Tensile Properties and Biocompatibility of Indonesian Wild Silk Cricula Trifenestrata: A Preliminary Study

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***Abstract*—As it is reported in many publication about successful work of many research in developing biomaterial from *Bombyx mori* silk which is attract many researchers to observe the possibilities in using different type of the silk other than *Bombyx mori* for biomaterial. Wild silks which is a silk that is not obtained from domesticated species are become object to explorer for better result for biomaterial. This research focused on Wild silk cocoon of Cricula trifenestrata obtained from Indonesian source. In this study the degumming process to separate the fiber from the cocoon was explored and the result is a separated fiber that is tested for its tensile strength and biocompatibility. It can be concluded that the silk obtained from cocoon of Cricula trifenestrata has good biocompatibility properties. The silk can be prepared by degumming method of boiling in 0.01 M NaOH for 1 hour. The modulus elasticity of the single fiber of Cricula trifenestrata is about 3681 MPa. The ultimate tensile strength is obtained about 162 MPa together with value of failure strain about 0.12.**

Index Terms*—tensile, biocompatibility, silk, *Cricula trifenestrata

I. Introduction

The *Cricula trifenestrata* in Indonesia is a pest for plantation of *Anacardium occidentale L.* (local name: *jambu mente*) as reported by Rojak [1]. But not only in Indonesia, in India also reported *Cricula trifenestrata* as a pest for *som* plant [2]. Fortunately that the cocoon of *Cricula trifenestrata* have interesting color that gold like as presented in Fig.1. The cocon of *Cricula trifenestrata* now day is spinning as a yarn and exported to Japan as valuable product for silk fabrics.

To increase the added value of the Cocoon of *Cricula trifenestrata*, the effort should be done in order possible to be introduced as valuable product such as biomaterial, following the success of the domesticated *Bombyx mori* silk as biomaterial. It is reported that *Bombyx mori* is suitable for 3-D scaffolding material which is make possible for the cell spread along the fiber and after that covered all the surface and grow to fill the gap to form the structure of the tissue [3]. It is well known that *Bombyx mori* silk is biocompatible as biomaterial and has been used commercially as sutures in biomedical [4]. More interesting properties of the silk is that the mechanical properties exceed all natural polymer and synthetic materials [5].

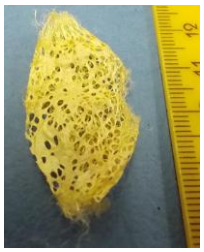

Figure 1. The cocoon of wild silk Cricula trifenestrata obtained from Indonesian source

Before further processing for biomaterial, the silk usually should be degummed first in order to fine fiber will be obtained. It is common for *Bombyx morri* cocoon to be degummed by boiling in hot water bath [6]. But our experience proofed that degumming technique by boiling in water is not working well for *Cricula trifenestrata* cocoon as can be seen in Fig. 2. Other protocols for degumming the silk which is succesfull for the *Bombyx mori* such as by using LiBr solution [5], [7], [8], Formic acid [9], and urea [10]. It was found in our initial research that all protocol above mentioned were not suitable for degumming the wild silk cocoon of *Cricula trifenestrata*. Therefore deguming method should be studied as first step in order possible to use the *Cricula trifenestrata* as future biomaterial instead *Bombyx mori*.

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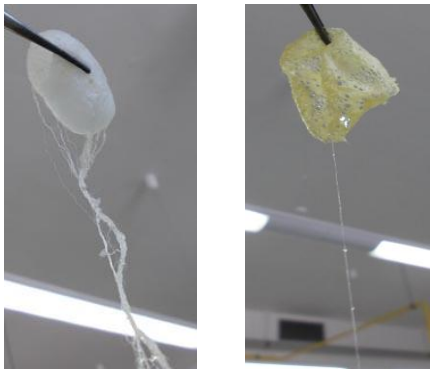


(a) (b)

Figure 2. (a) The cocoon of *Bombyx mori* that is easily degummed in boiling water. (b) The cocoon of *Cricula trifenestrata* that is not degummed well in boiling water. Only small portion of the fiber from the cocoon that can be degummed or release as single fiber

This article introduces a success work on degumming of wild silk cocoon of *Cricula trifenestrata* by using alkaline treatment. This work is continuation in detail our previous work [11] in effort to utilize wild silk cocoons as biomaterial.

II. Experimental

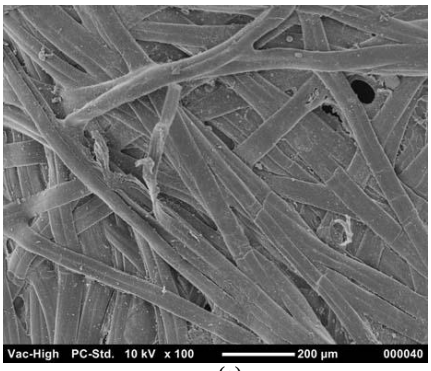
The cocoon of *Cricula trifenestrata* obtained from local collector in Central Java Indonesia (Fig. 1) was incubated in water and NaOH solution in concentration range from 0.001 to 1 M and boiled for 1 1hour. The samples then were shaken for about 10-20 seconds. The insoluble fiber then was collected and washed in water (70^{o}C) intensively. Scanning Electrom Microscope (SEM) was utilized to observe the structure of the fiber before and after degumming. The results of unwoven fiber were sterilized with 70% ethanol for 1 day at room temperature. The samples were washed by PBS and suspension of human osteosarcoma cell line (U2OS) cell in DMEM (Dulbecco's Modified Eagle's medium) cultivation medium and then was soaked (in the atmosphere with 5% CO_2 at 37^{o}C and 95% humidity) for 2 hours. The cultivated medium was supplemented with 10% FBS. The cells were cultivated for 2 days. For observation of the cell, the sample was taken out from cultivation medium and transferred to PBS and washed. Fluorescence microscope was utilized to observe the cell growth (IX-71, Olympus Japan) as characteristic of biocompatibility.

The tensile test was carried out as described else where [12], [13] and [14]. The tensile test was performed with screw test stand (ALX-S, INA) at a constant cross head speed. A balance (Gewinn, INA, resolution ± 10 mg) attached to the lower end of the sample was used to measure load as replacement of conventional load cell. Single fibers about 5 cm long was taken from degummed material and were glued across cardboard frame with distance length 30 cm as defining a gauge length. The test was performed under condition of 30^{o}C and 60% relative humidity.

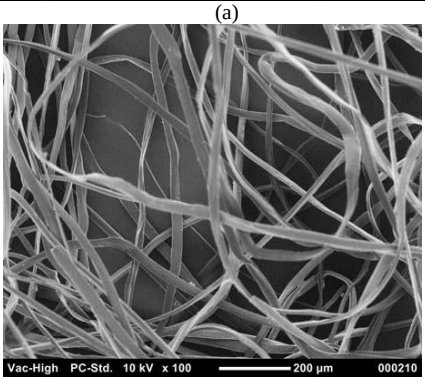
III. Result and Discussion

It is found that the efficient removal of the sericin coat was observed after treatment by 0.01 M NaOH as presented in Fig. 3 which well corresponded with releasing fine fibers (Fig 4). The degumming above 0.01 M NaOH resulted in strong hydrolysis of fibers, there fore not recommended

Figure 3. Result from degumming process by using 0.01 M NaOH which well corresponded with releasing fine fibers



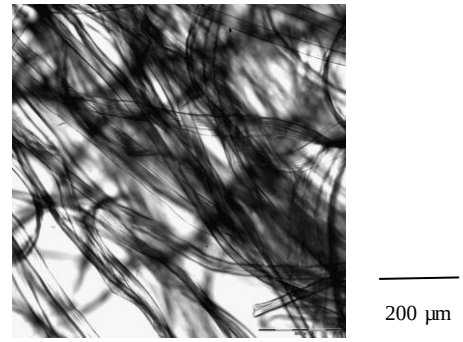
(a)



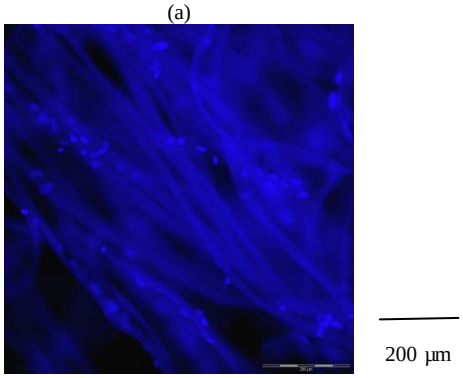
(b)

Figure 4. SEM micrograph of the degumming process of the silk. (a) condition before degumming as obtained from our previous publication [11]. (b) after degumming with 0.01m NaOH

Observation under microsccope after 2 days indicate that the *Cricula trifenestrata* fiber released from cocoon by 0,01 M NaOH was found that the cells are able to attach and grow during following two days. This is and indication that the fiber having good biocompatibility (fig. 5). For better visibility were the cell nuclei and actin microfilaments stained with DAPI and phalloidin-TRITC conjugate, respectively. Previous publications informed that some type of the silks from the cocoon of *Bombyx mori* [15], *Attacus atlas* [16], *Antheraea mylitta* [17] and *Samia cynthia* [18] were proofed have good biocompatibility properties as also silk from the cocoon of *Cricula trifenestrata* as reported in this article



(a)



(b)

Figure 5. Cell growth on *Cricula trifenestrata* fibers obtained by degumming of cocoon by using 0.01 M NaOH. (a) bright field. (b) After cell fixation, nuclei were stained using DAPI and analyzed by fluorescence microscopy

Tensile properties of *Cricula trifenestrata* fiber degumming by 0.01 M NaOH as stress-strain curves is presented in Fig. 6, which is average value can be observed at Table I. The modulus elasticity is about 3681 MPa. The ultimate tensile strength is obtained about 162 MPa together with value of failure strain about 0.12. The value of Ultimate tensile strength of *Cricula trinesentrata* obtained from this research is about lower than the value of ultimate tensile strength of *Bombyx mori* fiber which is the value around 200-600 MPa [6] and [14].

TABLE I. TENSILE PROPERTIES OF DEGUMING SILK WITH 0.01M NAOH FOR *CRICULA TRIFENESTRATA* FIBER

Properties	Value	Unit
Modulus elasticity (E)	3681	MPa
Ultimate tensile strength (σ_ε)	162	MPa
Failure strain (ε_u)	0.12	dimensionless

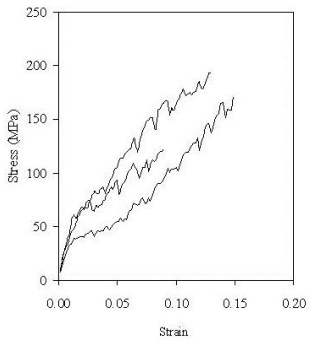


Figure 6. The representative of stress-strain curve of 3 single fibers of *Cricula trifenestrata* degummed from the Cocoon by using 0.01 M NaOH

IV. CONCLUSION

The silk obtained from cocoon of *Cricula trifenestrata* has good biocompatibility properties. The silk can be prepared by degumming method of boiling in 0.01 M NaOH in 1 hour. The modulus elasticity of the single fiber of *Cricula trifenestrata* obtained by using deguming process above mentioned is about 3681 MPa together with value of failure strain about 0.12. The ultimate tensile strength is obtained about 162 MPa.

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