Study of the antimicrobial activity of silk sericin from silkworm *bombyx mori*

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Abstract

Back ground: In this research, it is found that the natural silk sericin, the gummy substance that holds the fibroin stands in the silk filaments together which is produced by the silk worm *Bombyx mori*, has a good antimicrobial, antioxidant, and anti-tumor activity.

Materials & Methods: this is achieved by Tensile strength and growing of spores colonies methods before and after treatment of the cotton fabric with 2% sericin solution and burying the treated samples in garden soil for 7 days to achieve the spores test (according to Iraqi standard 147/ 1990)[(1)].

Results: The results showed that the spores colonies are reduced from (146) before treatment with sericin to (29) only after treatment. Also the Tensile strengths are increased about (5) times after treatment with 2% sericin. The *micrococcus leuteus* used to detect the effect of (10-20) mg/ml sericin to inhibit this kind of bacteria, by using a well diffusion method seeded nutrient agar, then incubated at (35-37) ^{o}C for 24 hours, zones of inhibition were observed between 9-12 mm in diameter.

Conclusion: Results above indicate the presence of antimicrobial and antibacterial activity of silk sericin; it is possible to use the silk sericin for medical applications after isolation and identification of some pathogenic bacteria like *Streptococcus pneumonia, Pseudomonas aeroginosa Escherichia coli* to produce medical bandages, mouth wash, and antibacterial soaps & teeth paste.

Key words: antimicrobial activity, silk sericin, *bombyx mori*

Introduction:

The natural gum, sericin, derived from Bombyx mori is normally left on the silk during reeling, throwing and weaving. It acts as a size which protects the fibers from mechanical injury; the gum is removed from the finished yarns or fabrics, usually by boiling with soap and water. And removed sericin is mostly discarded in silk degumming process. [2, 3].

Sericin constitutes (25–30) % of silk protein, this protein is made of 18 amino acids, and amino acids serine& aspartic acid constitute approximately (33.4) and (16.7) % of sericin respectively [4].

Low molecular weight sericin peptides or sericin hydrolysates are used in cosmetic. High molecular weight sericin peptides are used as medical biomaterial, degradable biomaterials, functional biomaterial and functional fibers [5, 6, 7].

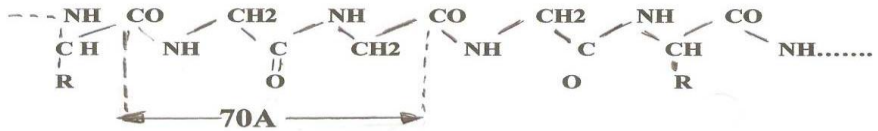
The use of natural silk sericin in textile processing has been reported by M. Nouri, P. Heidari. [2]

Blending of sericin with other polymers such as polyurethanes has been reported for production of biodegradable polymers, Magraphan and coworkers have studied the use of sericin as an antioxidant & antimicrobial for polluted air treatment [5].

A cross linked film made of sericin for use as a separating membrane for water & ethanol described was by Mizoguchi & coworkers. [8]

Takai[9] prepared a hydrophilic fiber by coating the surface of rayon & cotton fibers with sericin , these sericin coated fibers were absorbed and did not cause skin rash, In spite of a great number of papers which were published about sericin , but few are published about sericin for medical application.

The sericin structure is not known completely but scientists Mayer and Maric [10] suggested the following structure:



Where R= $-CH_3$ or $-CH_2C_6H_4OH$.

Materials & Methods:

1-Bacterial culture
2-Cotton fabric 135 gm / m.
3-Wetting agent (Sandoz comp. Swiss).
4-Tensile strength instrument, Lloyd instrument, AMETEK, UK.

Treatments:

Sericin extraction:

Many silk cocoons are placed in a beaker with 200 ml distilled water, temperature (80) ^{o}C for (30) minutes with stirring, then remove the cocoons, the solution was centrifuged for (15) minutes & The sericin was separated & dried at 60 ^{o}C, [(11)] the

weight of sericin obtained was (10 gm) as a powder.

Coating of cotton fabric with 2% sericin solution:

Five samples of (5×25) cm cotton fabric are scoured by anion detergent (ECE Liquid Sandoz company, Swiss) and this detergent is used usually for researches, It is free of optical brighter and has no effect on bacterial and fungal growth to increase the ability of absorption of sericin solution of cotton fabrics for both treated and untreated samples before treatment of sericin [12].

2% of this detergent is used for scouring the sample of cotton fabrics at room temperature for 10 min. then removed it from the beaker & dried the samples in the air.

Then the samples were placed in a beaker that contains 200 ml of 2% sericin solution at (80) ^{o}C for 15 min. with stirring, after that the treated samples were taken out the beaker & dried in the air.

The treated & untreated cotton fabric samples were buried in the garden soil (30 to 50 mm depth) for 7days for growing the spores (according to the Iraqi standard 147/1990), then the samples taken out from soil, the soil removed carefully from it, & cleaned gently by water.

To disinfect the samples were put it in 70% ethanol for 4 hours at room temperature, then dried it in air.

To know the effect of sericin as antimicrobial activity, tensile strength & number of spore's colonies were checked before & after treatment with sericin solution of cotton fabric pieces.

Agar diffusion assay:-

Modified agar wells diffusion method was used & checked the antibacterial activity of sericin (Sen *et al*, 1995, Mohammed *et al*. 2007) [13].

24 hours fresh culture of *Micrococcus leutus* growth on Nutrient agar slant were diluted with presterilized normal saline & bacterial concentration Equivalent to 3 × 10^{8} /ml turbidity was adjusted with (0.5) Mcfarland. Then mixed with nutrient agar at 32 ^{o}C to keep the bacterial viability before solidification of seeded agar. (Clutterbuck, *et al*, 2007) [14] Poured in disposable plate until the media becomes solid. The wells (5) mm in diameter on neutrient agar plate were prepared [15].

About 50 micro liter of diluted silk sericin at conc. 10 & 20 mg/ ml from powder extracted were added in wells & incubated at 37 ^{o}C for 24 hours. [16].

Results:

Table (1) & figure (1) shows that the average of tensile strengths before & after treatment with 2% sericin solution are 14.25 & 96 NIT respectively, (P<0.05) so the tensile strength increased (6.5) times after treatment with 2% sericin solution. The tensile strengths are increased about (5) times after treatment with 2% sericin. Results above are improved microbial property.

Table (1) Shows the effect of sericin on tensile strength of cotton fabric samples which indicated a significant increase (P<0.05) in tensile strength.

Samples	Tensile strength before treatment with sericin (NIT)	Tensile strength after treatment with sericin (NIT)
1	**10**	**80**
2	**15**	**85**
3	**12**	**90**
4	**14.5**	**115**
5	**20**	**110**
Average	**14.25**	**96**

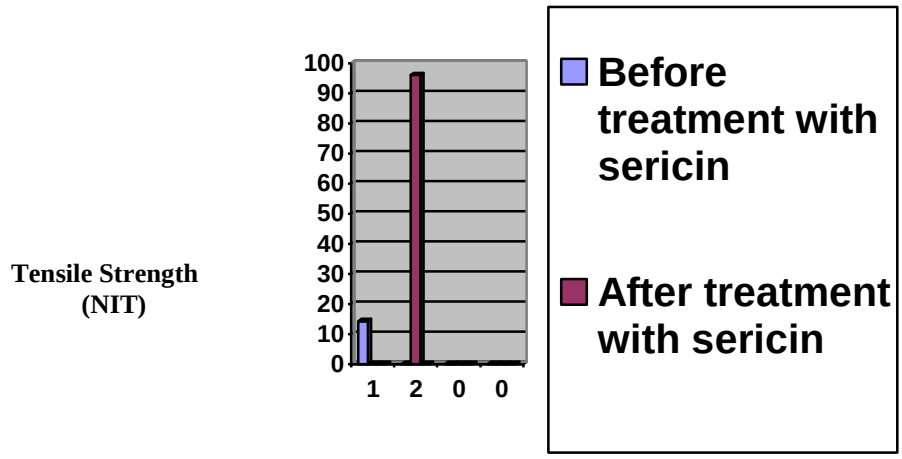


Figure (1): Effect of sericin on tensile strength.

Table (2) & (figures 2 and 3) shows that the numbers of growing of spores colonies are reduced from (146) spores colonies on the cotton fabric (5×25) cm samples before treatment with sericin to (29) spores colonies ($P<0.05$) on sericin coated fabric.

The zones of inhibition observed & measured (9-12) mm in diameters respectively. (Fig 4).

Table (2) the effect of sericin on the growing of spores colonies which indicated a significant increase ($P<0.05$) in spore colonies.

Samples	Numbers of spores colonies before treatment with sericin	Numbers of spores colonies after treatment with sericin
1	150	30
2	145	35
3	145	25
4	140	27
5	150	29
Average	146	29

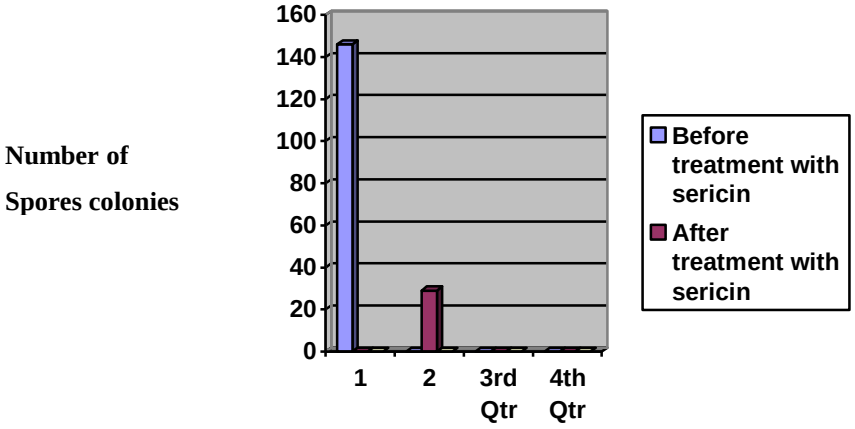


Figure: 2 the effect of Sericin on growing of spores colony

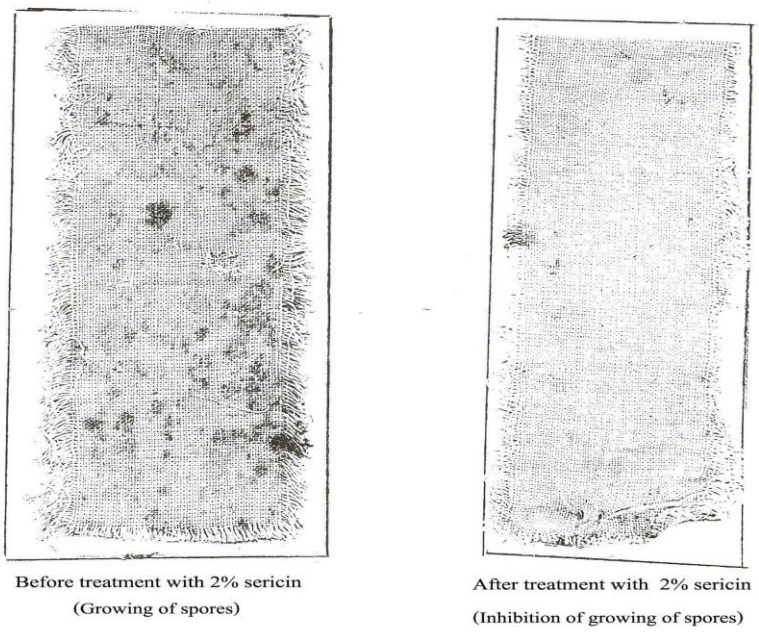


Figure (3) The effect of sericin solution as antibacterial on cotton fabric.

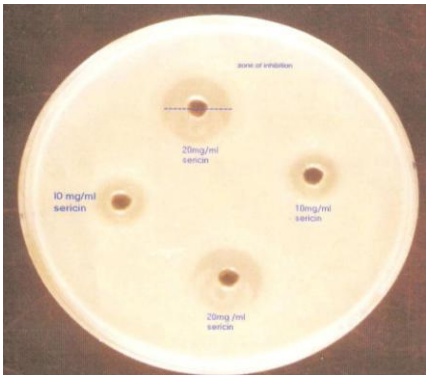


Figure: (4) the zone of inhibition (9 & 12 mm) in diameter of growing *Micrococcus leuteus* at concentration 10 &20 mg/ ml respectively.

Discussion:

Silk sericin is the gummy protein that is isolated from the silk produced by the silk worm *Bombyx mori*, that holds the fibroin stand in the silk filament together, usually is discarded in waste water during silk processing, was examined for antibacterial property & for this purpose growing of spores colonies & tensile strength tests are achieved before & after treatment of the sample with 2% sericin solution & burying in the garden sand for [7] days.

Sericin was examined for antibacterial property, and for this purpose growing of the *Micrococcus leutus* bacteria by modified agar diffusion method has been shown in (fig 4). This figure shows the zone of inhibition & measured (9-12) mm in diameter for 10 & 20 mg/ml concentration, the antibacterial efficiency increases when the concentration of sericin increases [(5)]. According to these results above the silk sericin has antimicrobial & antibacterial properties & can inhibit the growing of them.

This research has explained that the protein sericin has good antimicrobial properties, it can inhibit the growing of bacteria, & it is possible to use the silk sericin for medical applications after isolation and identification of some pathogenic bacteria like *Pseudomonas aeruginosa*, *Staphyllococcus aureous Escherchia coli* to produce medical bandages, mouth wash, antibacterial soaps & teeth paste.

We recommended doing more researches about the silk sericin to use it in a mouth & face wash solutions, teeth paste and antibacterial soaps according to its antimicrobial properties.

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