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# STUDY ON THE IMPACT OF MULBERRY AND NON-MULBERRY WASTE (SERICIN) AS ANTIFUNGAL ACTIVITY

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## Abstract

*Cocoon waste products are produced in great quantities in the silk processing process, especially in the degumming process, when sericin, a water-soluble protein of silk, is extracted from the fibroin fibers. Sericin has long been treated as waste by the industries, and the recent research shows that it is biologically important (antimicrobial and antifungal). The current study intends to explore the antifungal effectiveness of sericin derived from mulberry and non- mulberry cocoon wastes. The extraction of sericin was done by the process of degumming, and the agar diffusion was done against the selected fungal species. As the results show, both sericin sources have a considerable level of antifungal activity. Non-mulberry sericin was, however, reported to exhibit more inhibition on particular strains of fungi because of variations in amino acid composition and structure. It recommends that sericin, which is the waste in the cocoon, can be used as a natural antifungal agent and can help in the sustainable use of the wastes in the sericulture industry.*

**Keywords:** *Sericin, Cocoon Waste, Mulberry Silk, Non-Mulberry Silk, Antifungal Activity, Sericulture Waste Utilization.*

## 1. Introduction

Sericulture is a notable agro-based industry, which is a vital contributor to the rural economy of most countries, especially in India. The process of producing silk covers a series of activities that include the rearing of silkworms, harvesting of cocoon, as well as, reeling of silk. In the processes, huge quantities of waste products like pierced cocoons, faulty cocoons and degumming wastes are produced. Such wastes are usually not fully utilized even though they contain valuable biomolecules that can be used in various fields of science and industry.Sericin, a natural protein glue that covers fibroin fibers and binds them all together, is one of the major components of the cocoons of silk. The sericin contributes about 2030% of the weight of the whole cocoon. The cocoons are then boiled in water, and psylla or sericin is extracted in this process, called degumming, during silk processing. Historically, the sericin extracted is usually thrown away as industrial waste, which may cause some environmental pollution, as well as the loss of potentially valuable biological resource (Aramwit et al., 2012; Kundu et al., 2008). Recent scientific research has unveiled the fact that sericin has many biological attributes, such as antioxidant, antibacterial, antifungal, anti-inflammatory, and moisturizing effects. These properties have shown more interest in the sericin as a natural biomaterial to be used in biomedicine, pharmaceuticals, cosmetics and agriculture (Dash et al., 2008; Aramwit et al., 2010). Due to its biodegradation and biocompatibility, sericin is also a promising substance to use in tissue engineering, wound care, and drug delivery systems. The mulberry silkworm Bombyx mori is the primary producer of silk, although a number of non-mulberry silkworm species are also producers of silk with different properties. Other notable non-mulberry silks are Eri silk of Samia ricini, Tasar silk of Antheraea mylitta and Muga silk of Antheraea assamensis. The chemical makeup of the amorphous framework of sericin differs among species, which could influence their biological and antimicrobial actions (Mondal et al., 2007). The fungal infection is a significant issue in medicine, agriculture and food preservation. Pathogenic fungi may result to health risks and economic losses by causing severe diseases to plants, animals and humans. In addition, the growing resistance of fungi to synthetic antifungal medicines has generated an urgent demand for natural and environmentally friendly antifungal drugs (Perfect, 2017).

## 2. Objectives of the Study

1. To extract sericin from mulberry cocoon waste.
2. To extract sericin from non-mulberry cocoon waste.
3. To evaluate the antifungal activity of extracted sericin.
4. To compare the antifungal activity of mulberry and non-mulberry sericin.

### 2.1 Extraction of Sericin from Mulberry Cocoon Waste

Bombyx mori is the most prevalent producer of Mulberry silk and of the silk industry all over the world. In the process of silk processing, a huge amount of cocoon waste is produced, and it contains sericin protein that holds fibroin filaments. Sericin constitutes about 20 percent of the cocoon weight and is generally eliminated when undergoing the degumming process (Nayak et al., 2018; Haddad et al., 2016).Removal of the sericin in the waste of mulberry cocoons is normally performed by a procedure of the degumming of the cocoons, which is accomplished by boiling the cocoons in distilled water or weak bases. Under this technique, the cocoon waste is washed in order to eliminate impurities, and thereafter, boiled in distilled water at approximately 100o C and 30-60 minutes. Sericin is dissolved in water and becomes dissolved during boiling and detaches the fiber of fibroin (Tsubouchi et al., 2005).

**Table 1: Extraction Procedure of Mulberry Sericin**

| Step | Procedure | Purpose |
|---|---|---|
| **1** | Collection of mulberry cocoon waste | Raw material preparation |
| **2** | Washing with distilled water | Removal of impurities |
| **3** | Boiling in distilled water (100°C) | Degumming process |
| **4** | Filtration | Separation of dissolved sericin |
| **5** | Concentration and drying | Obtaining crude sericin extract |

The resulting solution of boiling has dissolved sericin with fibre-size particles of fibroin. Thus, the solution is filtered with filter paper to get rid of insoluble substances. The filtrate is subsequently concentrated or dried to get crude sericin extract with which to proceed with analysis (Rao et al., 2010). Mulberry cocoons yield sericin, which has amino acids including serine, glycine, aspartic acid, and threonine that make it have biological activities. These amino acids have polar functional groups that have the ability to react with microbial membranes and biological tissues (El-Sayed et al., 2015). The recent research highlighted the need to use sericin in silk waste as a biomaterial in biomedical and pharmaceutical products, such as wound healing, antimicrobial preparations, and drug delivery platforms (Wang et al., 2017). Hence, the transformation of sericin in waste materials of mulberry cocoon is a viable solution in transforming sericulture waste into useful bioactive materials.

### 2.2 Extraction of Sericin from Non-Mulberry Cocoon Waste

There are a number of wild or semi-domesticated species of silkworms that give rise to non-mulberry silk, which are very different from the more commonly domesticated mulberry silkworm. Other noteworthy non-mulberry silkworms are Samia ricini, Antheraea mylitta and Antheraea assamensis, which give birth to Eri, Tasar and Muga silk, respectively. The production of these silks is largely done in tropical areas, and they are characterized by special physical characteristics, including increased durability, naturalness in color, and increased fiber. In contrast to mulberry silk that is produced under rearing conditions, non-mulberry silks are usually sourced through silkworms that feed on a range of various host plants and are sometimes reared in semi-wild rearing conditions (Singh et al., 2015).

**Table 2: Non-Mulberry Silk Sources**

| Silkworm Species | Silk Type | Characteristics |
|---|---|---|
| *Samia ricini* | Eri silk | Soft texture, wool-like fiber |

| **Silkworm Species** | **Silk Type** | **Characteristics** |
|---|---|---|
| *Antheraea mylitta* | Tasar silk | Strong and coarse fiber |
| *Antheraea assamensis* | Muga silk | Natural golden color and durability |

Like the mulberry cocoons, non-mulberry cocoons are also composed of two major proteins, namely, fibroin and sericin. Silk fiber is made up of fibroin as the structural core of the fiber and sericin as a natural adhesive that glues pieces of fibroin filaments together. Even though sericin is usually discarded in silk processing, it possesses a lot of biologically active compounds that can be applied in biomedical and industrial practice. Silkworm species vary in their molecular composition of sericin, which leads to differences in amino acid content, molecular weight, and biological activity (Aramwit et al., 2012).

In this experiment, the non-mulberry cocoon waste was gathered and washed with a lot of distilled water to eliminate any impurities, such as dust and any plant remains. The washed cocoon waste was then taken through the degumming process to obtain sericin. The process of degumming was done by boiling the waste of the cocoon in distilled water, 30-60 minutes at 100 °C. In this process, sericin protein is dissolved in water, and the fibroin fibers are not dissolved. The insoluble fibroin residues were removed by filtering the sericin-containing solution after being boiled using filter paper. The filtrate was next evaporated or freeze-dried to get crude sericin extract. This extract was kept under controlled laboratory conditions, where it was to be tested further in antifungal tests (Das et al., 2014).

Research has indicated that non-mulberry sericin tends to have a higher molecular weight of proteins and amino acid composition than mulberry sericin. The differences could improve its biological characteristics, such as antimicrobial and antioxidant effects (Ghosh et al., 2018). Thus, the production of sericin by extracting the non-mulberry cocoon waste not only gives a useful biomaterial but also aids in the sustainable use of waste in the sericulture industry.

**Table 3: Antifungal Test Conditions**

| **Parameter** | **Description** |
|---|---|
| Method | Agar well diffusion method |
| Culture medium | Potato Dextrose Agar |
| Incubation temperature | 28°C |
| Incubation time | 24–48 hours |

### 2.3 Evaluation of Antifungal Activity of Extracted Sericin

To measure the antifungal activity of sericin of the mulberry and non-mulberry cocoon waste, a commonly accepted technique of identifying the antimicrobial activity of natural compounds was used, namely, the agar well diffusion method. Through this technique, the researchers are able to quantify the inhibitory ability of a substance on the growth of fungi by monitoring the clear area that is formed around the sample in an agar test (Fujii et al., 2010).

In the current experiment, Potato Dextrose Agar (PDA) was utilized in the preparation of fungal cultures, citing that it is a nutritious medium used to develop fungi. PDA plates were then sterilized and inoculated with the chosen fungal species. The fungal strains that were involved in the experiment were Aspergillus niger, Candida albicans and the Penicillium species. The rationale behind the choice of these fungi is that they are commonly linked to human colonization, food poisoning, and plant poisoning (Gupta et al., 2013).

**Table 3: Antifungal Test Conditions**

| **Parameter** | **Description** |
|---|---|
| Method | Agar well diffusion method |
| Culture medium | Potato Dextrose Agar |
| Incubation temperature | 28°C |
| Incubation time | 24–48 hours |

After spreading the fungal cultures in the agar plates, sterile wells were made with the help of a cork borer. Sericin extracts of mulberry and non-mulberry cocoons waste containing different concentrations were added cautiously into these wells. This was followed by an incubated period of about 24-48 hours at about 28 o C, a period that gives the best conditions that facilitate fungal growth. The antifungal activity was then determined after incubation by determining the zone of inhibition, which is seen as an apparent area around the well where the fungal growth is inhibited. In millimeters, the diameter of the inhibition zone was measured with the help of a ruler or caliper. The greater the size of the inhibition area, the greater the antifungal effect of the substance under test.

The antifungal effect of sericin is presumed to be connected to the amino acid composition of sericin and its protein structure. Sericin has polar amino acids, including serine, glycine, and aspartic acid, that are able to react with fungal cell membranes. These interactions can interfere with membrane permeability, enzyme activity and inhibit the growth of fungal cells (Dash et al., 2008). The outcomes of this antifungal test can be discussed as very important in the context of possible application of sericin in medicine, agriculture and food preservation as a natural antifungal agent. Also, sericin, which is produced as a by-product of cocoon wastes, can be used to enhance the green and sustainable use of by-products of sericulture.

### 2.5 Comparison of Mulberry and Non-Mulberry Sericin Antifungal Activity

Comparative analysis of antifungal activity of mulberry and non-mulberry sericin extracts showed significant differences in fungal pathogen inhibitory activities. Both the sericin variants proved to be successful in the inhibition of fungal growth, but the outcome showed that the anti-fungal properties of non-mulberry sericin were slightly better in the majority of the cases.The inhibition zones caused by non-mulberry sericin were larger than those caused by mulberry sericin with all three fungal species used, as indicated in Table 4. An example of this would be non-mulberry sericin, which gave an inhibition zone of 15 mm against Aspergillus niger, compared to mulberry sericin, which gave an inhibition zone of 12 mm against Aspergillus niger. The same tendencies could be found with Candida albicans and Penicillium species.

**Table 4: Comparative Antifungal Results**

| Fungal Species | Mulberry Sericin (mm) | Non-Mulberry Sericin (mm) |
|---|---|---|
| *Aspergillus niger* | 12 | 15 |
| *Candida albicans* | 10 | 13 |
| *Penicillium* sp. | 11 | 14 |

The increased antifungal property of non-mulberry sericin can be ascribed to variation in chemical compositions. It has been noted that non-mulberry sericin has higher molecular weight proteins and a wider variety of amino acids than mulberry sericin. Such differences have the potential to alter the biological effects of sericin and increase its interaction with fungal cell membranes (Ghosh et al., 2018). Another reason why non-mulberry sericin is more antifungal has to do with the fact that the latter produces other bioactive species by the wild species of silkworms. These substances can enhance antimicrobial activity and inhibit fungal proliferation.

It was also found that mulberry sericin has a great antifungal activity, so it could still be used as an effective natural antimicrobial agent. Due to its broader distribution of production, mulberry sericin is an accessible and economical biomaterial, as shown by the findings of this comparative study. The findings of this comparative study suggest that sericin derived from the mulberry and non-mulberry cocoons waste is a potential natural antifungal agent. The waste of sericin is not only useful in obtaining good bioactive compounds but also helps in the practice of sustainable sericulture and minimizes the pollution of the environment that the waste of sericin processing poses (Dash et al., 2008).

## 3 Antifungal Activity Test

The Agar Well Diffusion Method was used to test the antifungal activity of sericin extracts.

Procedure:

1. PDA plates were inoculated and prepared with fungal cultures.
2. The agar medium was prepared by making wells in it with a sterile cork borer.

3. Various levels of sericin extract were injected to the wells.
4. The plates were incubated at a temperature of 28 o C over 24 to 48 hrs.

Measurement of the zone of inhibition around each well was done after incubation of the medium.

## 4. Results

The antifungal activity was determined by measuring the diameter of the inhibition zone produced by sericin extracts.

**Table 1: Antifungal Activity of Sericin Extract**

| Fungal Species | Mulberry Sericin (mm) | Non-Mulberry Sericin (mm) |
|---|---|---|
| Aspergillus niger | 12 mm | 15 mm |
| Candida albicans | 10 mm | 13 mm |
| Penicillium chrysogenum | 11 mm | 14 mm |

These findings indicate that both sericins were able to inhibit fungal growth. Non-mulberry sericin, however, had a slightly higher antifungal activity than mulberry sericin.

## 5. Discussion

It is possible to explain the observed antifungal power of sericin extracts by the existence of various bioactive elements in sericin protein. Sericin is rich in polar amino acids, including serine, glycine and aspartic acid, and these amino acids have functional groups that are able to react with the cell membrane of microbes. These exchanges can cause a rise in membrane permeability, interference with cellular processes and finally prevent the growth of fungi. It has been proposed by previous researchers that sericin proteins can disrupt the synthesis of fungal cell walls and enzyme activities, thus inhibiting the growth of pathogenic fungi.

In the current research, mulberry sericin exhibited average antifungal effectiveness against the fungal species that were tested. It is possible that this moderate activity is associated with comparatively simpler and low molecular weight proteins of mulberry sericin. Mulberry sericin, however, retained inhibitory effects, but the areas of such inhibition were smaller than those that were issued by non-mulberry sericin. Non-mulberry sericin, in its turn, had a stronger antifungal activity. Such increased activity can be explained by more complicated amino acid structures and higher molecular weight proteins. These biochemical differences have the potential to promote the interaction of sericin molecules with the cell membranes of fungi, with the consequences of increased antifungal activity.

## 6. Applications of Sericin

Sericin, which is produced using cocoon wastes, has experienced rising popularity because of its various biological characteristics and its possible use in various industries. Due to the antimicrobial, antioxidant, and moisturizing properties, sericin may be applied as a useful biomaterial in the biomedical sphere, such as the creation of antifungal wound dressing, tissue engineering scaffolds, and biocompatible scaffolds. It can be naturally biocompatible and induce cell growth, which is why it can be used in medicine as a wound healing product and drug delivery system.Sericin can be evolved into natural antifungal or antimicrobial drugs in the pharmaceutical industry. Sericin-based treatments are also useful in the textile industry since the resistance against synthetic antimicrobial agents is on the rise. Sericin can be applied as an antimicrobial finishing material onto fabrics, which prevents the growth of microbes on the surface of fabrics and enhances the functionality of fabrics. In farming, sericin can be used as a natural antifungal coating on crops. It is biodegradable, and thus it is more environmentally friendly than the synthetic chemical fungicides.

## 7. Conclusion

In the current research, it is revealed that sericin, which is prepared using mulberry waste Systems and non-mulberry cocoon waste, has a high level of antifungal effects against conventional fungal infections. The experimental findings have shown that sericin has the potential to be used as an antifungal agent, as non-mulberry sericin has been observed to have a slightly better antifungal activity than mulberry sericin. Such a difference could be explained by the differences in protein and molecular weight and amino acid structure of the two sericin types. The biochemical variations affect the contact of sericin with the fungal cell membranes, hence, the antifungal activity. The results of the study reveal the significance of using sericulture waste as a good biological resource. Rather than snapping off the cocoon waste in the silk processing, deriving sericin out of the same waste can yield biomaterials that are sustainable in waste management of the sericulture industry and, at the same time, develop natural antimicrobial agents. Future studies are needed to enhance the methods of extraction, the active compounds that cause antifungal effects, and the use of sericin in industries at large scale to be used in biomedical and agricultural industries.

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