

# Study of Sericin Sequences from *Bombyx mori* as Antiaging through ROS with Molecular Simulation and DPPH Evaluation

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## ABSTRACT

The presence of ROS is associated with aging, which is damage caused by free radical reactions. ROS causes oxidation of low density lipoprotein (LDL), which builds up in plaque and contributes to inflammation. With aldehyde secondary products of lipid peroxidation such as Malondialdehyde (MDA), lipoxygenase, and xanthine oxidase as markers of oxidative stress, oxidized LDL causes endothelial dysfunction and cell apoptosis. The antioxidant 1,1 diphenyl-2-picrylhydrazyl (DPPH) sericin from *Bombyx mori* was tested *in silico* and *in vitro* in this study. The *Bombyx mori* peptide sequences QAYADYHSDPNGGSA (SP4) and ASSSFDAASSA (SP7) had lower Gibbs energy for lipoxygenase (LOX) than native ligands, with values of -23.1044, -21.0056, and -10.3275 kcal/mol, respectively. Hydrogen bonding to Gln289, Asp293, and Gly569. While ASSSFDAASSA (SP7) has a higher Gibbs energy for xanthine oxidase (XOX), SEASSSTQATTVS (SP 5) has a lower Gibbs energy with values of -20.1839, -17.8952, and -11.8921 kcal/mol, respectively. While the cavity binding of the xanthine oxidase peptide binding SP5 and SP7 is located at the Glu802, Asp872, and Ser876 binding sites, the DPPH test confirmed *in vitro* that the 10% sericin Gel had an IC50 of 19.7394 ppm compared to 3.71 ppm ascorbic acid. The findings of the preceding study demonstrate that sericin, as an antioxidant, is one of the candidates for antiaging.

**Key words:** Sericin, ROS, LOX, DPPH.

## INTRODUCTION

Aging is the accumulation of progressive changes over time associated with increased susceptibility to disease and death as one gets older, as well as the amount of damage caused by free radical reactions to cells and tissues. Aging is defined by structural and functional damage. This damage results in pathological conditions and can result in death.<sup>1</sup> Interactions between free radicals, antioxidants, and cofactors are critical in the maintenance of health, the aging process, and the development of age-related diseases. Free radicals cause oxidative stress, which is balanced by the body's endogenous antioxidant system via cofactors or by exogenous antioxidants obtained through diet.

The pathological process of vascular occlusion that causes the cardiovascular disease is thought to involve free radicals. ROS causes the oxidation of low density lipoprotein (LDL), which accumulates in plaque and contributes to the pathogenesis of atherosclerotic inflammation. Oxidized LDL causes endothelial dysfunction, which leads to cell apoptosis and vasoconstriction.<sup>2,3</sup> Secondary products of lipid peroxidation aldehyde as markers or markers of oxidative stress Malondialdehyde (MDA), lipoxygenase is the most commonly used lipid marker for oxidative stress, which is formed by peroxidation of polyunsaturated fatty acids (PUFA; polyunsaturated fatty acids) and increased ROS.<sup>2</sup> They have been reported to act through single or combined mechanisms; particularly, by neutralizing radicals (as radical scavengers), as singlet oxygen quenchers; through synergism with other antioxidants; through complexing of pro-oxidants that catalyze the generation of radicals;

and finally, as inhibition of pro-oxidant enzymes that generate radicals (i.e., lipoxygenase, xanthine oxidase, and NADPH oxidase).<sup>3-6</sup> Oxidative stress caused by xanthine oxidoreductase hyperreactivity is associated with gout,<sup>7</sup> whereas the oxidizing activity of lipoxygenase plays a significant role in oxidative stress.<sup>8</sup>

Endogenous oxidative stress can be reduced in two ways: by preventing ROS formation or by reducing ROS effects with antioxidants. Endogenous antioxidants become insufficient under certain conditions caused by oxidative stress, necessitating the use of exogenous antioxidants to maintain optimal cellular function. Antioxidants are molecules that can stabilize or deactivate free radicals before they attack cells. Glutathione peroxidase, catalase, and superoxide dismutase are examples of enzymatic antioxidants. Vitamin E, vitamin C, thiol antioxidants (glutathione, thioredoxin, and lipoic acid), melatonin, carotenoids, natural flavonoids, and other non-enzymatic antioxidants are examples.<sup>2</sup>

Animal models' lives can be extended by bioactive compounds derived from natural ingredients. Ascorbic acid (vitamin C) is a powerful hydrophilic antioxidant and lipid peroxidation inhibitor. Sericin from *Bombyx mori* is one of the natural ingredients used. Sericin is an amino acid sequence from *Bombyx mori* that has a specific sequence with the amino acid component serine. Takasu et al. were successful in sequencing ten proteins from sericin, including SRDGSVSTG, SRDENVSTTGSSDNT, DGSVSTGSSS, SDAASSEDGF QAYADYHSDPNGGSA, and SEASSSTQATTVS.<sup>9</sup> Sericin derived from *Bombyx mori* cocoons and its hydrolysate have been studied

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for DPPH scavenging, tyrosinase and -glucosidase inhibitory activity, anti-tumor, and mitogenic functions.<sup>10,11</sup> In these experiments, a sericin solution was prepared by degumming the silkworm cocoon in boiling water followed by dialysis against water for 24 h. These stocks were shown to be able to eliminate the DPPH free radical and inhibit tyrosinase.<sup>12</sup> An *in silico* test using a molecular docking approach was performed as a mechanical test for markers of oxidative stress.

## MATERIALS AND METHODS

This study uses a computer with specifications Intel® Inside CORE i5 CPU 2.00 GHz, 6,00 GB of RAM dan operation system Windows 8.1, AutoDock Tools, AutoDock, DiscoveryStudio, Protein Data Bank [www.rcsb.org/pdb](http://www.rcsb.org/pdb) and ligan at [www.uniprot.com](http://www.uniprot.com). 3D Structure lipoxygenase, xanthine oxidase, with PDB access: 1HU9 dan 3NVY and sequence structure from *Bombyx mori* SRDGSVSSTG, SRDENVSTTGSSDNT, DGSVSSTGSSS, SDAASSEDGF, QAYADYHSDPNGGSA, SEASSSTQATTVS. In-vitro test using DPPH.

### In-silico molecular simulation

Protein Structure Preparation Download the protein structure from [www.rcsb.org/pdb](http://www.rcsb.org/pdb), selected lipoxygenase enzyme, xanthine oxidase, PDB: 1HU9 and 3NVY. Using Python Molecule Viewer (PMV) software, the lipoxygenase enzyme receptors, xanthine oxidase, and the receptors were cleaned of other components.

The *Bombyx mori* sequence's ligand structure was designed. The structure was designed using Marvin Sketch software, which will convert the 3D structure in.ent format to.pdb format.

Preparation of docking files, Ligands, and receptors are converted to.pdbqt format using the AutoDock Tools software. The number of torsions on the ligands is changed, and the grid box on the receptor is determined. Docking and visualization are performed using the AutoDock Vina software, which is run at the command prompt. Docking analysis is visible on the output in notepad format, and docking visualization is visible using MOE software.

### In-vitro method with DPPH 1,1 diphenyl-2-picrylhydrazyl

Antioxidant activity evaluation (DPPH method) The DPPH method was used to assess sericin's antiradical activity. DPPH reagent solution preparation 15.77 mg of DPPH were weighed and dissolved in 100.0 mL of ethanol at a concentration of 0.4 mM. The maximum wavelength (max) of 0.7 mL of DPPH in a 5.0 mL volumetric flask was determined by adding ethanol and measuring absorbance at 450-545 nm against a blank of 5.0 mL of ethanol. In a 10.0 mL volumetric flask, sericin gel 10%, pure sericin, and gel-based stock solutions were weighed and added with p.a ethanol solvent, vortexed until homogeneous. The ascorbic acid stock solution was prepared by weighing plus solvent, vortexed until homogeneous, pouring into a 10.0 mL volumetric flask, adding solvent to mark, and obtaining a solution with a concentration of 0.1 percent. IC50 values of sericin gel 10%, pure sericin, and gel-based were determined. Six concentration series of sericin stock solutions are 6.25; 12.5; 25; 50; 100; and 200 g/mL, placed in a 5.0 mL volumetric flask. The sample was then treated with 0.7 mL of 0.4 mM DPPH and ethanol. After vortexed for 30 seconds, the mixture was incubated for 30 minutes. The absorbance of the sample was measured against a blank consisting of several stock solutions in ethanol at maximum concentrations of ascorbic acid, namely 0.39; 0.78; 1.56; 3.12; 6.25; 12.5, and 25 g/mL and 0.7 mL of 0.4 mM DPPH in ethanol p.a. up to the mark. The percentage of antiradical activity was then calculated. Making a linear regression curve between concentration and percent antiradical activity to obtain a linear regression equation for determining sample concentration at 50% activity. Three times, the antiradical activity test was repeated. Each test, stock preparation, and

sample dilution were also three times replicated. Analysis calculating the inhibitory concentration was used to determine antioxidant activity (IC50). The IC50 value is the concentration of sericin and ascorbic acid that provides 50% anti-radical activity when compared to the control using a linear regression line equation between concentration and percent radical scavenging.<sup>13</sup>

$$\% \text{ antioxidant activity} = \frac{(\text{abs. control} - \text{abs. sample})}{\text{abs. control}} \times 100\%$$

## RESULTS AND DISCUSSION

### Molecular docking validation

The 3D structure used in this study is a 3D lipoxygenase structure downloaded from PDB at <https://www.rcsb.org/structure/1HU9> with a resolution of 2.2A and binding sites at positions Gln289, Phe292, Asp293, Arg299, Pro759, Lys760, Phe761, Gln762, Arg252, Ser253, Asn254, Ser281, Gln282, Leu285, Leu563, Ser564, Asn567, Gly569, Gly570, Val571, Gln574, Gln574, Gln574, Gln574, Gln574.

Figure 1b shows an active site protein that acts as a binding site for ligands and has inhibitory activity or activation of receptors or enzymes. Following molecular simulation, it is discovered that the binding site has undergone a conformational change with RMSD 0.66A Figure 1C. The change in binding activity was caused by the inhibition of compounds with lipoxygenase activity (Figure 1d). A change in the cavity before docking is shown in yellow, and a change in the loop after the molecular simulation is shown in green.

The 3D structure used in the study is a 3D Xanthine oxidase structure obtained from the PDB using the site <https://www.rcsb.org/structure/3NVY>. Gln112, Cys113, Cys150, Gln767, Gly796, Phe798, Gly799, Glu802, Asp872, Leu873, Ser876, Arg880, Ala910, Phe911, Arg912, Gly914, Ser1008, Phe1009, Thr1010, Val 1011, Leu1014, Met1038 Figure 1b shows an active site protein that serves as a binding site for ligands and has inhibitory or activating activity from receptors or enzymes. Following molecular simulation, it is discovered that the binding site has undergone a conformational change with RMSD 0.20A Figure 1C. The change in binding activity was caused by the inhibition of compounds with lipoxygenase activity (Figure 1d). A change in the cavity before docking is shown in yellow, and a change in the loop after molecular simulation is shown in red.

### Sericin peptide sequence analysis with *Bombyx mori*

Sericin is a protein with important properties such as biocompatibility and biodegradability. Because sericin is widely used in biomedical applications, it is thought to be a promising natural resource as an alternative medical ingredient, medical active ingredient. Three types of sericin form a layer on top of the fibroin. Sericin A is insoluble in water and contains 17 percent nitrogen, as well as amino acids like serine, threonine, aspartic acid, and glycine. Sericin B is the same as sericin A, but with tryptophan added. Sericin C is found in the deepest layer, next to fibroin. Sericin C, which is insoluble in water, can be separated from fibroin by adding hot and weak acids. Similar to sericin A, but with the addition of tryptophan and proline. The sequences of amino acids that make up sericin are as shown in table 1. Physicochemical and toxicity tests are used to characterise new inhibitor candidates. using the Adme1 Swiss webserver <http://www.swissadme.ch/index.php>.

The analysis results are shown in Table 2. Through molecular anchoring, the structure-based approach is screened. The herbal polypeptide compounds *Bombyx mori*, namely SRDGSVSSTG, SRDENVSTTGSSDNT, DGSVSSTGSSS, SDAASSEDGF, QAYADYHSDPNGGSA, SEASSSTQATTVS as test ligands and native ligands LOX and XOX as positive controls, were used in this test process.<sup>9</sup> Based on the toxicity analysis results shown in Table 1.

**Table 1: Analysis of physicochemical properties of peptide sequences from *Bombyx mori*.**

Amino Acid of Sericin Sequences	AMES	Oral Acute Toxicity	FDAMDD	Skin sensitization	Carcinogenicity	Respiratory Toxicity	LC50FM	LC50DM
SRDGSVSSTG	---	---	++	+++	--	---	3.353	3.319
SRDENVSTTGSSDNT	---	---	++	+++	-	+++	4.426	4.268
DGSVSSTGSSS	---	---	++	+++	---	+++	4.423	3.702
QAYADYHSDPNGGSA	---	---	+++	+++	+	+++	4.322	4.638
SEASSSTQATTVS	---	---	+++	+++	---	+++	4.309	4.147
SDAASSEDGF	--	---	++	+++	--	+++	4.535	3.501
ASSSF DASSA	--	---	++	+++	--	+++	4.568	4.087
XQESRPRRTGY	---	---	+++	+++	+	+++	4.545	3.957
QIFEDKFEN	--	---	+++	+++	+	+++	4.767	4.012
SILNPVLNTLGLG	--	---	+++	+++	+	+++	5.395	5.772
Control LOX	-	---	---	-	--	---	3.353	3.319
Ascorbic Acid	---	---	---	++	--	---	2.389	3.606
Control XOX	-	---	---	-	--	---	3.353	3.319

**Table 2: Docking of sericin amino acid sequences against LOX and XOX.**

Compounds	Lipoxygenase LOX (1HU9)		Xanthine Oxidoreductase XOX (3NVY)	
	Gibbs energy (kcal/mol)	Hydrogen bond	Gibbs energy (kcal/mol)	Hydrogen bond
SRDGSVSSTG (SP1)	-17.8059	Asp293, Glu300	-15.0873	Thr803, Asp872
SRDENVSTTGSSDNT (SP2)	-20.7580	Gln289, Asp293, Glu300, Gly570, Gln574	-14.8091	-
DGSVSSTGSSS (SP3)	-19.2177	Ser444, Asn567, Asp568, Gly569	-10.1817	-
QAYADYHSDPNGGSA (SP4)	<b>-23.1044</b>	Asp212, Ser281, Gln289, Asp293, Glu300, Ser444, Asn567, Asp568, Gly569, Gly570, Gln574	-17.5604	Asp872, Val1011
SEASSSTQATTVS (SP5)	-20.9953	Ser281, Gln289, Asp293, Asp568, Gly569, Gly570	<b>-17.8952</b>	Ser876, Asn768, Val1011
SDAASSEDGF (SP6)	-16.3397	Ser444, Asn567,	-15.8768	Thr803, Asp872
ASSSF DASSA (SP7)	<b>-21.0056</b>	Ser444, Asn567, Asp568, Gly569	<b>-20.1839</b>	Glu802, Thr803, Asp872, Ser876, Asn768, Val1011
XQESRPRRTGY (SP8)	-18.8932	Asp293, Glu300, Gly570, Gln574	-16.0981	Asp872, Ser876
QIFEDKFEN (SP9)	-17.4995	Glu300, Gly570, Gln574	-12.3174	-
SILNPVLNTLGLG (SP10)	-14.3889	Ser444, Asn567, Asp568	-14.8092	-
Native XOX	-8.3990	-	-11.8921	-
Native LOX	-10.3275	Ser444, Asn567	-10.9434	-

**Table 3: IC50 sericin dan control.**

Concentration	Inhibition		Concentration	Inhibition		
	Ascorbic Acid			Gel Based	Sericin 10%	Pure Sericin
0.390625	17.528		6.25	15.606	5.08	41.236
0.78125	19.359		12.5	16.613	9.565	48.467
1.5625	31.716		25	16.887	15.972	59.085
3.125	46.178		50	20.091	25.263	84.348
6.25	72.173		100	23.576	35.194	92.037
12.5	94.965		200	24.027	44.347	93.990
25	94.874		400	28.573	92.219	95.972
<b>IC50</b>	3.7587			1052.149	19.739	14.933
R	0.9952			0.927	0.900	0.990

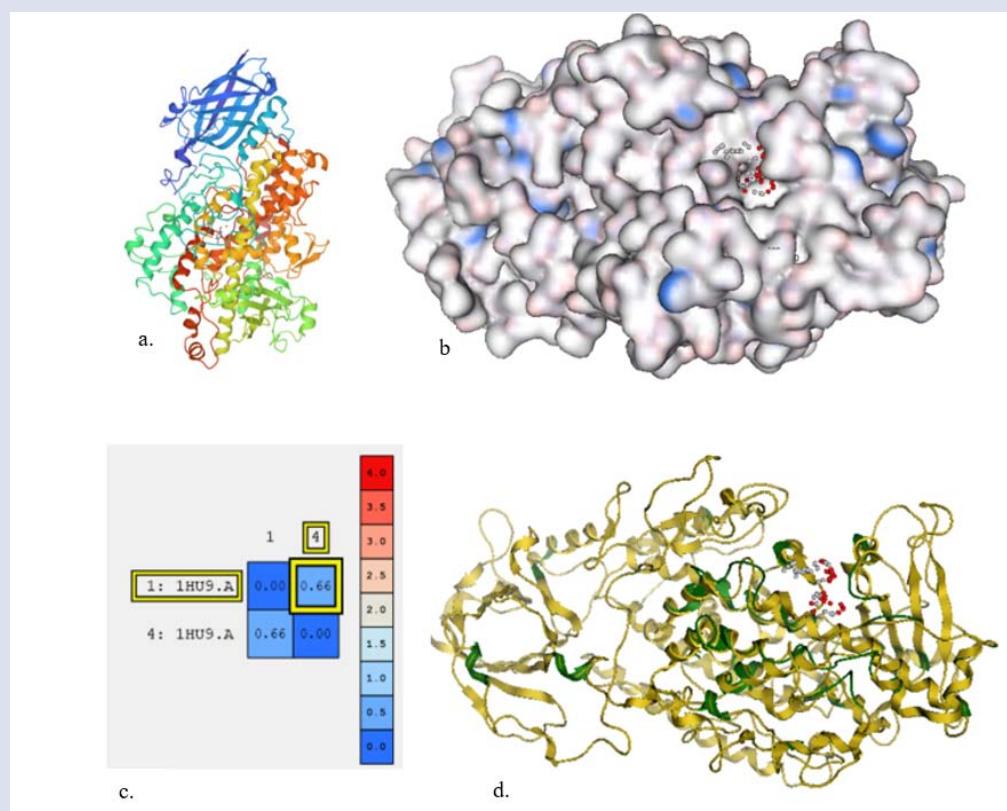
All peptides tested positive for skin sensitization in cytotoxicity tests. Skin sensitization is a response of the adaptive immune system, characterised by a delayed T-cell-mediated allergic response to chemically modified skin proteins haptens or sensitizers<sup>14</sup> are chemicals that can covalently modify skin proteins and cause an allergic reaction.

### Binding energy molecular docking

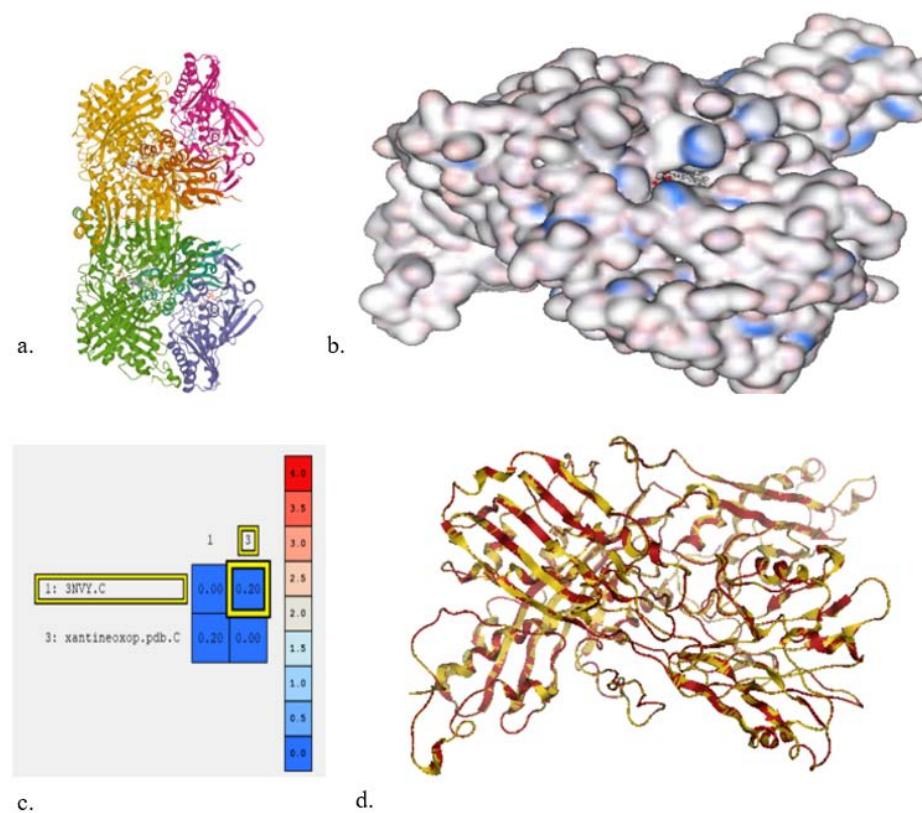
Amino acid residues, hydrogen bonds, predicted inhibition constants, and bond free energies were among the parameters studied. According to the molecular docking results, the peptide sequence 4 compound, peptide sequence 7, is close to having a ligand conformation as the

native ligand binding cavity on the protein lipoxygenase (LOX). These two compounds were then examined in 2 and 3 dimensions for interactions and anchoring at the active site. The validation results show that the RMSD (Root Mean Square Deviation) value 2A at the ligand position is improving as it approaches the original conformation. While the docking results for Xanthine Oxidase (XOX) revealed a peptide sequence compound 5, Peptide Sequence 7 was close to having a ligand conformation as the native ligand's binding cavity with RMSD 2A.

LOX molecular docking. The docking results of two Sericin sequences SP4 and SP7 against lipoxygenase (LOX) were compared with native ligands with values of -23.1044, -21.0056, and -10.3275 kcal/mol, with



**Figure 1:** The 3D structure of oxidative stress markers a) Lipoxygenation (1HU9), b) LOX active site, c) superposition of LOX before and after docking, d) backbone 3D LOX before docking (yellow), after docking (green).



**Figure 2:** The 3D structure of oxidative stress markers a) Xanthine oxidase (3NVY), b) XOX active site, c) superimpose of XOX before and after docking, d) backbone 3D XOX before docking (yellow), after docking (red).

hydrogen binding to Gln289, Asp293, and Gly569. The native ligand lipooxygenase has the highest binding energy value against 1HU9, as shown in Table 2. Docking results show that the *Bombyx mori* peptide sequences QAYADYHSDPNGGSA (SP4) and ASSSFDAASSA (SP7) have lower Gibbs energy for lipooxygenase (LOX) than the native ligand.

LOX molecular docking. Based on the docking results of ten Sericin sequences against the 3NVY (Table 2). The peptide sequences ASSSFDAASSA (SP7) and SEASSSTQATTVS (SP 5) had lower xanthine oxidase (XOX) Gibbs energies than the native ligand, with values of -20.1839, -17.8952, and -11.8921 kcal/mol, respectively. While xanthine oxidase cavity binding SP5 and SP7 are found at the binding sites of Glu802, Asp872, and Ser876.

Table 2 shows that peptide sequences 7 and 5 have a lower binding energy (-11.8921 kcal/mol) than the native ligand. Oxidation of xanthine oxidase is a superoxide-producing enzyme found in serum and the lungs that increases in activity during influenza A infection.<sup>15</sup> Hydroxyl free radicals and hydrogen peroxide, both by products of XO activity, can cause oxidative stress.

L-serine has antioxidant and cytoprotective impacts through the rise of a few vital antioxidant variables such as Nrf2, HO-1 and NO.<sup>16</sup> With the presence of a substrate from this peptide sequence supporting XOX activity, it is hoped that Sericin can become an antiaging candidate via an antioxidant mechanism.

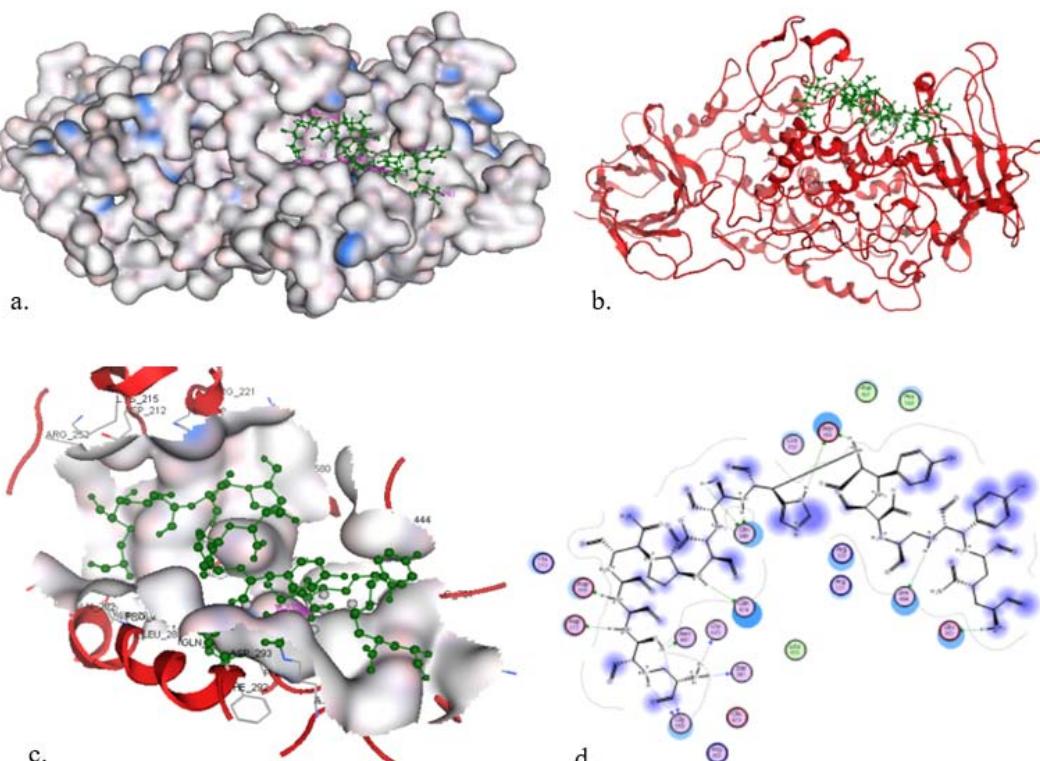
### Visualization and analysis

Docking results are obtained by determining the conformation of the docking ligands in the best pose, which is accomplished by selecting the ligand conformation with the lowest bond energy that binds to the binding cavity. The best docking results are then analysed using the Autodock analyzer, as shown in Figures 3 and 4.

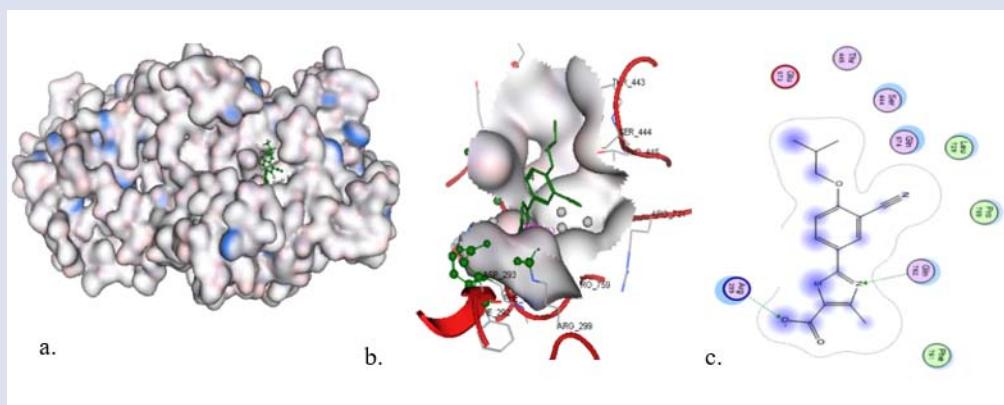
Figure 3 shows that the activity of the peptide sequences QAYADYHSDPNGGSA (SP4) and ASSSFDAASSA (SP7) on lipooxygenase enters the binding site with a conformational pose similar to Figure 3a, which shows that SP4 forms a cavity area as well as the lock and Key location of the substrate in the LOX surface topology area (TPSA). Figure 3b shows that SP4 is in the LOX loop with a significant change, as shown in Figure 1d. SP4 can occupy the cavity area in the 2D data of Asp212, Ser281, Gln289, Asp293, Glu300, Ser444, Asn567, Asp568, Gly569, Gly570, Gly574 supported by 3d Figure. Figures 4a and 4b show that when compared to the native ligand LOX, the native ligand occupies a portion of the TPSA and cavity. Binding activity is a property of the enzyme's active site that increases the substrate's binding affinity towards an enzyme. A binding site is a region on a macromolecule, such as a protein, that specifically binds to another molecule. The macromolecule's binding partner is commonly referred to as a ligand. Other proteins can act as ligands (resulting in a protein-protein interaction).<sup>17</sup>

Figure 5 shows that the activity of the SEASSSTQATTVS (SP5) and ASSSFDAASSA (SP7) peptide sequences on xanthine oxidase (XOX) enters the binding site in a conformational pose similar to Figure 5a, which shows that SP7 in the XOX surface topology area (TPSA) forms a cavity area as well as the lock location. Figure 5b shows that SP7 is in the XOX loop with a significant change, as shown in Figure 2d. SP7 can occupy the cavity area around Gln112, Cys113, Cys150, Gln767, Gly796, Phe798, Gly799, Glu802, Asp872, Leu873, Ser876, Arg880, Ala910, Phe911, Arg912, Gly914, Ser1008, Phe1009, Thr1010, 10 Val14 Met1038 as shown in Figure 5d. Figures 6a and 6b show that when compared to the native ligand XOX, the native ligand occupies a portion of the TPSA and cavity.

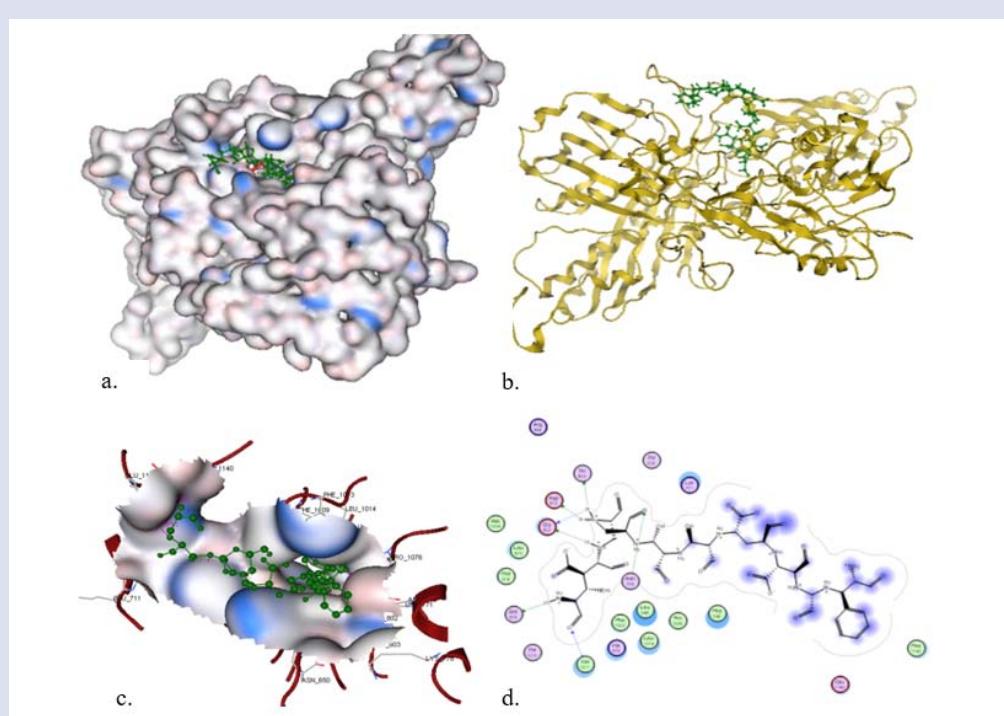
Our results show that the SP 4, Sp5, and SP7 have a clear tendency to act as xanthine oxidoreductase (PDB ID: 3NVY) inhibitors. 6 structures gave docking scores higher than the native ligand hypoxanthine.



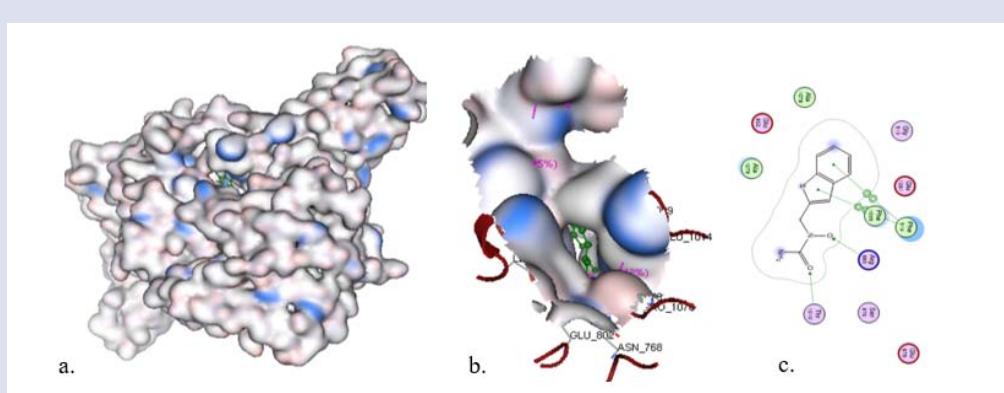
**Figure 3:** Complex docking SP4-LOX, a) structure 3D SP4-LOX, b) backbone of SP4-LOX, c) cavity site of SP4-LOX, d) structure 2D SP4-LOX.



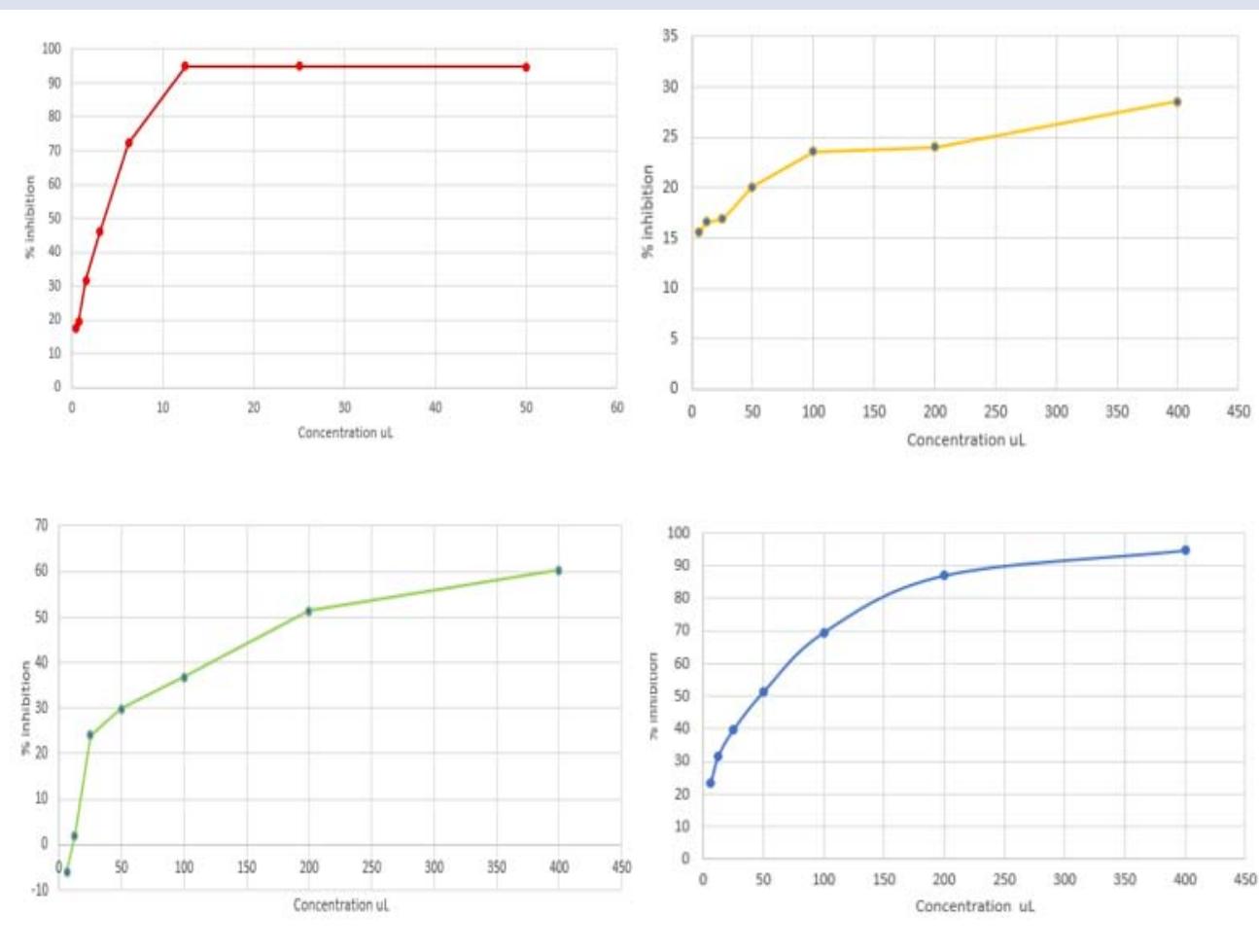
**Figure 4:** Visualization of docking LOX results, a) complex 3D structure nativeligand-LOX, b). cavity site native ligand-LOX, c) hydrogen bonding, and 2D structure of native ligand-LOX.



**Figure 5:** Complex docking SP7-XOX, a) structure 3D SP7-XOX, b) backbone of SP7-XOX, c) cavity site of SP7-XOX, d) structure 2D SP7-XOX.



**Figure 6:** Docking visualization XOX, a) structure 3D complex native ligand-XOX, b). cavity site native ligand-XOX, c) hydrogen bond, dan structure 2D native ligand-XOX.



**Figure 7:** DPPH (1,1 diphenyl-2-picrylhydrazyl) extrapolation against antioxidants a) ascorbic acid (red); b) gel-based (yellow); c) pure sericin (green); d) sericin gel 10% (blue).

Xanthine oxidoreductase is the enzyme that catalyzes the oxidation of hypoxanthine to xanthine and the subsequent transformation of xanthine to uric acid. In addition to this biological role, mammalian xanthine oxidoreductase is a physiological source of ROS, such as superoxide ions or hydrogen peroxide, which can trigger the activation of various pathways.<sup>18</sup> Therefore, the inhibition of this particular enzyme could induce a significant *in vitro* antioxidative effect. According to the obtained docking scores, among the most active compounds are various structures. Xanthine oxidoreductase inhibitors are usually researched for their potential effect in reducing the oxidative stress present in gout. Published data showed that the assessed compounds showed superior *in silico* inhibitory activity of xanthine oxidoreductase

Direct inhibition of the xanthine oxidase and lipoxygenase pathways also inhibits eicosanoids and leukotriene biosynthesis, which are end products of the xanthine oxidase and lipoxygenase pathways. The antioxidant effects of natural ingredients such as peptide sequences indirectly support anti-inflammatory effects. Free radicals can attract a variety of inflammatory mediators. Peptide sequences can stabilise Reactive Oxygen Species ROS by reacting with reactive radical compounds, rendering radicals inactive.

#### DPPH 1,1 diphenyl-2-picrylhydrazyl antioxidant *in vitro* test

The activity of herb-origin natural extracts is often evaluated for their proposed antioxidant activity using established methods such as those used in our current study. The ability of a natural compound or extracts

to scavenge free radicals such as DPPH· or ABTS· reflects its ability to act similarly in the presence of ROS at the cellular/mitochondrial levels. Numerous studies have shown a clear correlation between the ability of an extract to scavenge free radicals assessed by the DPPH or ABTS method and the ability of the same product to decrease ROS production *in vitro*.<sup>15</sup>

The average (mean) value of sericin compound with sericin gel 10%, pure sericin and gel-based at concentrations of 6.25; 12.5; 25; 50; 100; 200; 400 g/mL. The effects of extrapolation inhibition Figure 7 depicts the extrapolation of sericin gel 10% with an inhibition value as Table 4 shows that sericin gel 10% at concentrations ranging from 6.25 to 400 g/mL is increasing; the higher the concentration of the test compound, the greater the inhibitory power. The inhibition results were then used to calculate the IC (inhibitor concentration) value. The linear equation  $y = mx + C$  is used to calculate the IC50 value.

IC50 results revealed that sericin pure sericin, sericin gel 10%, and gel-based all had very high IC50 values of 14.933, 19.739, and 1052.49, respectively. The fact that pure sericin has an IC50 close to that of ascorbic acid indicates that sericin preparations as antioxidants require an *in vivo* mechanism approach and *in silico* confirmation. Our findings show that 10% sericin has significantly high antioxidant levels. The non-sericin component was primarily responsible for the high biological activity of the sericin 10% in gel. In addition to the sequence peptide, bioactive compounds such as flavonoids and other phenolic compounds can be found in sericin cocoon. Flavonoids, such

as those found in the extracts, have numerous beneficial properties, including antioxidant activity against a wide range of easily oxidizable compounds. Flavonoids have several known properties, including free radical scavenging, antioxidation, inhibition of hydrolytic and oxidative enzymes, and anti-inflammatory action.<sup>19</sup> Chili's flavonoids, ascorbic acid, and total phenolic content have been shown to correlate well with antioxidant activity.<sup>20</sup> Similarly, in this experiment, 10% sericin has an IC<sub>50</sub> close to ascorbic acid control, with no obvious relevance to the amino acids content from the *in-silico* study.

## CONCLUSIONS

According to molecular docking and DPPH analysis, sericin is a potential compound from *Bombyx mori* that has a higher binding affinity than the native ligand. *In vitro*, the DPPH test demonstrates that the sericin gel 10% has an IC<sub>50</sub> of 19.7394 ppm compared to 3.71 ppm ascorbic acid. The findings of the preceding study demonstrate that sericin, as an antioxidant, is one of the candidates for antiaging. The sericin obtained from cocoons protects against oxidative stress and restores cell viability to that of control on antioxidants with the sericin.

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## AUTHOR'S CONTRIBUTIONS

Fitria Agustina: Conceptualization, Investigation, Methodology, Software, Validation, Data curation, Resources, Formal analysis, Visualization. Fadilah Fadilah: Conceptualization, Investigation, Resources, Software, Formal analysis, Visualization, Supervision. Wimpie Pangkahila, Anak Agung Gde Putra Wiraguna, I Gusti Ayu Sri Mahendra Dewi: Conceptualization, Methodology, Formal analysis, Data curation, Review and editing and Supervision

## DECLARATION OF COMPETING INTEREST

The authors declare "No conflicts of interest".

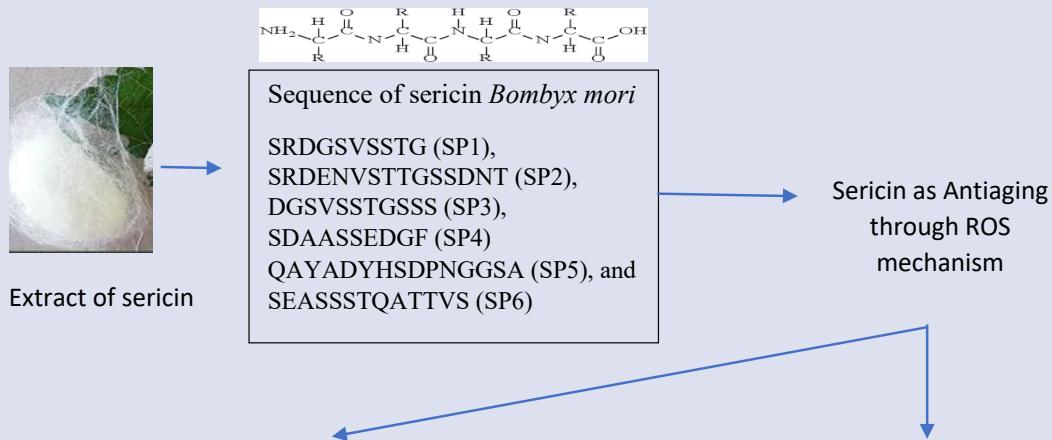
## LIST OF ABBREVIATIONS

PDB	Protein Data Bank
RBD	Receptor Binding Domain
RMSD	Root Mean Square Deviation
IC <sub>50</sub>	Inhibitory Concentration
Nrf2	Nuclear Factor- erythroid 2- related factor 2
HO-1	Heme Oxygenase-1
NO	Nitric Oxide.

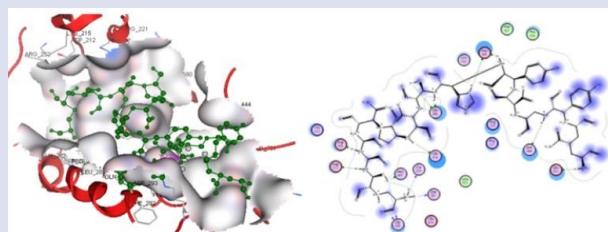
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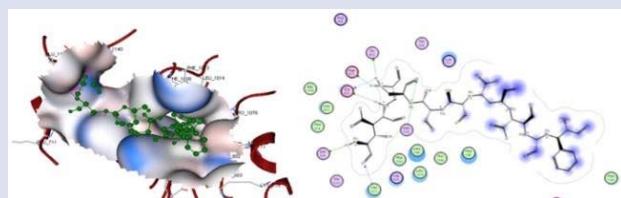
## GRAPHICAL ABSTRACT



### In-silico molecular simulation



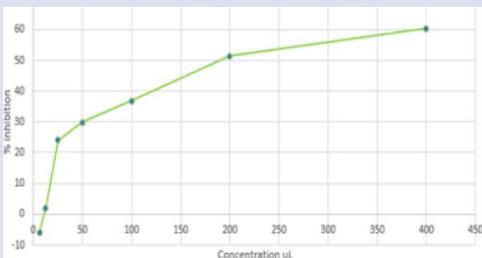
Cavity site of SP4-LOX and structure 2D SP4-LOX



Cavity site of SP7-XOX and structure 2D SP7-XOX

### In-vitro method with DPPH

Concentration	Inhibition		
	Gel Based	Sericin 10%	Pure Sericin
6.25	15.606	5.08	41.236
12.5	16.613	9.565	48.467
25	16.887	15.972	59.085
50	20.091	25.263	84.348
100	23.576	35.194	92.037
200	24.027	44.347	93.990
400	28.573	92.219	95.972
IC <sub>50</sub>	1052.149	19.739	14.933
R	0.927	0.900	0.990



DPPH (1,1 diphenyl-2-picrylhydrazyl) extrapolation against antioxidants of sericin

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