Final Report of the work done on the Major Research Project

1. Project report No. 1st /2nd /3rd/Final: FINAL


3. Period of report: from 01/07/2015 to 30/06/2018

4. Title of research project: Antioxidant and antityrosinase activities of sericin and non-protein fractions from oak tasar (Antheraea proylei J.) silkworm cocoons

5. (a) Name of the Principal Investigator: Dr. Sanjenbam Kunjeshwori Devi
   (b) Deptt.: Biochemistry Department
   (c) University/College where work has progressed: Manipur University

6. Effective date of starting of the project: 11/09/2015 (Date on which the first instalment of the grant was received by Manipur University)

7. Grant approved and expenditure incurred during the period of the report:
   a. Total amount approved Rs. 12,42,171.00 (Twelve lakhs forty two thousands one hundred seventy one) only
   b. Total expenditure Rs. 11,97,676.00 (Eleven lakhs ninety seven thousands six hundred seventy six) only
   c. Report of the work done: The final report on the work done under the project is given below:

   (i) Brief objective of the project: (i) Sericin from oak tasar (Antheraea proylei J.) cocoons will be extracted and fractionated by different methods to obtain crude sericin, purified sericin and non-protein fraction. Same extraction and fractionations will be carried out taking the mulberry (Bombyx mori L.) cocoons as standard. (ii) The resulting sericin preparations and non-protein fraction will be assessed for antioxidant activity by chemical and cell culture methods and will be compared with corresponding sericin and non-protein fractions obtained from the mulberry cocoons as reference. (iii) The sericin and non-protein fractions will also be assessed for antityrosinase and antimelanogenic activities by enzymatic and cell culture methods and will be compared with the corresponding sericin and non-protein fractions obtained from the mulberry cocoons as reference.

   (ii) Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication: The details are given as follow:

   1. Extraction of sericin: The silk protein sericin was extracted (degummed) from silkworm cocoon shell
(simply known as cocoon) of oak tasar (Antheraea proylei J.) silkworm by the following three methods, viz. the hot water extraction according to Wang et al. (2012), the urea extraction according to Dash et al. (2006), and sodium chloride extraction according Dash et al. (2006). After adopting the three methods of degumming, the weight of the resulting sericin powder obtained was determined and the degumming ratio \( D \) was calculated as \( \frac{W_{S}}{W_{I}} \times 100 \), where \( W_{S} \) is the weight of the extracted sericin powder and \( W_{I} \) is the initial weight of the cocoon sample. The results are given in Table 1:

**Table 1:** The degumming ratios of different degumming methods applied to the oak tasar (Antheraea proylei J.) cocoon

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Initial weight of silk cocoon shell pieces (g)</th>
<th>Weight of sericin powder after degumming (g)</th>
<th>Degumming ratio (% sericin yield)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot water extraction</td>
<td>5</td>
<td>0.473±0.017</td>
<td>9.460±0.342</td>
</tr>
<tr>
<td>Extraction by urea</td>
<td>5</td>
<td>0.103±0.004</td>
<td>2.080±0.081</td>
</tr>
<tr>
<td>Extraction by sodium chloride</td>
<td>5</td>
<td>0.062±0.010</td>
<td>1.240±0.204</td>
</tr>
</tbody>
</table>

It is clear that among the three degumming methods tested under this investigation, the hot water degumming method, when applied to the oak tasar (Antheraea proylei J.) cocoon, gives the highest degumming ratio, i.e. highest sericin yield. Therefore, this method of degumming the silk cocoon was adopted for the rest of the present investigation. For gaining a better understanding, the hot water sericin extraction performed according to Wang et al. (2012) is described here. The oak tasar (Antheraea proylei J.) cocoon shell was cut into small square pieces. 5g of the cocoon pieces was mixed with 150mL of distilled water, cooked by pressure cooking 1hr, and the resulting aqueous fraction was collected. This process was repeated two more times with the same cocoon sample. The resulting aqueous fractions were pooled, centrifuged to collect the supernatant, and then lyophilised to obtain sericin extract. The same degumming procedure was applied to the mulberry cocoon which was taken as a standard for present investigation. The comparative results of the hot water degumming method are given in Table 2:

**Table 2:** The degumming ratios for oak tasar and mulberry cocoons subjected to hot water sericin extraction

<table>
<thead>
<tr>
<th>Type of cocoon</th>
<th>Initial weight of cocoon (g)</th>
<th>Weight of sericin extract (g)</th>
<th>Degumming ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antheraea proylei J.</td>
<td>5</td>
<td>0.473±0.017</td>
<td>9.460±0.342</td>
</tr>
<tr>
<td>Bombyx mori L.</td>
<td>5</td>
<td>1.155±0.111</td>
<td>23.100±2.220</td>
</tr>
</tbody>
</table>

Based on the above results, the oak tasar cocoon yielded about 2.4-times less sericin extract as compared to the mulberry cocoon. The higher degumming sericin yield from mulberry cocoon is compatible with the literature reports that the mulberry cocoon contains more sericin than the non-mulberry cocoons. The oak tasar sericin extract was analysed with respect to antioxidant, antityrosinase and antimelanogenic activities. In all these studies, the mulberry sericin extract was
included as the standard as far as possible. To make things convenient in analysis and reporting of the experimental outcomes, the sericin extract obtained by hot water degumming of the oak tasar \((Antheraea proylei\ J.)\) cocoon was designated as APHWE (an acronym of \(Antheraea proylei\ J.\) cocoon hot water sericin extract). Similarly, the sericin extract obtained by hot water degumming of the mulberry \((Bombyx mori\ L.)\) cocoon was designated as BMHWE (an acronym of \(Bombyx mori\ L.\) cocoon hot water sericin extract).

**2. Fractionation of APHWE according to Wang et al. (2012):** As a part of the present investigation, the sericin extract APHWE was fractionated by ethanolic precipitation into ethanolic APHWE sericin fraction and ethanolic APHWE non-protein fraction by a procedure following that of Wang et al. (2012). Similarly, the sericin extract BMHWE was also fractionated ethanolic BMHWE sericin fraction and ethanolic BMHWE non-protein fraction. To 0.2mL of aqueous solution containing 2 mg sericin extract, 1.6 mL absolute ethanol was mixed and then kept overnight at -20°C. Next day, the mixture was centrifuged at 15,000g for 30 min at -4°C, the pellet collected as the ethanolic sericin fraction was air dried, and kept for further biochemical investigation. The resulting supernatant containing the non-protein fraction was concentrated in the hot air oven at 60°C and then kept also for further biochemical analysis.

3. Characterization of sericin extracts (APHWE/BMHWE), ethanolic APHWE/BMHWE sericin fractions and ethanolic APHWE/BMHWE non-protein fractions

**3.1. Protein content, total phenolic content, flavonoid content, and electrophoretic behaviour on SDS-PAGE:** The sericin extracts APHWE and BMHWE were characterized with respect to protein content, total phenolic content, and flavonoid content. The protein contents were estimated according to the method of Lowry et al. (1951) using crystalline bovine serum (BSA) as the standard. The total phenolic content was determined according to the procedure of Mathew and Abraham (2006) using the Folin’s Ciocalteu reagent and gallic acid as the standard. The flavonoid content was determined according to Sahu and Saxena (2013) adopting the aluminium chloride method and quercetin as the standard. The sericin extracts APHWE and BMHWE and their corresponding ethanolic sericin fractions were analysed by SDS-PAGE (12% gel) according to the procedure of Laemmli, (1970) using medium range protein markers (Mr 6.6 to 97.4 kDa). The separated protein bands were visualized by silver staining procedure of Merril (1990). The results are given in Table 3 and Fig.1.

**Table 3:** Protein content, and total phenolic and flavonoid contents of the sericin extracts APHWE and BMHWE

<table>
<thead>
<tr>
<th>Sericin extract</th>
<th>Protein content mg/g extract</th>
<th>Total phenolic content mg GAE/g extract</th>
<th>Total flavonoid content mg Quercetin Equivalent/g extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>APHWE</td>
<td>380.600±3.231</td>
<td>111.300±2.936</td>
<td>255.220±1.010</td>
</tr>
<tr>
<td>BMHWE</td>
<td>251.050±6.394</td>
<td>33.160±1.153</td>
<td>18.460±0.530</td>
</tr>
</tbody>
</table>

APHWE from the oak tasar \((Antheraea proylei\ J.)\) cocoon was found to have 1.5-fold higher protein content \((380.600±3.231\ mg/g)\), 3-fold higher total phenolic content \((111.300±2.936\ mg\ GAE/g)\) and 14-fold higher flavonoid content \((255.220±1.010\ mg\ Quercetin\ Equivalent/g)\) than the corresponding values of BMHWE extract from the mulberry \((Bombyx mori\ L.)\) cocoon, viz. 251.050±6.394 mg/g, 33.160±1.153 mg GAE/g and 18.460±0.530 mg Quercetin Equivalent/g, respectively.
**Fig.1:** SDS-PAGE (12%) gel. Lane 1: medium range protein markers, Lane 2: APHWE from oak tasar (*Antheraea proylei* J.) cocoon, Lane 3: purified sericin (ethanolic APHWE) fraction, Lane 4: BMHWE from mulberry (*Bombyx mori* L.) cocoon, Lane 5: purified sericin (BMHWE) fraction

APHWE and corresponding ethanolic APHWE sericin fraction from oak tasar (*Antheraea proylei* J.) cocoon were found to contain protein species having a wide range molecular weight, 6.6 to >97.4 kDa, with high contents of sericin species at around 80kDa, 40kDa, 35kDa, 20kDa, 15kDa and 6 kDa. Similarly, BMHWE and its corresponding ethanolic BMHWE sericin fraction from mulberry (*Bombyx mori* L.) cocoon were found to contain sericin protein species having a wide range molecular weight. The protein bands became more well defined after purification of the sericin extract by ethanol precipitation which lead to part removal non-protein components.

### 3.2. Free radical scavenging activity:

The free radical scavenging activities of the sericin extracts (APHWE and BMHWE), corresponding ethanolic sericin fractions and corresponding ethanolic non-protein fractions were determined using different methods, viz. DPPH scavenging activity analysis, Hydroxyl radical scavenging activity assay, \( \text{H}_2\text{O}_2 \) scavenging activity assay, and Ferric reducing antioxidant power (FRAP) assay.

#### 3.2.1. DPPH scavenging activity:

The DPPH scavenging activity was measured according to Chen *et al.* (2013) using the stable free radical DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), the violet colour of which turns colourless on reduction by samples having free radical scavenging activity. Ascorbic acid and gallic acid were used as the positive controls. The results in terms of EC\(_{50}\) (sample concentration at which 50% DPPH scavenging effect is observed) are given in Table 4 to 5.
Table 4: EC₅₀ values for DPPH scavenging effect of the sericin extracts (APHWE and BMHWE) and standard free radical scavengers, viz. ascorbic acid and gallic acid

<table>
<thead>
<tr>
<th>Sample</th>
<th>EC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APHWE</td>
<td>169.600±2.773</td>
</tr>
<tr>
<td>BMHWE</td>
<td>618.670±7.563</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>5.150±0.610</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>3.280±0.469</td>
</tr>
</tbody>
</table>

Examination of the above EC₅₀ values indicated that APHWE from oak tasar (Antheraea proylei J.) cocoon has about four times higher free radical scavenging activity than BMHWE from mulberry (Bombyx mori L.) cocoon.

Table 5: EC₅₀ values for DPPH scavenging effect of the ethanolic APHWE/BMHWE sericin fractions, and ethanolic APHWE/BMHWE non-protein fractions

<table>
<thead>
<tr>
<th>Sample</th>
<th>EC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic APHWE non-protein fraction</td>
<td>119.371±2.645</td>
</tr>
<tr>
<td>Ethanolic APHWE sericin fraction</td>
<td>422.270±2.809</td>
</tr>
<tr>
<td>Ethanolic BMHWE non-protein fraction</td>
<td>1450.651±37.621</td>
</tr>
<tr>
<td>Ethanolic BMHWE sericin fraction</td>
<td>4136.000±54.671</td>
</tr>
</tbody>
</table>

The ethanolic APHWE non-protein fraction was found to have about 3.5 times lower EC₅₀ value (119.371±2.645 µg/mL) than the ethanolic APHWE sericin fraction (422.270±2.809 µg/mL), i.e. the non-protein fraction from oak tasar (Antheraea proylei J.) cocoon has 3.5 times higher DPPH scavenging activity than the corresponding sericin fraction purified by ethanol treatment (ethanolic APHWE sericin fraction).

The ethanolic BMHWE non-protein fraction was found to have about 2.8 times lower EC₅₀ value (1450.651±37.621 µg/mL) than the ethanolic BMHWE sericin fraction (4136.000±54.671 µg/mL), i.e. the non-protein fraction from mulberry (Bombyx mori L.) cocoon has 2.8 times higher DPPH scavenging activity than the corresponding sericin fraction purified by ethanol treatment (ethanolic BMHWE sericin fraction).

3.2.2. Hydroxyl radical scavenging activity: The Fenton Reaction Based Radical (FRBR) assay was performed according to Gayathri et al. (2014). Hydroxyl radical scavenging activity was measured by the ability of the sample at varying concentrations to scavenge the hydroxyl radicals generated by the Fe³⁺-ascorbate-EDTA-H₂O₂ system (Fenton Reaction). Hydroxyl radical scavenging assay was carried out for APHWE and BMHWE, ethanolic APHWE/BMHWE sericin fractions, and ethanolic APHWE/BMHWE non-protein fractions. Ascorbic acid at varying concentration (200-1000 µg/mL) was used as a positive control. The results obtained are given Fig.2 and Table 6 to 7:
Fig. 2: Comparison of hydroxyl radical scavenging activities of APHWE and BMHWE and ascorbic acid (as standard) at increasing concentrations under the standard assay conditions.

The corresponding EC\textsubscript{50} values of hydroxyl radical scavenging activities are given in Table 6.

**Table 6: EC\textsubscript{50} values of hydroxyl radical scavenging activities of APHWE, BMHWE and ascorbic acid**

<table>
<thead>
<tr>
<th>Sample</th>
<th>EC\textsubscript{50} (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APHWE</td>
<td>1.136±0.015</td>
</tr>
<tr>
<td>BMHWE</td>
<td>1.724±0.247</td>
</tr>
<tr>
<td>Ascorbic acid (Standard)</td>
<td>0.926±0.021</td>
</tr>
</tbody>
</table>

As inferred from this table, significantly lower value (1.136±0.015 mg/mL) than that of BMHWE (1.724±0.247 mg/mL), i.e. the sericin extract from oak tasar (Antheraea proylei J.) cocoon has significantly higher hydroxyl radical scavenging activity than the BMHWE from mulberry (Bombyx mori L.) cocoon. Ascorbic acid as the standard antioxidant has higher hydroxyl radical scavenging activity than both the sericin extracts (APHWE and BMHWE).

**Table 7: Hydroxyl radical scavenging activities in terms of EC\textsubscript{50} value of the ethanolic sericin fractions and ethanolic non-protein fractions obtained from sericin extracts APHWE and BMHWE**
<table>
<thead>
<tr>
<th>Sample</th>
<th>EC₅₀ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic APHWE non-protein fraction</td>
<td>4.610±0.040</td>
</tr>
<tr>
<td>Ethanolic APHWE sericin fraction</td>
<td>5.002±0.312</td>
</tr>
<tr>
<td>Ethanolic BMHWE non-protein fraction</td>
<td>3.527±0.250</td>
</tr>
<tr>
<td>Ethanolic BMHWE sericin fraction</td>
<td>4.210±0.072</td>
</tr>
</tbody>
</table>

As inferred from this table, the ethanolic APHWE/BMHWE non-protein fractions have significantly higher hydroxyl radical scavenging activity (EC₅₀ values 4.610±0.04 mg/mL and 3.527±0.025 mg/mL) than the corresponding ethanolic APHWE/BMHWE sericin fractions (EC₅₀ values 5.002±0.0312 mg/mL and 4.210±0.072 mg/mL).

The ethanolic BMHWE non-protein and ethanolic BMHWE sericin fractions have significantly lower EC₅₀ values (3.527±0.025 mg/mL and 4.210±0.072 mg/mL) than the corresponding ethanolic APHWE non-protein and ethanolic APHWE sericin fractions (4.610±0.04 mg/mL and 5.002±0.312 mg/mL), i.e. the ethanolic BMHWE non-protein and ethanolic BMHWE sericin fractions from mulberry (Bombyx mori L.) cocoon have significantly higher hydroxyl radical scavenging activity than the corresponding ethanolic APHWE non-protein and ethanolic APHWE sericin fractions from oak tasar (Antheraea proylei J.) cocoon.

3.2.3. H₂O₂ scavenging activity: H₂O₂ scavenging activity assay was performed according to Gayathri et al. (2014). The results are given in Fig.3 and Table 8 to 9.

![Fig.3: Hydrogen peroxide scavenging activity as a function of concentration of sericin extracts APHWE and BMHWE](image)

Table 8: The EC₅₀ values for %H₂O₂ scavenging activities of APHWE and BMHWE and ascorbic acid as the standard
Ascorbic acid has higher H$_2$O$_2$ scavenging activity than both the sericin extracts APHWE and BMHWE. However, APHWE extracted from oak tasar (*Antheraea proylei* J.) cocoon has higher H$_2$O$_2$ scavenging activity than BMHWE extracted from mulberry (*Bombyx mori* L.) cocoon.

**Table 9**: H$_2$O$_2$ scavenging activities in terms of EC$_{50}$ of the ethanolic sericin fractions and ethanolic non-protein fractions obtained from the sericin extracts APHWE and BMHWE

<table>
<thead>
<tr>
<th>Sample</th>
<th>EC$_{50}$ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic APHWE non-protein fraction</td>
<td>0.704±0.014</td>
</tr>
<tr>
<td>Ethanolic APHWE sericin fraction</td>
<td>1.090±0.013</td>
</tr>
<tr>
<td>Ethanolic BMHWE non-protein fraction</td>
<td>4.170±0.167</td>
</tr>
<tr>
<td>Ethanolic BMHWE sericin fraction</td>
<td>10.000±0.396</td>
</tr>
</tbody>
</table>

The ethanolic APHWE/BMHWE non-protein fractions have EC$_{50}$ values lower than the corresponding EC$_{50}$ values of the ethanolic APHWE/BMHWE sericin fractions, i.e. the non-protein fractions from both the non-mulberry and mulberry cocoons have higher H$_2$O$_2$ scavenging activity than the corresponding ethanol-precipitated sericin fractions from both cocoon types. The ethanolic APHWE sericin and ethanolic APHWE non-protein fractions have EC$_{50}$ values (1.090±0.013 mg/mL and 0.704±0.014 mg/mL) lower than those of the corresponding mulberry cocoon fractions (10.000±0.396 mg/mL and 4.170±0.167 mg/mL), i.e. the ethanol fractionated sericin and non-protein fractions from the oak tasar (*Antheraea proylei* J.) cocoon have higher H$_2$O$_2$ scavenging activity than the corresponding ethanol fractionated sericin and non-protein fractions from mulberry (*Bombyx mori* L.) cocoon.

3.2.4. Ferric reducing antioxidant power (FRAP): The ferric reducing antioxidant power (FRAP) assay was performed according to Uddin et al. (2014). The FRAP assay uses an antioxidant as a reductant in a redox-linked colorimetric method employing an easily reduced oxidant, Fe (III). Reduction of a ferric tripyridyltriazine complex to ferrous-(2,4,6-tripyridyltriazine)$_2$ i.e. reduction of Ferric (III) [colourless] to Ferrous (II) [blue] can be monitored by measuring absorbance at 593 nm. The FRAP was expressed in terms of μg Fe$^{3+}$/mg of the sample using FeSO$_4$.7H$_2$O as the standard, or μg Ascorbic Acid Equivalent [AAE]/mg of the sample using ascorbic acid as the standard. The results are given in **Table 10**.

**Table 10**: FRAP values of sericin extracts APHWE and BMHWE

<table>
<thead>
<tr>
<th>Sample</th>
<th>μg Fe$^{3+}$/mg of sample</th>
<th>μg [AAE]/mg of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>APHWE</td>
<td>33.000±1.360</td>
<td>10.987±0.450</td>
</tr>
<tr>
<td>BMHWE</td>
<td>7.615±2.001</td>
<td>2.530±0.383</td>
</tr>
</tbody>
</table>
Higher the FRAP value in the FRAP assay indicates higher antioxidant potential of the sample for reducing Fe$^{3+}$ to Fe$^{2+}$. The values were expressed both in terms of Ferrous Equivalent and Ascorbic Acid Equivalent. The sericin extract APHWE from oak tasar (Antheraea proylei J.) cocoon has higher FRAP value (33.000±0.136 μg Fe$^{2+}$/mg of sample or 10.987±0.045 μg [AAE]/mg of sample) than the sericin extract BMHWE from mulberry (Bombyx mori L.) cocoon (7.615±0.102 μg Fe$^{2+}$/mg of sample or 2.530±0.038 μg [AAE]/mg of sample).

### Table 11: FRAP values of ethanolic APHWE/BMHWE sericin and ethanolic APHWE/BMHWE non-protein fractions

<table>
<thead>
<tr>
<th>Sample</th>
<th>μgFe$^{2+}$/mg of sample</th>
<th>μg[AAE]/mg of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic APHWE non-protein fraction</td>
<td>174.560±10.580</td>
<td>56.000±0.460</td>
</tr>
<tr>
<td>Ethanolic APHWE sericin fraction</td>
<td>28.971±0.257</td>
<td>9.300±0.081</td>
</tr>
<tr>
<td>Ethanolic BMHWE non-protein fraction</td>
<td>15.641±0.376</td>
<td>4.970±0.127</td>
</tr>
<tr>
<td>Ethanolic BMHWE sericin fraction</td>
<td>2.440±0.145</td>
<td>0.783±0.049</td>
</tr>
</tbody>
</table>

Amongst the four different fractions, the Ethanolic APHWE non-protein fraction from oak tasar (Antheraea proylei J.) cocoon has the highest FRAP value (174.560±10.58 μgFe$^{2+}$/mg of sample and 56.000±0.460 μg[AAE]/mg of sample). The ethanolic APHWE/BMHWE non-protein fractions from both the non-mulberry and mulberry cocoons have higher FRAP values than the corresponding ethanolic APHWE/BMHWE sericin fractions. The ethanolic APHWE sericin fraction has higher FRAP value (28.971±0.257 μgFe$^{2+}$/mg of sample and 9.300±0.081 μg[AAE]/mg of sample) than the ethanolic BMHWE sericin fraction (2.440±0.145 μgFe$^{2+}$/mg of sample and 0.783±0.049 μg[AAE]/mg of sample) (Table 11).

#### 3.3. Antityrosinase activity: The antityrosinase activities of the silkworm cocoon extracts were determined according to Kim et al. (2005). The amount of dopachrome produced was measured by a spectrophotometer at 480 nm. The absorbance of the reaction mixture without the sample was taken as the control of the experiment. The percentage inhibition of the tyrosinase activity was calculated using the formula: 

\[
\% \text{Inhibition} = \frac{A_c - A_s}{A_c} \times 100, \text{ where } A_c = \text{Absorbance of the control at 480 nm, } A_s = \text{Absorbance in presence of the test sample at 480 nm. Kojic acid (1mg/mL in 100 mM phosphate buffer, pH 6.8) was used as a positive control. The results obtained are given in Table 12 and Fig.4:}

### Table 12: IC$_{50}$ values for tyrosinase inhibition

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APHWE</td>
<td>8.803±0.419</td>
</tr>
<tr>
<td>BMHWE</td>
<td>2.574±0.333</td>
</tr>
<tr>
<td>Kojic acid</td>
<td>0.006±0.002</td>
</tr>
<tr>
<td>Ethanolic APHWE non-protein fraction</td>
<td>3.808±0.920</td>
</tr>
<tr>
<td>Ethanolic BMHWE non-</td>
<td>3.307±0.613</td>
</tr>
</tbody>
</table>
Though sericin extract APHWE from oak tasar (*Antheraea proylei* J.) cocoon was found to exhibit antityrosinase activity (*IC_{50} = 8.803±0.419 mg/mL*), the similar activity exhibited by the sericin extract BMHWE from mulberry (*Bombyx mori* L.) cocoon (*IC_{50} = 2.574±0.333 mg/mL*) was found to be higher. The standard antityrosinase agent Kojic acid (*IC_{50} = 0.006±0.002 mg/mL*) showed relatively much higher antityrosinase activity when compared to both of the sericin extracts (Table 12). The ethanolic APHWE non-protein fraction was found to exhibit a significant antityrosinase activity with *IC_{50} = 3.808±0.920 mg/mL* which compares well with the antityrosinase activity of ethanolic BMHWE non-protein fraction with *IC_{50} = 3.307±0.613 mg/mL*. On other hand, the ethanolic BMHWE) sericin fraction exhibited relatively higher antityrosinase activity than the ethanolic APHWE sericin fraction as shown in Fig. 4.

3. **Isolation of lymphocytes:** Blood was collected by a medically trained person and transferred to a BD Vacutainer K2EDTA. The anticoagulated blood was slowly and carefully layered over an equal volume of Histopaque-1077, which has already been brought to room temperature. It was then centrifuged at 400 × g (2000 rpm) for 30 minutes at room temperature. Following centrifugation, an opaque lymphocyte layer formed at the plasma-Histopaque-1077 interface was recovered using a pipette and transferred to a clean and fresh tube. It was then washed with 3 volumes of PBS and centrifuged at 250 × g (1500 rpm) for 10 minutes at room temperature. The supernatant was aspirated and the cells were again washed in PBS at a speed of 250 × g (1500 rpm) for 10 minutes at room temperature. The supernatant was aspirated and the cells were suspended in RPMI-1640 media. After uniform mixing of the cells, 10 µL of the cell suspension was taken for counting of the cells.

4. **Counting of cells:** Cells were uniformly mixed with Trypan blue 0.4 % solution in Dulbecco’s PBS in the ratio of 1:1.10 µL of the mixture was mounted on the Neubauer chamber and observed under an inverted microscope. The total number of cells was determined using the formula:

\[
\text{Total number of cells} = \text{Cell number} \times \text{DF} \times \frac{\text{Volume of the sample used}}{\text{Total volume of the cell suspension}}.
\]

*DF= Dilution Factor*
5. Cytotoxicity of sericin preparation on human peripheral blood lymphocytes: The cytotoxic effect of sericin extract APHWE/BMHWE was tested according to the procedure of Dash et al. (2007). The isolated human peripheral blood lymphocytes (hPBL) were seeded in RPMI in a 96 well microtitre plate at a concentration of $1 \times 10^6$ cells/mL per well. The cells were treated with different concentrations of APHWE/BMHWE (50 μg/mL, 100 μg/mL, 200 μg/mL, 400 μg/mL, 800 μg/mL and 1600 μg/mL) and incubated for 48 hours at 37°C. After 48 hours, cell viability was assessed using MTT assay. The results are shown in Fig.5.

![Fig.5](image_url)

Fig.5: Assay of viability of human Peripheral Blood Lymphocytes exposed to sericin extract APHWE/BMHWE by MTT test.

The sericin extract APHWE/BMHWE up to a concentration of 1600 μg/mL was found to neither increase nor decrease the viability of the human peripheral blood lymphocytes significantly ($p \leq 0.5$) (Fig 5). Hence the sericin extract from oak tasar (*Antheraea proylei* J.) coccon or from mulberry (*Bombyx mori* L.) cocoon was found to be non-cytotoxic on human peripheral blood lymphocyte.

6. Cytotoxicity of hydrogen peroxide on human peripheral blood lymphocytes: The cytotoxic effect of hydrogen peroxide on human peripheral blood lymphocytes was assessed according to the procedure of Dash et al. (2007). Isolated human peripheral blood lymphocytes were seeded in RPMI complete media. The cells were then incubated at 37°C. After 24 hours of incubation, the cells were treated with hydrogen peroxide at increasing concentrations and then incubated for 24 hours. After the incubation, cell viability was assessed using MTT assay. The results are shown in Fig.6.
7. **Protective effect of sericin extract against hydrogen peroxide-induced cytotoxicity of lymphocytes:** The protective effect of sericin extract against hydrogen peroxide-induced cytotoxicity of lymphocytes was determined by a procedure adopted by Dash et al. (2007). Isolated human peripheral blood lymphocytes were seeded in RPMI-1640 complete media in a 96-well microtitre plate. The cells were mixed with sericin extract APHWE/BMHWE) at increasing concentration and then incubated for 24 hours at 37°C. After the incubation, the cells were exposed to hydrogen peroxide and incubated for another 24 hours. The cell viability was assessed by MTT assay. The corresponding results of the experiment are shown in **Fig.7:**
Fig. 7: Protective effect of sericin extract (APHWE or BMHWE) on the lymphocyte cytotoxicity induced by hydrogen peroxide

The sericin extract APHWE from oak tasar (Antheraea proylei J.) cocoon at concentration range 50 to 800 µg/mL protects the lymphocyte from cytotoxicity induced by hydrogen peroxide. In this concentration range, the sericin extract enhances the cell viability significantly as compared to the negative control. On the other hand, the cell viability enhancement was observed only at 800 µg/mL of the sericin extract BMHWE from mulberry (Bombyx mori L.) cocoon thereby indicating that the sericin extract APHWE can provide greater protection to lymphocyte against cytotoxicity induced by hydrogen peroxide as compared to the sericin extract BMHWE.

8. Assessment of antioxidant activity of the sericin extract (APHWE/BMHWE) using human peripheral blood lymphocyte: The antioxidant activity of the extract was determined at cellular level using human peripheral blood lymphocytes. The human peripheral blood lymphocytes were seeded in a volume of 5 mL in RPMI in T flask. The cells were treated with sericin extract APHWE/BMHWE at increasing concentrations and kept incubated at 37°C. After 24 hours of incubation, the cells were exposed to hydrogen peroxide and then incubated further for 24 hours. At the end of the incubation period, the cells were harvested, lysed in a lysis buffer using a homogenizer, and centrifuged to collect the supernatant for analysis of the following parameters:

(i) Superoxide dismutase activity: The superoxide dismutase (SOD) activity was determined following the method of Roberto et al. (2009). The supernatant obtained from lysed lymphocyte was mixed with a solution containing sodium carbonate buffer (50 mM, pH 10.2) and 50 mM epinephrine prepared in 0.1 N HCl. The autoxidation of epinephrine producing adrenochrome was monitored at 480 nm as a function of time. The SOD activity was assessed in terms of the inhibition of the autoxidation of epinephrine. Results were expressed as units (U) of SOD/mg of protein, where 1 U of SOD induces the inhibition of autoxidation of adrenaline by 50%. The results of assessment of antioxidant activity of the sericin extract APHWE/BMHWE using hydrogen peroxide-exposed human peripheral blood lymphocyte in terms of its SOD activity are shown in Fig. 8.
The sericin extract APHWE/BMHWE at increasing concentrations significantly increased SOD activity (production) in the hydrogen peroxide-treated lymphocyte. With the APHWE treatment, the highest SOD activity in the lymphocyte was observed at the treatment dose of 100 \( \mu \text{g/mL} \), while the highest SOD activity (production) in the cell was observed at the treatment dose of 200 \( \mu \text{g/mL} \) with the BMHWE treatment (Fig.8). These results indicated that sericin extract from oak tasar (\textit{Antheraea proylei} J.) cocoon exhibit higher antioxidant activity than the sericin extract from mulberry (\textit{Bombyx mori} L.) cocoon.

(ii) **Glutathione peroxidase activity:** Glutathione peroxidase activity was determined by a procedure adopted by Wendel, (1981). Phosphate buffer along with appropriate amounts of glutathione reductase, GSH, and NADPH were transferred respectively into a 1 mL cuvette. The cell lysate was added and incubated for 10 minutes at 37°C. The reaction was started by addition of hydrogen peroxide. The linear decrease in NADPH absorption was recorded at 366 nm. The corresponding blank reading due to the spontaneous reaction was determined without the enzyme and then it was subtracted. One unit of glutathione peroxidase was the amount of enzyme required for oxidation of 1 micromole of reduced glutathione to oxidised glutathione per minute at pH 7 at 25°C. The specific activity of glutathione peroxidase was expressed as U/mg protein. It was calculated using the formula:

\[
\text{Specific activity} = \frac{\Delta \text{abs} \times \text{Total volume of reaction mixture}}{\text{Molar extinction coefficient} \times \text{Total volume of sample} \times \text{Protein concentration}}
\]

The results are shown in Fig.9. Glutathione peroxidase activity was significantly increased compared to the negative control in lymphocytes treated with a dose ranging from 100 to 800 \( \mu \text{g/mL} \) of the sericin extract APHWE/BMHWE.
Catalase activity: The catalase activity was determined by a procedure adopted by Aebi (1984). The activity of the antioxidant enzyme catalase was monitored by its ability to decompose H$_2$O$_2$. The reaction mixture contained 500 µL of H$_2$O$_2$, 440 µL of phosphate buffer and 60 µL of the cell lysate. The reaction was initiated with the addition of hydrogen peroxide. The decrease in absorbance was recorded at 240 nm against a blank containing 60 µL sample and 940 µL phosphate buffer. One unit of catalase activity was the amount of enzyme required to decompose 1mmol of H$_2$O$_2$/min at 25°C and pH 7. The specific activity was calculated using the formula given below and it was expressed as U/mg protein.

$$\text{Specific activity} = \frac{\Delta | \times | \frac{\text{Total volume of reaction mixture}}{\text{Molar extinction coefficient} \times \text{Total volume of sample}}} \text{Protein concentration}$$

The results are shown in Fig.10. Catalase activity of the hydrogen peroxide-exposed human peripheral blood lymphocyte was significantly increased as compared to the that of the negative control when treated with a dose of 50 µg/mL of the sericin extract APHWE/BMHWE.
(IV) **GSH content:** The GSH content was determined by the procedure described by Khan et al. (2010). The Glutathione content of the cytosolic lysates of the lymphocytes was determined using 5,5-dithiobis-2-nitrobenzoic acid (DTNB). DTNB combines with reduced glutathione (GSH) to form 5-thio-2-nitrobenzoic acid (TNB). The reaction mixture contains 100 µL of the cell lysate, 750 µL of phosphate buffer and 150 µL of 1 mM DTNB. The reaction mixture was mixed well and incubated for 5 minutes. After the incubation, absorbance was read at 412 nm. The GSH content was estimated from a standard curve using pure GSH as the standard. The results are shown in **Fig.11.** The content of the non-enzymatic antioxidant glutathione in the hydrogen peroxide-exposed human peripheral blood lymphocytes was significantly increased as compared to that of the negative control when treated with sericin extract (APHWE/BMHWE) at increasing concentrations (50, 100, 200 and 800 µg/mL).
Lipid peroxidation assay: The lipid peroxidation assay was carried out according to procedure of Ohkawa et al. (1979). The extent of lipid peroxidation inhibition induced by the sericin extract APHWE/BMHWE on the hydrogen peroxide-exposed human peripheral lymphocytes was measured using malondialdehyde (MDA) as the standard. The results are shown in Fig.12.

Fig.12: Lipid Peroxidation assay of human Peripheral Blood Lymphocytes

The results are shown in Fig.12. Increased lipid peroxidation was observed in the hydrogen peroxide-exposed human peripheral lymphocytes of the negative control. This increase of the peroxidation was significantly reduced by treatment of the lymphocytes with the sericin extract APHWE/BMHWE at increasing doses.

9. Anti-melanogenic activity: The anti-melanogenic activity assay was carried out in zebrafish following the procedure of Chen et al. (2019). The wild-type zebrafish was reared in a temperature and light controlled culture facility. The zebrafish embryos were obtained by breeding male and female adult zebrafish populated in the ratio 1:2 in a breeding chamber overnight at room temperature. The embryos were cultured in E3 medium. The 24 hpf embryos were collected and then distributed in a 12-well plate @ 25 embryos per well. The embryos in separate wells were treated with 2 mL E3 medium with 0.5% DMSO containing increasing concentration of the sericin extract APHWE. After 48 hr of exposure to the sericin extract, the 72 hpf embryos were collected and analysed for pigmentation level and tyrosinase activity. Triplicates at each concentration of the sericin extract were run. The same experiment was repeated twice replacing the sericin sample by BMHWE/kojic acid. The value for the control was taken as 100% and other similar experimental values were calculated as percentage relative to the control. The results are given in Fig.13, 14 & 15.
Fig. 13 Changes in the relative melanin content in zebrafish embryo exposed to increasing concentrations of sericin extract APHWE/BMHWE and standard anti-melanogenic compound Kojic acid. The data are presented as percentages compared with the control group (set to 100%) and are the means ± SD, n = 3. Different alphabetical letters indicate significant differences among the conditions as assessed by one-way ANOVA followed by the Tukey’s test (Pb 0.05).

The sericin preparation from oak tasar (Antheraea proylei J.) cocoon (APHWE) had a decreasing effect on melanin synthesis in zebrafish embryo. The anti-melanogenic activity increased with an increase in concentration in the range 0 to 200 g/µmL. However, the sericin preparation from mulberry (Bombyx mori L.) cocoon (BMHWE) and kojic acid had greater anti-melanogenic activity than APHWE. The treatment with 10 μg/mL APHWE was not able to significantly decrease the melanin content, whereas BMHWE and kojic acid could significantly decrease melanin content at the same concentration with reference to that of the untreated zebrafish embryos which served as the control.
Fig. 14: Decrease of pigmentation in zebrafish embryo exposed to increasing concentration of the sericin extract (APHWE/BMHWE) or the standard anti-melanogenic compound Kojic acid

To determine the inhibitory effect on tyrosinase activity, the 72 hpf embryos of above, already exposed to the sericin extract, were sonicated in cold lysis buffer, and centrifuged at 4°C to collect the supernatant. For determining the tyrosinase activity, 80 μL of the supernatant was taken in a 96-well microtiter plate, mixed with 20 μL L-DOPA (2 mg/mL), incubated for 1 hr, and the absorbance was read at 490 nm. The ‘specific tyrosinase activity’ was estimated as the absorbance divided by the protein content estimated by Bradford method. The relative tyrosinase activities were calculated by taking the specific tyrosinase activity of the control as 100%. The results are shown in Fig. 15.
Fig.15: Inhibition of tyrosinase activity in zebrafish embryo at increasing concentrations of the sericin extract APHWE/BMHWE or the standard tyrosinase inhibitor Kojic acid. The data are presented as percentages compared with the control group (set to 100%) and are the means ± SD, n = 3. Different alphabetical letters indicate significant differences among the conditions as assessed by one-way ANOVA followed by the Tukey’s test (P < 0.05).

The anti-melanogenic activity was assessed in terms of the inhibition of the tyrosinase activity of the zebrafish embryo. With increasing concentration, APHWE showed increasing inhibitory effect on tyrosinase activity. BMHWE and the standard tyrosinase inhibitor kojic acid had greater tyrosinase inhibitory effect than that of APHWE. Corresponding to the decreasing effect on melanin production, the treatment with 10 μg/mL APHWE was not able to significantly decrease the tyrosinase activity. On the other hand, BMHWE and kojic acid could significantly decrease the tyrosinase activity at the same concentration with reference to the tyrosinase activity level in the untreated zebrafish embryos as the control.

Conclusion:

Till recently, silk protein sericin was considered as a waste material in the sericulture industry and it was discarded causing environmental issues and loss of an important bioresource. Thus, the recovery and reuse of the proteinaceous material not only minimize environmental issues but also have a high scientific and commercial value. The physicochemical properties of the proteinaceous material are such that it is suitable for numerous applications in biomedicine. The presence of highly hydrophilic amino acids in it and its antioxidant potential make it useful in the food and cosmetic industry. The moisturizing power allows it to be used as a therapeutic agent for wound healing, stimulating cell proliferation, protection against ultraviolet radiation, and formulating creams and shampoos. The antioxidant activity associated with low digestibility finds its applications in medical field as antitumour, anticoagulant, antimicrobial and anti-inflammatory agent. The protein also finds its applications in formulation of cell culture media and cryopreservation, tissue engineering and drug delivery, demonstrating its effective use as an important biomaterial. Most of these findings were accrued from the extensive study conducted on the sericin extracted by degumming of the cocoon produced by the most-widely spread and fully domesticated mulberry (Bombyx mori L.) silkworm. However, very little study has been conducted so far on the sericin extracts obtained from the cocoons produced by many different species.
of non-mulberry silkworms found in the forest habitats. The sericin serves as the gummy agent which binds the silk (fibroin) fibres together to form the cocoon which serves as the protective envelope to the metamorphosing larva (pupa) of the silkworm. It is likely that sericin in the cocoon helps it in protecting the pupa from harsh environmental stresses including sunshine (UV light), rain (moisture), disease causing-microbes, predators, etc. As compared to the sericin extract isolated from the cocoon produced by the fully domesticated mulberry silkworm reared indoor, the sericin extract from the cocoons, produced by the non-mulberry silkworm found in the harsher outdoor environment, is likely to possess better protective properties. With this idea in mind, the present project was undertaken to study the antioxidative, antityrosinase and antimelanogenic activities of sericin extracts from cocoon produced by the non-mulberry oak tasar (Antheraea proylei J.) silkworm and to compare the results with those of the sericin extract from the mulberry cocoon. It was found that sericin extract from oak tasar cocoon has higher antioxidant potential than that of the mulberry cocoon. This could be at least partly because of the high total phenolic and flavonoid contents in the oak tasar sericin preparation. However, the sericin extract from mulberry cocoon showed higher antityrosinase and antimelanogenic activity than the oak tasar sericin extract. Further, no cytotoxic effect on lymphocytes was exhibited by sericin extract from the oak tasar cocoon. Hence, we may conclude that the sericin extract from oak tasar (Antheraea proylei J.) cocoon could be an antioxidant, antityrosinase and antimelanogenic agent which may find applications in the pharmaceutical and cosmetic industry.

REFERENCES:
Dash et al. (2007) Antioxidant potential of silk protein sericin against hydrogen peroxide-induced oxidative stress in skin fibroblasts. BMB reports, 236-241.
Lowry et al. (1951) Protein measurement with Folin phenol reagent. The Journal of Biological Chemistry 193, 265-275.


iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons: The progress has been according to original plan of work and towards achieving the objective except that the planned experiments took longer time than expected.

iv. Please indicate the difficulties, if any, experienced in implementing the project: Not much difficulties were faced except that the planned experiments took longer time than expected because of the large scale disturbances in Manipur University campus, the place of the project work.

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet: Not applicable as the project has already been completed.

vi. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission:

A summary of the findings of the study is given below and one bound copy of the final detailed report is sent herewith.

SUMMARY

Out of the three methods for extraction of sericin (degumming) from silkworm cocoon shell (simply known as cocoon), viz. the hot water extraction according to Wang et al. (2012), the urea extraction according to Dash et al. (2006), and sodium chloride extraction according to Dash et al. (2006), the hot water degumming method, when applied to the oak tasar (Antheraea proylei J.) cocoon, gave the highest degumming ratio, i.e. highest sericin yield. Therefore, this method of degumming the silk cocoon was adopted for the rest of the present investigation. In this method, the oak tasar (Antheraea proylei J.) cocoon shell was cut into small square pieces. 5g of the cocoon pieces was mixed with 150mL of distilled water, cooked by pressure cooking for 1hr, and the resulting aqueous fraction was collected. This process was repeated two more times with the same cocoon sample. The resulting aqueous fractions were pooled, centrifuged to collect the supernatant, and then lyophilised to obtain sericin extract. The same degumming procedure was applied to the most well studied mulberry (Bombbyx mori L.) cocoon type which was taken as a standard for the present investigation. To make things convenient in analysis and reporting of the experimental outcome, the sericin extract obtained by hot water degumming of the oak tasar (Antheraea proylei J.) cocoon was designated as APHWE (an acronym of Antheraea proylei J. cocoon hot water sericin extract). Similarly, the sericin extract obtained by hot water degumming of the mulberry (Bombbyx mori L.) cocoon was designated as BMHWE (an acronym of Bombbyx mori L. cocoon hot water sericin extract).

As a part of the present investigation, APHWE was fractionated by ethanolic precipitation into ethanolic APHWE sericin fraction and ethanolic APHWE non-protein fraction by a procedure following that of Wang et al. (2012). Similarly, BMHWE was also fractionated ethanolic BMHWE sericin fraction and ethanolic BMHWE non-protein fraction. APHWE and BMHWE were characterized with respect to protein content, total phenolic content, and flavonoid content. The protein contents were estimated according to the method of Lowry et al. (1951) using crystalline bovine serum (BSA) as the standard. The total phenolic content was determined according to the procedure of Mathew and Abraham (2006).
using the Folin’s Ciocalteu reagent and gallic acid as the standard. The flavonoid content was determined according to Sahu and Saxena (2013) adopting the aluminium chloride method and quercetin as the standard. APHWE was found to have 1.5-fold higher protein content (380.600±3.231 mg/g), 3-fold higher total phenolic content (111.300±2.936 mg GAE/g) and 14-fold higher flavonoid content (255.220±1.010 mg Quercetin Equivalent/g) than BMHWE (251.050±0.394 mg/g, 33.160±1.153 mg GAE/g and (18.460±0.530 mg Quercetin Equivalent/g), respectively. On running SDS-PAGE coupled with silver staining of protein, APHWE and corresponding ethanolic APHWE sericin fraction from oak tasar (Antheraea proylei J.) cocoon were found to contain protein species having a wide range molecular weight, 6.6 to >97.4 kDa, with high contents of sericin species at around 80kDa, 40kDa, 35kDa, 20kDa, 15kDa and 6 kDa. Similarly, BMHWE and its corresponding ethanolic BMHWE sericin fraction from mulberry (Bombyx mori L.) cocoon were found to contain sericin protein species having a wide range molecular weight. The protein bands became more well defined after purification of the sericin extract by ethanol precipitation which lead to part removal non-protein components.

The free radical scavenging activities of APHWE and BMHWE, and corresponding ethanolic sericin and non-protein fractions were determined using different methods, viz. DPPH scavenging activity analysis, Hydroxyl radical scavenging activity assay, H₂O₂ scavenging activity assay, and Ferric reducing antioxidant power (FRAP) assay. Based on experimentally determined EC₅₀ values, APHWE has about four times higher free radical scavenging activity than BMHWE. Similarly, the sericin extract APHWE has significantly higher hydroxyl radical scavenging activity than the BMHWE. Also, APHWE has higher H₂O₂ scavenging activity than BMHWE. APHWE was also found to have higher FRAP value (indicative of antioxidant potential) than BMHWE.

The antityrosinase activities of the silkworm cocoon extracts were determined according to Kim et al. (2005). Though APHWE was found to exhibit antityrosinase activity, the similar activity exhibited by BMHWE was found to be higher. The standard antityrosinase agent Kojic acid (IC₅₀ = 0.006±0.002 mg/mL) showed relatively much higher antityrosinase activity when compared to both of the sericin extracts. The ethanolic APHWE non-protein fraction was found to exhibit a significant antityrosinase activity which compares well with the antityrosinase activity of ethanolic BMHWE non protein fraction. On other hand, the ethanolic BMHWE sericin fraction exhibited relatively higher antityrosinase activity than the ethanolic APHWE.

The cytotoxic effect of sericin extract APHWE or BMHWE was tested according to the procedure of Dash et al. (2007). The isolated human peripheral blood lymphocytes (hPBL) were seeded in RPMI in a 96 well microtitre plate at a concentration of 1×10⁶ cells/mL per well. The cells were treated with different concentrations of APHWE or BMHWE (50 µg/mL, 100 µg/mL, 200 µg/mL, 400 µg/mL, 800 µg/mL and 1600 µg/mL) and incubated for 48 hours at 37°C. At the end of the incubation period, the cell viability was assessed using MTT assay. The sericin extract APHWE orBMHWE up to a concentration of 1600 µg/mL was found to neither increase nor decrease the viability of the human peripheral blood lymphocytes significantly (p≤0.5). Hence the sericin extract from oak tasar (Antheraea proylei J.) cocoon or from mulberry (Bombyx mori L.) cocoon was found to be non-cytotoxic on human peripheral blood lymphocyte.

The cytotoxic effect of hydrogen peroxide on human peripheral blood lymphocytes was assessed assessed according to the procedure of Dash et al. (2007). Isolated human peripheral blood lymphocytes were seeded in RPMI complete media. The cells were then incubated at 37°C. After 24 hours of incubation, the cells were treated with hydrogen peroxide at increasing concentrations and then incubated further for 24 hours. After the incubation, cell viability was assessed using MTT assay. Up to 2.5 µM hydrogen peroxide in the test cell culture medium, there was no decrease in the
lymphocyte viability. However, the gradual decrease in the cell viability was observed in the concentration range 125 to 1000 µM of hydrogen peroxide. The viability of lymphocytes was about 50% when the concentration of hydrogen peroxide was 250 µM under the experimental conditions adopted. Hence this condition of cytotoxic stress was selected for use in subsequent experiments.

The protective effect of sericin extract against hydrogen peroxide-induced cytotoxicity of lymphocytes was determined by a procedure adopted by Dash et al. (2007). Isolated human peripheral blood lymphocytes were seeded in RPMI-1640 complete media in a 96-well microtitre plate. The cells were mixed with sericin extract APHWE or BMHWE at increasing concentration and then incubated for 24 hours at 37°C. After the incubation, the cells were exposed to 250 µM hydrogen peroxide and incubated for another 24 hours. The cell viability was assessed by MTT assay. The sericin extract APHWE from oak tasar (Antheraea proylei J.) cocoon at concentration range 50 to 800 µg/mL protects the lymphocyte from cytotoxicity induced by hydrogen peroxide. In this concentration range, the sericin extract enhances the cell viability significantly as compared to the negative control. On the other hand, the cell viability enhancement was observed only at 800 µg/mL of the sericin extract BMHWE from mulberry (Bombyx mori L.) cocoon thereby indicating that the sericin extract APHWE can provide greater protection to lymphocyte against cytotoxicity induced by hydrogen peroxide as compared to the sericin extract BMHWE.

The antioxidant activity of the sericin extracts was determined at cellular level using human peripheral blood lymphocytes. The cells were seeded in a volume of 5 mL in RPMI in T flask and were treated with sericin extract APHWE or BMHWE at increasing concentrations and kept incubated at 37°C. After 24 hours of incubation, the cells were exposed to hydrogen peroxide as done earlier and then incubated further for 24 hours. At the end of the incubation period, the cells were harvested, lysed in a lysis buffer using a homogenizer, and centrifuged to collect the supernatant for analysis of superoxide dismutase (SOD) activity, glutathione peroxidase activity, catalase activity, GSH content, and lipid peroxidation inhibition. The sericin extract APHWE or BMHWE at increasing concentrations significantly increased SOD activity (production) in the hydrogen peroxide-treated lymphocyte. With the APHWE treatment, the highest SOD activity in the lymphocyte was observed at the treatment dose of 100 µg/mL, while the highest SOD activity (production) in the cell was observed at the treatment dose of 200 µg/mL with the BMHWE treatment. These results indicated that sericin extract from oak tasar (Antheraea proylei J.) cocoon exhibit higher antioxidant activity than the sericin extract from mulberry (Bombyx mori L.) cocoon. Glutathione peroxidase activity was significantly increased compared to the negative control in lymphocytes treated with a dose ranging from 100 to 800 µg/mL of the sericin extract APHWE or BMHWE. Catalase activity of the hydrogen peroxide-exposed human peripheral blood lymphocyte was significantly increased as compared to the that of the negative control when treated with a dose of 50 µg/mL of the sericin extract APHWE or BMHWE. The content of the non-enzymatic antioxidant glutathione in the hydrogen peroxide-exposed human peripheral blood lymphocytes was significantly increased as compared to that of the negative control when treated with sericin extract (APHWE or BMHWE) at increasing concentrations (50, 100, 200 and 800 µg/mL). Increased lipid peroxidation was observed in the hydrogen peroxide-exposed human peripheral lymphocytes of the negative control. This increase of the peroxidation was significantly reduced by treatment of the lymphocytes with the sericin extract APHWE or BMHWE at increasing doses.

The anti-melanogenic activity assay for the sericin extracts was carried out in zebrafish following the procedure of Chen et al. (2019). The wild-type zebrafish was reared in a temperature and light controlled culture facility. The zebrafish embryos were obtained by breeding male and female adult zebrafish populated in the ratio 1:2 in a breeding chamber overnight at room temperature. The embryos were cultured in E3 medium. The 24 hpf embryos were collected and then distributed in a 12-well plate @ 25 embryos per well. The embryos in separate wells were treated with 2 mL E3 medium with 0.5% DMSO containing increasing concentration of the sericin extract APHWE. After 48 hr of exposure to the
sericin extract, the 72 hpf embryos were collected and analysed for pigmentation level and tyrosinase activity. Triplicates at each concentration of the sericin extract were run. The same experiment was repeated replacing the sericin sample by BMHWE/kojic acid. The sericin preparation from oak tasar (Antheraea proylei J.) cocoon (APHWE) had a decreasing effect on melanin synthesis in zebrafish embryo. The anti-melanogenic activity increased with an increase in concentration in the range 0 to 200 g/µmL. However, the sericin preparation from mulberry (Bombyx mori L.) cocoon (BMHWE) and kojic acid had greater anti-melanogenic activity than APHWE. The anti-melanogenic activity was also assessed in terms of the inhibition of the tyrosinase activity of the zebrafish embryo. With increasing concentration, APHWE showed increasing inhibitory effect on tyrosinase activity. BMHWE and the standard tyrosinase inhibitor kojic acid had greater tyrosinase inhibitory effect than that of APHWE.

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph.D. awarded (c) Publication of results (d) other impact, if any:

(a) Manpower trained: The Research Fellow appointed under the project was trained in teaching and research activity.

(b) Ph.D. awarded: The Research Fellow is registered as a PhD scholar w.e.f. and she is trying to submit the PhD thesis within about six months.


(d) other impact, if any: Nil