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In vitro evaluation of antioxidant activity of sericin

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Abstract
Sericin is a silk protein produced by the silkworm, *Bombyx mori* along with fibroin protein and it is removed during reeling process. The present study investigated the antioxidant properties of sericin. The ability of sericin to scavenge DPPH, superoxide, catalase and glutathione reductase was evaluated. The results indicated better anti-oxidant activity of sericin in comparison to ascorbic acid, a known anti-oxidant. The IC50 value of sericin for DPPH radical scavenging activity was found to be 0.0741 mg; whereas for ascorbic acid, it was found to be 0.213 mg. The IC50 value of sericin was found to be 0.0779, 0.0733 and 0.0701 mg for superoxide dismutase, catalase and glutathione reductase respectively. The IC50 value of ascorbic acid was found to be in the range of 0.201 to 0.226 mg. These observations indicated that sericin is able to mimic these antioxidant enzymes at very low concentration in comparison to ascorbic acid. Hence, from this study it is revealed that sericin was proved to be a better anti-oxidant in the tested parameters.

Keywords: Sericin, antioxidant, DPPH, superoxide dismutase, catalase, glutathione reductase, IC50

Introduction
Sericin is a silk protein produced by the silkworm, *Bombyx mori* along with fibroin protein [1, 2]. Sericin is removed called degumming and mostly discarded in waste water during reeling process. It is estimated that 400,000 tons of dry cocoons worldwide, producing 50,000 tons of sericin [3]. The sericin is a natural polymer and highly hydrophilic with a molecular weight ranges from 20 to 400 kDa and composed of 18 amino acids with strong polar groups (carboxyl, hydroxyl, and amino groups) and combinations with other polymers, which convey unique properties to sericin as an antioxidant, moisturizing, healing, antibacterial, antimicrobial protection against ultraviolet radiation, antitumour [4, 5, 6] and vehicle for drug delivery, food and cosmetic industries [7-11].
Kato *et al.* [4] evaluated, that sericin inhibits lipid peroxidation in rat brain homogenate for the first time. Cocoons of *B. mori* provide natural pigments typically flavonoids and carotenoids accumulated in sericin layers [12] known for their biological properties as antioxidants and antityrosinase. Aramwit *et al.* [13] demonstrated that the antityrosinase activity of sericin. Chlapanidas *et al.* [14] working with cocoons of 20 lineages of *B. mori* and demonstrated its influence on the antioxidant properties (antityrosinase, antielastase, and elimination of reactive oxygen species) of sericin.
Dash *et al.* [15] analysed the antioxidant potential of sericin in *Antheraea mylitta*, against ultraviolet light B (UVB) in irradiated human keratinocytes.
Li *et al.* [16] observed a protective effect of sericin in hepatic and gastric injuries caused by alcohol in mice. Micheal and Subramanyam [17] suggested that the main constituent amino acids of sericin protects the midgut epithelial cells of *B. mori* and haemocytes from oxidative damage. The antioxidant properties of sericin could be related to high serine and threonine content. Keeping this view, the present investigation was carried out to study the antioxidant activity of sericin under *in vitro* conditions.

Materials and Methods
Free radical scavenging activity (DPPH)
The scavenging effect of sericin on DPPH radical was examined using the modified method described by Shimada *et al.* [18]. The free radical scavenging activity of the sericin was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH

radical method. Different concentrations *viz.*, 0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml of sericin were used for the study. The DPPH solution (0.1mM) in ethanol was made and 1.0ml of this solution was added to 3.0 ml of extracts solution in water at different concentrations. The mixture was shaken vigorously and left to stand for 30min in the dark and the absorbance was then measured at 517nm against a blank. Ascorbic acid was used as standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation. The mean values were obtained from triplicate experiments. DPPH of radical scavenging activity of sericin was calculated using the formula (%) = (Control OD- Sericin sample OD / Control OD) × 100

Super oxide anion radical scavenging activity

Assay of Superoxide dismutase was carried according to the method of Beauchamp and Fedovich [19]. Superoxide radicals were generated in a PMS-NADH system by oxidation of NADH and assayed through reduction of NBT. 200µM NBT (1.5mg in 10ml PBS), 468µM NADH solution (3.32mg in 10ml PBS) and 60µM PMS (183.04mg in 10 ml of PBS) was prepared. 100µl of NBT solution, 100µl of NADH solution, 10µl of different concentrations *viz.*, 0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml of sericin and 10µl of PMS solution were added together and incubated at 25 ºC for 5 minutes and absorbance was read at 560 nm. L-Ascorbic acid was used as the positive control. The decrease in the extent of NBT reduction, measured by the absorbance of the reaction mixture, correlates with the superoxide radical scavenging activity of sericin. The percentage of superoxide radical scavenging was calculated using the following formula: Superoxide radical scavenging activity (%) = $[(A_0-A_1)/A_0 \times 100]$, where A_0 is the absorbance of the control and A_1 is the absorbance of sericin sample.

Catalase

Catalase activity was determined by the titrimetric method described by Chance [20]. 7mg of catalase in 700µl of PBS, 394.17µl of different concentrations (0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml) of sericin in 20ml PBS, 3.4 µl H_2O_2 in 10ml PBS (10mM), 10.203µl H_2O_2 in 10ml PBS (30mM) was prepared. The assay mixture consisted of phosphate buffer, hydrogen peroxide, followed by the addition of sericin and mixed thoroughly. The time required for the decrease in absorbance by 0.05 units was recorded at 450nm in a spectrophotometer.

Glutathione Reductase

The activity of glutathione reductase (GR) was assayed using the method that was described by Carlberg and Mannervik [21]. Different concentrations *viz.*, 0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml of sericin were added to the incubation medium contained 0.4 ml of buffer, 0.2 ml of sodium azide, 0.2 ml of EDTA, 0.2 ml of Hydrogen peroxide and 0.2 ml of reduced glutathione. The incubation medium was made upto a final volume of 2.0 ml with water. The tubes were incubated at 37°C for 90 and 180 minutes. This was terminated by the addition of 1.0 ml of the precipitating agent. The reaction mixture was centrifuged and to the supernatant, 6.0ml of disodium hydrogen phosphate was added. 1.0ml of DTNB reagent was added just prior to the calorimetric analysis. The absorbance was read at 412nm against a blank, had only 6.0 ml of disodium phosphate and 1.0 ml of DTNB reagent. The activity was expressed in terms of µgm of glutathione utilized/minute/mg protein. The formula was follows: Glutathione (U/mg protein) = OD of blank-OD of sample x 1/0.001x mg protein x 1

Statistical analysis

All experiments were performed in triplicate and the results were expressed as mean standard deviation (SD). One-way ANOVA using SPSS software was used to compare the mean values of each treatment. Significant differences ($p<0.05$) among the means were determined by using Duncan's multiple range Test.

Results and Discussion

The anti-oxidant activity of sericin was determined by *in vitro* methods. The sericin's antioxidant activity is one of the most significant property and showed positive effects on people health and as a natural food preservative in the food industry [22]. The antioxidants maintain a balance between formation and elimination of reactive oxygen species (ROS) and nitrogen. High ROS levels can be detrimental for the cell, affecting proteins, lipids and DNA, and consequently to the physiological functions of the organism. The human body possess an antioxidant system that involves enzymatic complexes, vitamins and other specialized molecules. In addition, there are exogenous antioxidants provided by food such as fruits, vegetables and dietary supplements, which contribute to the body antioxidant defense [23-25].

The standard anti-oxidants such as DPPH, superoxide desmutase (SOD), catalase (CAT) and glutathione reductase (GSH) were measured to elucidate the radical scavenging activity of sericin and its effect was compared with the standard ascorbic acid (Vit C).

Free radical scavenging activity (DPPH)

DPPH is one of the free radicals widely used for testing radical scavenging activity and it is a direct, quick and reliable method for determining *in vitro* antioxidant activity of pure compounds [26-29]. These antioxidants either transfer an electron or a hydrogen atom to DPPH thus neutralizing its free radical character [30]. In the present study, it was observed that 0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml of sericin inhibited 8.1, 19.3, 34.6, 55.6 and 73.4 percent respectively (Fig 1). The IC50 value of sericin for DPPH radical scavenging activity was found to be 0.0741 mg; whereas for ascorbic acid, it was found to be 0.213 mg (Fig 2). The results indicated that better anti-oxidant activity of sericin in comparison to ascorbic acid, a known anti-oxidant. Takechi's *et al.* [31] studied the methods of 1,1-diphenyl- 2-picrylhydrazyl (DPPH), chemiluminescence, and oxygen radical absorbance capacity (ORAC) proved a major antioxidant potential of sericin obtained from the yellow-green cocoon and revealed that the flavonoid pigments present in the sericin layers is responsible for antioxidant characteristics.

Super oxide anion radical scavenging activity

It was noticed that 0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml of sericin inhibited 11.2, 15.3, 31.2, 53.6 and 69.3 percent respectively (Fig 1). The IC50 value of superoxide dismutase radical scavenging activity for sericin was found to be 0.0779 mg; whereas for ascorbic acid, it was found to be 0.201 mg (Fig 2). Increased concentration of sericin showed increased

antioxidant activity on superoxide radicals.

Catalase

The antioxidant potential of silk protein sericin from the non-mulberry tropical tasar silkworm *Antheraea mylitta* cocoon has been assessed by Dash *et al.* [32]. The results showed that the sericin obtained from tasar cocoons offers protection against oxidative stress and cell viability is restored to that of control on pre-incubation with the sericin. Fibroblasts pre-incubated with non-mulberry sericin had significantly lower levels of catalase activity when compared with control. In the present study the results indicated that IC 50 value of sericin for catalase radical was found to be 0.0733 mg when compared to ascorbic acid (0.214 mg) (Fig 2.). It was observed that 0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml of sericin inhibit 11.2, 19.5, 31.4, 58.6 and 74.3 percent respectively (Fig 1). Catalase is involved in the decomposition of hydrogen peroxide to water and oxygen and is therefore important in protecting cells against oxidative stress [33].

Glutathione Reductase

The percentage of scavenging effect was concomitantly increased with the increase in the concentration of sericin 0.01 to 0.1 mg/ml. The percent of inhibition was 7.2, 16.7, 33.5, 60.8 and 82.3 for 0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml of sericin (Fig 1). From the results it is known that the sericin possess hydrogen donating capabilities and does scavenging free radicals. Furthermore, it was noticed that sericin has more pronounced scavenging activity than that of the standard, ascorbic acid. IC 50 value of sericin for GSH radical was found to be 0.0701 mg when compared to ascorbic acid (0.226 mg) (Fig 2). Devi *et al.* [34] studied the antioxidant activity of sericin extracted from *Antheraea assamensis.* Prasong [35] compared the silk of *Samia ricini* with *B. mori* concluded that the presence of polyphenols and flavonoids in sericin is responsible for its antioxidant ability.

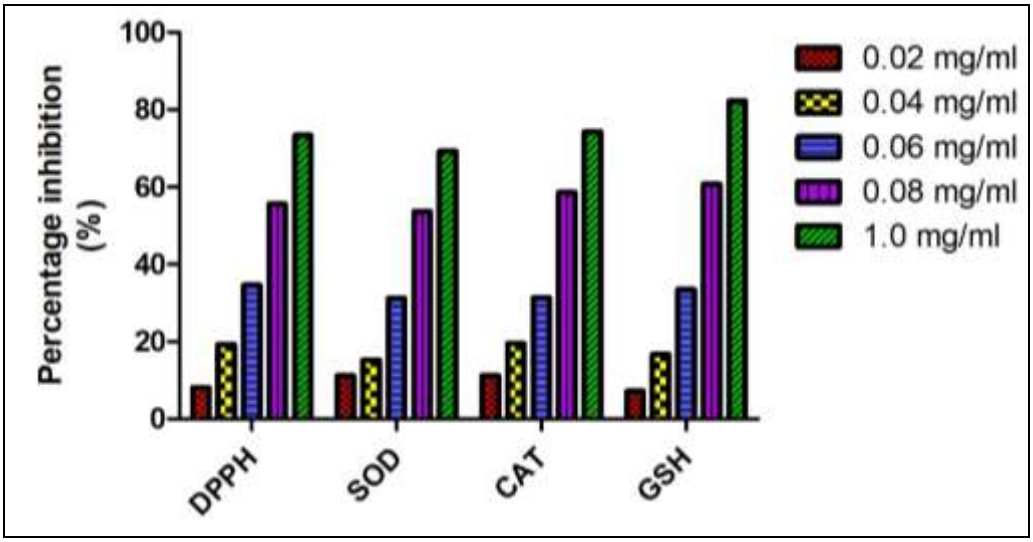

Fig 1: Inhibitory activity of sericin against DPPH, SOD, CAT & GSH radicals

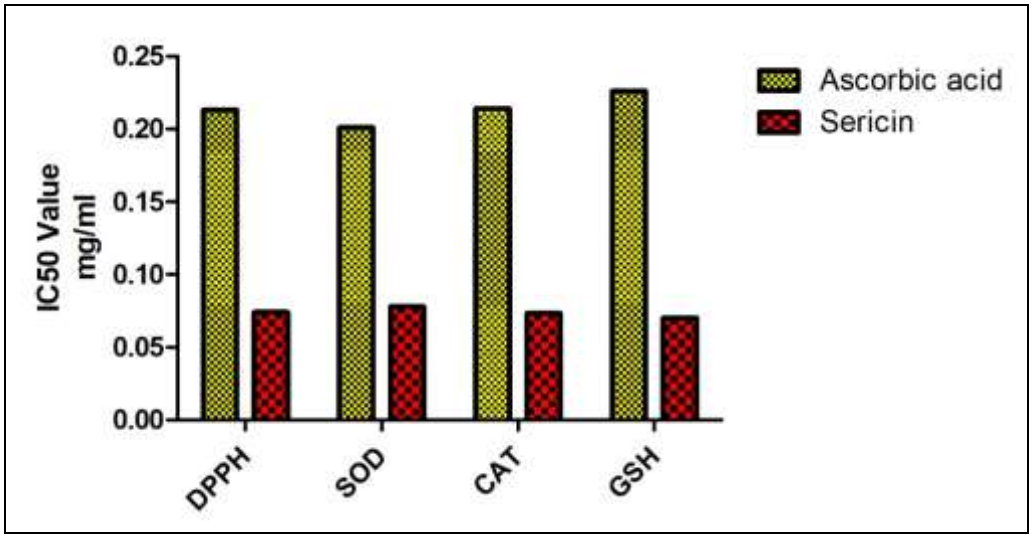

Fig 2: IC 50 value of Sericin and ascorbic (Standard) acid

Conclusion

The present study was carried out to investigate the antioxidant ability of sericin. The results indicated that the sericin exhibited strong antioxidant activity than standard ascorbic acid might be due to the presence of amino acids and flavonoids in sericin. Due to this property, sericin can be well utilized in food and pharmaceutical industries.

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