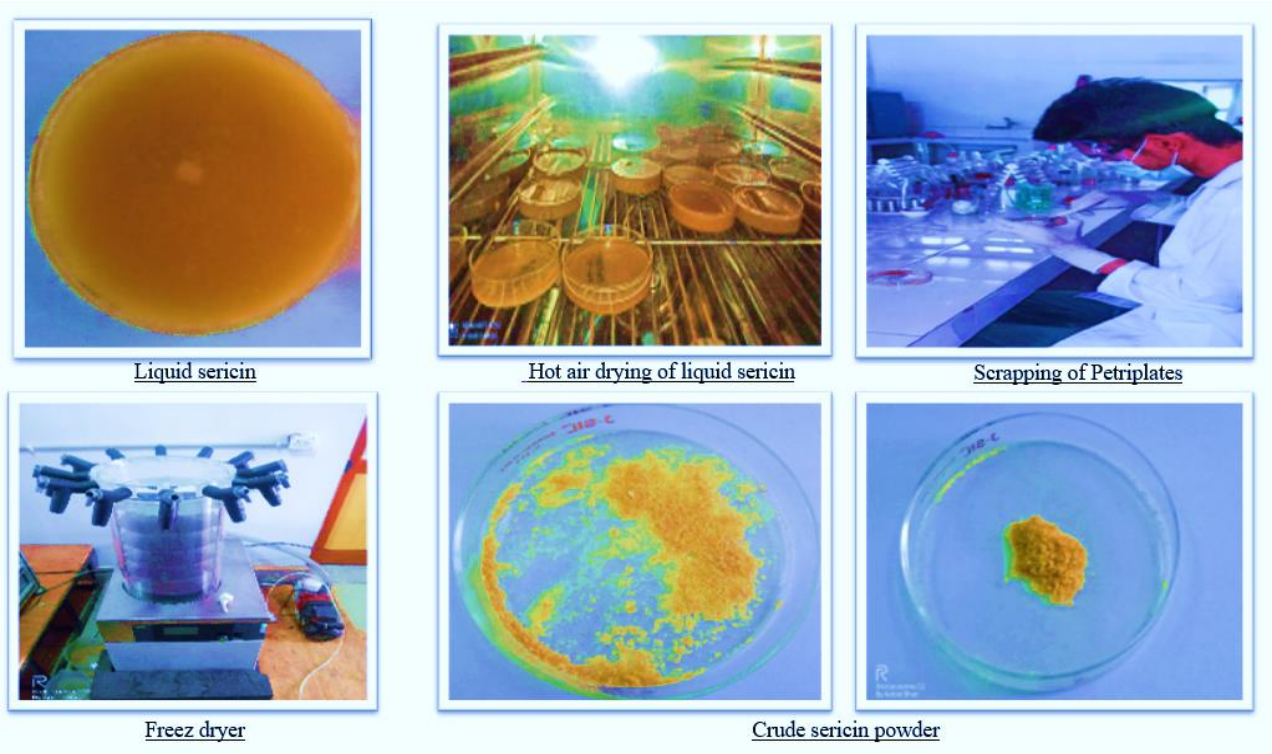

Fig. 1. Overview of recovery of crude sericin powder

Qualitative estimation of sericin:

A- ratio Indicates the level of purity in protein samples. Purity of sericin was found to be maximum in autoclave extraction (A-ratio =1.117) as compared to that of open boiling extraction (A-ratio =1.096). The purity ratio showed an increasing trend in both open boiling and autoclave method (1.026 -1.096) and (1.063 -1.117) respectively (Table, 3).

Table 3. Purity of sericin extracted by open boiling and autoclave method.

Method / Treatment time (min)	Open boiling (A-ratio) Mean ± SD	Autoclave (A-ratio) Mean ± SD
30	1.026 ± 0.02	1.063± 0.01
60	1.066 ± 0.01	1.133 ± 0.02
90	1.096 ± 0.02	1.117 ± 0.01
LSD (P value)	0.003 (0.025)	0.081 (0.504)

Protein quantification:

Maximum protein quantity was observed in general when extraction was carried out using autoclave compared to that of open boiling. Among the three-time regimes tested extraction for 90 mins showed maximum sericin quantity. According to Table 4 it is evident that protein quantity increased with increase in extraction time from 2.060 mg/mL to 5.300 mg/mL in open boiling method and 2.760 mg/mL to 6.310 mg/mL in autoclave method, respectively.

Table 4. Estimation of protein quantity in the extracted liquor obtained by open boiling and autoclave method.

Method / Treatment time (min)	Protein quantity (mg/mL) Open boiling Mean ± SD	Protein quantity (mg/mL) Autoclave Mean ± SD
30	2.060 ±0.05	2.760 ± 0.07
60	3.640 ±0.04	4.830 ± 0.15
90	5.300±0.3	6.310 ± 0.11
LSD (P value)	0.361 (0.027)	0.254 (0.024)

Electrophoretic Profiling of sericin:

Among the systems tested for three times, the 30-min and 60-min extractions showed a clear banding pattern with a molecular weight of 15–25 KDa. While the banding was not clear in the samples extracted for 90 mins with decreased molecular weight ranging from 10-15 KDa (Table, 5). Sericin extracts obtained by two extraction methods i.e., open boiling and autoclave method did not display different molecular weight ranges. The effect of extraction process on the electrophoretic patterns of sericin is shown in (Fig, 2).

Table 5: Determination of molecular weight of samples extracted by open boiling and autoclave method

Method / Treatment time (min)	Open boiling (Molecular weight)	Autoclave (Molecular weight)
30	15-25KDa	15-25 KDa
60	15-25 KDa	15-25KDa
90	10-15 KDa	10-15 KDa

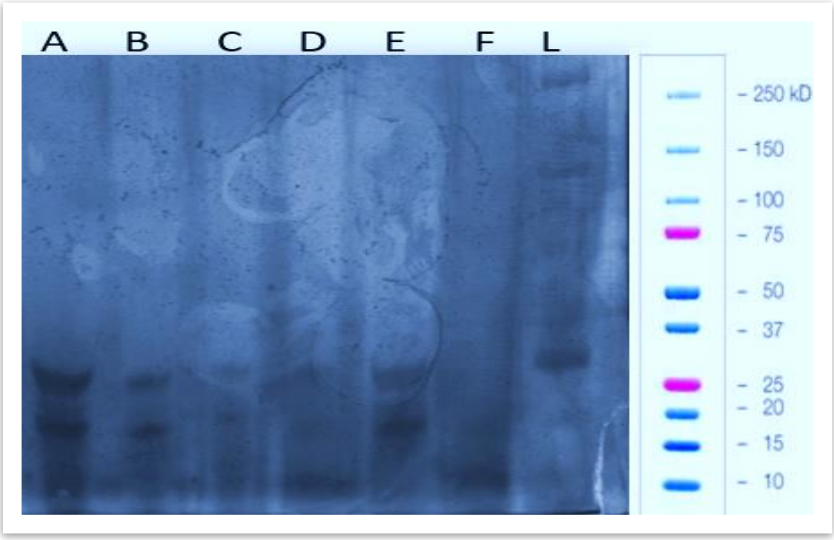

Fig. 2. Electrophoretic profiling of sericin

A= Open boiling 30 min
B= Open boiling 60 min
C= Open boiling 90 min
D= Autoclave method for 30 min
E= autoclave method for 60 min
F= Autoclave method for 90 min
L = molecular ladder

The gelation property:

Among the three-time regimes tested extraction for 90 min did not show gelation property (Fig, 3 and Table 6). In both methods, it was observed that sericin showed good gelling property at 60 min followed by 30 min within 1 hour after extraction. It was also observed that the gellying was more in the samples extracted by autoclave method as compared to the open boiling method.

Table 6: Gelation of sericin solution extracted by open boiling and autoclave method

Method / Treatment time (min)	Open boiling method (Gelation)	Autoclave method (Gelation)
30	Yes	Yes
60	Yes	Yes
90	No	No

Fig. 3. Sericin gel

Measurement of radical scavenging activity (DPPH assay):

The DPPH radical scavenging activity of sericin varied over the range of doses from 10 to 40 mg/mL. At 10, 20, and 40 mg/mL of the samples, sericin's concentration-dependent antioxidant activity ranged from 35%, 49% and 64% (Table,7).

Table 7: Antioxidant potential of Sericin using DPPH method.

Concentration (mg/mL)	Radical scavenging activity (RSA)
10	35 ± 6
20	49 ± 2
40	64 ± 7

Antibacterial activity:

The Cefroflaxin was used as positive control. The sericin did not show any resistance against the *B. Cereus* and *S. aureus*. While as Cefroflaxin showed resistance against the both pathogens and the reading recorded was (0.362 ± 0.09) and (0.368 ± 0.06), respectively (Table, 8.) In this study, it was observed that that sericin extracted from *Bombyx mori* cocoons does not have any antibacterial activity against S*taphylococcus aureus* and *Bacillus cereus.*

Table 8. Antibacterial activity of sericin.

Compound	Bacteria	Reading at 620nm
Sericin	*B. cereus*	0.00
Cefroflaxin (Positive control)	*B. cereus*	0.362 ± 0.04
Sericin	*S. aureus*	0.00
Cefroflaxin (Positive control)	*S. aureus*	0.368 ± 0.05

Anti-fungal activity:

The sericin did not show zone of inhibition against the fungus, and was similar to that of negative control DMSO while as positive control Nystatin showed the zone of inhibition against the *Aspergillus flavus. A. flavus* which is a highly sporulated fungal pathogen, is mostly found in soil as a saprophyte. It has a wide host range as an opportunistic pathogen (Fig. 4).

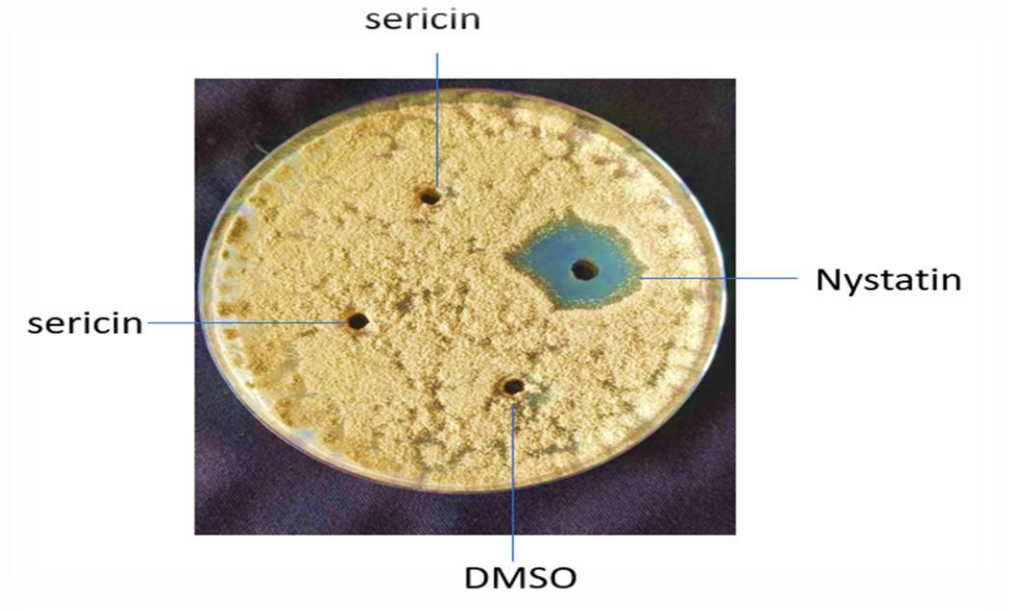

Fig. 4. Effect of sericin on growth pattern of *Aspergillus flavus.*

DISCUSSION

The maximum recovery of crude sericin powder was observed when drying was carried out using freeze drying compared to that of hot air drying. Although the recovery of sericin followed the same increasing trend as that of hot air drying in both extraction methods viz., open boiling and autoclave method but the total content of crude sericin powder recovered was more comparatively. Sericin recovery showed an increasing trend from 3.467 % to 11.133 % in open boiling and 4.557 % to 14.12% in autoclave method with increase in extraction time from 30 min to 90 mins. Similarly, Srinivas *et al*., (2014) reported that with increase in time duration the yield of sericin increased. P value (≤0.05) was obtained that signifies there is a significant difference among the treatments. Freeze drying is certainly the most suitable drying method as it yields superior product quality and enhances the microstructure of the powder compared to that of conventional hot air drying as reported by Jangam *et al.,* (2016). Moreover, it is not possible to scrape crude sericin powder from the petri plates completely and due to fluctuation in temperature sericin may get burn in the hot air drying which causes loss of crude sericin powder.

It was observed that sericin extracted by autoclave method showed higher protein purity ratio as compared to the open boiling. One way analysis of variance revealed there was overall statistically significant difference between treatments P value (≤0.05). The results are in line with the results obtained by Deepti *et al.,*

(2013) who stated that sericin extracted from cocoons showed A-ratio to the tone of 1.25. Better is the quality of protein if higher is the A-ratio. By autoclave method good quality and pure product is attained as reported by Lamboni *et al.,* (2015).

The current study revealed that maximum protein quantity was found in the samples extracted by the autoclave method as compared to open boiling method due to the high temperature. High temperature decreases the hydrogen bonding stability, formed by the binding between the various hydroxyl groups, allowing the interaction of water with hydroxyl groups of the polar amino acid (Damodaran *et al*., 2010). P value ≤0.05 indicating there is a significant difference among the treatments. According to Agrawal *et al*., (2013) the protein quantity in liquor extracted by autoclave method was higher as compared to the open boiling method.

It was observed that bands were not clear at 90 min of extraction due to denaturation of protein. A, B, D, and E samples show broader bands due to the mixture of different molecular weight peptides. Contrary to *B. mori* silk fibroin (BMSF), the number of the subunits in *B. mori* silk sericin (BMSS) is still unclear, and not much is known about their molecular mass either (Traian *et al.,* 2016). Various numbers of polypeptide subunits in BMSS have been reported over nearly a century of research, including three polypeptides by, Takasu *et al.,* (2002), one polypeptide by Moorthy (2020) or more Fedic *et al*. (2002) while in between 20 and 400 kDa series of molecular masses has also been reported. There is a significant difference between the native BMSS and the refined ("regenerated") BMSS, and it is not always clear whether the values previously reported for the distribution of molecular masses should be attributed to the native dissemination of sericin proteins as secreted in the gland, or whether they reflect the dissemination after sericin extraction (isolation) from cocoons and further processing. For example, after degumming experiments with numerous proteolytic enzymes, the resulting sericin polypeptides have molecular weights ranging from 5 to 20 kDa (Freddi *et al.,* 2003). One can legitimately wrap up that if we need to obtain BMSS with values for the size and distribution of molecular mass similar to the native values, we shall extract BMSS directly from the silkworm's middle gland. Rationally, this course of action would be completely unworkable for a variety of reasons. Furthermore, the resulting material is unlikely to be uniform and would require further purification due to impurities with other natural substances, such as colours (carotenoids, flavonoids), lipids, paraffins, uric acid, and proteinase inhibitors (Traian *et al.,* 2016). Overall, the SDS-PAGE analysis's findings support the findings of multiple other researchers that the sericin protein is a family of proteins and that the extraction method determines its molecular weight.

Gelation is the important property of the sericin. The maximum gelation was observed in the samples extracted by open boiling as compared to autoclave method. In both the method, sericin showed virtuous gelling property when extracted at 60 min followed by30 min. It was reported that extraction for 90 minutes revealed no gellying property. Partially unfolded proteins enlarge uncoiled polypeptide segments, which interact at specific points to form a three-dimensional cross-linked network. As a result of protein-protein and protein solvent interactions, the gelation process is dependent on a 3D protein network. Because of the increased intensity of intermolecular contacts, these interactions and gelation are accelerated at high protein concentrations. Gel formation is a result of hydrogen bonding, ionic and hydrophobic interaction. According to Aramwit *et al.,* (2012). The aqueous sericin solution forms a gel when the protein's random coil structure swaps to the-given sheet. Upon cooling, sericin shows sol–gel properties due to its conversion from the random coil to the β–sheet form Kunz *et al*., (2016).

Sericin is well known for its strong antioxidant activity. Current study revealed that sericin showed incremental increase in the RSA (Radical scavenging activity) on increasing the sericin concentration to the tone of 64 ± 7%. The antioxidant activities of sericin are correlated with its high serine and threonine content, whose hydroxyl groups act as chelating trace elements such as copper and iron (Gilotra *et al*., 2018). The pigment molecules (e.g., flavonoids and carotenoids) accumulated in sericin layers may be one of the causes that bestow sericin with antioxidant properties.

It was observed that that sericin extracted from *Bombyx mori* cocoons does not have any antibacterial activity against S*taphylococcus aureus* and *Bacillus cereus.* The results obtained agree with Jasjeet *et al.* (2014). The results attained agree with Jasjeet *et al.* (2014). Hence, this study cleared uncertainty about the antibacterial property of sericin powder as reported by many other workers. The antimicrobial activities reported by earlier workers Jassim *et al.* (2010), Aramwit *et al*. (2020) could be due to the presence of extractants like Urea, Soap, NaOH etc the antibacterial activity of sericin could be due to the chemical extraction methods used to obtain sericin.

The sericin did not show zone of inhibition against the fungus and was similar to that of negative control DMSO while as positive control Nystatin showed the zone of inhibition against the *Aspergillus flavus. A. flavus* which is a highly sporulated fungal pathogen, is mostly found in soil as a saprophyte. It has a wide host range as an opportunistic pathogen (Fig, 4). It was reported in this study that sericin did not show any antifungal activity against the *A. flavus.* Analogous results were got by Srinivas *et al.,* (2015) who testified that sericin did not inhibit the growth of *A. flavus* and *Trichoderma harzianum.*

CONCLUSION

The present study envisages and recommends that the extraction of sericin from cocoons for the maximum duration of 60 min followed by 30 min time duration in order to retain the characteristic function of the protein like gelation and molecular weight. The present study re-affirms the potential antioxidant properties of silk sericin and thereby promising material for nutraceutical, pharmaceutical and cosmetic applications. In addition, the sericin could be used as promising and as an ingredient for moisturizing formulations and utilized in the formulation of hydrogels and in wound dressing owing to its good gelation property. The present study is a base work which can be explored further for development of value-added products from the otherwise waste sericin protein. Sequencing of bands of sericin from (FC1) cocoons will help in identification of sericin isoforms and further characterization for its biological properties which could be taken into consideration for further study.

Future scope:

Over a longer period of time sericin has been rejected as a left-over in the textile industry. Now a days sericin is being used as biomaterial and also for medical applications in pharmaceutical industries and it is also used as an ingredient in cosmetics and also in food industry. The present investigation will help researchers to recover sericin powder from degumming solution. Due to exceptional properties of this protein, sericin has a huge demand in the world market. Moreover, these findings will also help the researchers to draw up a protocol for assessing the quality and quantity of sericin protein obtained from different methods of extraction at different time regimes. In the future we are interested in developing different value-added products of sericin. Recycling of this high value protein will add value to sericulture industry and will help in becoming sustainable.

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Conflict of interest: - The authors affirm that they have not at all contending benefit.

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