



## **Antibacterial Activity of Silver Nano/Microparticles in Chitosan Matrix Prepared using *Mangifera Indica* and *Chrysophyllum Cainito* Leaf Extracts and its Application in Pineapple (*Ananas Comosus*) Polyester Fabric**

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### **ABSTRACT**

*This study reports the green synthesis of silver nano/microparticles (SN/M), its application in pineapple (*Ananas comosus*) polyester fabric as SN/M chitosan composite (SN/M-C), and its antibacterial activity. SN/M were prepared using leaf extracts of mango (*Mangifera indica*) and star apple (*Chrysophyllum cainito*) and were characterized by scanning electron microscopy (SEM) and energy dispersive X-ray (EDX). The SN/M were combined with chitosan and was applied to the fabric. The SN/M-C treated fabrics were evaluated for antibacterial activity by parallel streak assay. SEM analysis showed the smallest particles were produced using mango leaf extracts with size range of 37-138 nm. EDX analysis revealed an absorption peak at approximately 3 keV, characteristic of metallic nanocrystals. The result of parallel streak assay showed that SN/M-C treated fabrics inhibited the growth of *Staphylococcus aureus*, and the antibacterial property was still observed after five washing. The results indicate that SN/M-C could be a potentially useful textile finishing to impart antibacterial properties to natural fiber polyester blend fabric.*

**Key words:** *Chitosan, Chrysophyllum cainito, Mangifera indica, Pineapple-polyester, Silver nano/microparticles.*

### **1. INTRODUCTION**

Textiles made up of natural fibers could serve as a favorable environment for pathogenic and odor-producing microorganisms because of their large surface area and ability to retain oxygen and moisture. To control microbial growth on textiles, synthetic, and natural antimicrobial agents are applied to impart antimicrobial property. However, the use of synthetic antimicrobial agents is being discouraged due to their environmental toxicity and biological risk. Often, chemically prepared antibiotics cause biological side effects and the target microorganisms develop resistance which makes them less effective or less useful antimicrobial agents. Moreover, the production of synthetic antimicrobials uses organic solvents and chemical reagents which are harmful to the environment. With the increasing incidence of multi-drug resistance of bacterial pathogens and emerging environmental concerns, it is, therefore, necessary that the search for a new generation of eco-friendly, biocompatible, and non-toxic antimicrobial textile finishing should be explored to develop textile materials that can be used for biomedical, hygienic, and industrial applications to protect the public from

pathogenic microorganisms. The present study is part of this new line of research.

Green chemistry approach to nanomaterials and nanoparticles preparation is an eco-friendly, cost effective, and inexpensive method which employs environmentally benign materials, biodegradable, non-toxic, and natural substances. Recently, studies on the application of metal nanoparticles (particles with <100 nm) to fabrics are emerging rapidly and gaining research interest [1-4] due to their high surface to volume ratio and size-dependent properties such as catalytic, electronic, optical properties, and excellent biological activities as compared to bulk scale. Silver nanoparticles have been recognized by several researchers due to their low toxicity to normal cells [1,5], broad range antimicrobial properties, and easy application to fabrics as finishing material. The antimicrobial properties of silver nanoparticles depend on their nanoscale dimension [2,3,6]. Silver nanoparticles can be synthesized using the green chemistry method employing plant extracts [3,5,7-19] and biodegradable polymers [20-25]. Polyhydroxy and phenolic groups in plant extracts [11,26] and biological

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polymers such as alginate, carboxymethyl cellulose, chitosan, and pectin [20-25] can act as reducing and stabilizing agent for nanoparticle preparation. On the other hand, chitosan, a biodegradable polymer derived from chitin, is also known to possess antimicrobial activity. Its ability to kill microorganisms depends on the number of the available amino group [23,27,28]. Due to its polycationic nature and biocompatibility, chitosan and its structurally similar compounds have been used for various agricultural, biomedical, and pharmaceutical applications.

Mango (*Mangifera indica*) and star apple (*Chrysophyllum cainito*) are popular tropical fruit bearing trees in the Philippines. Previous phytochemical studies of mango leaves revealed alkaloids [29], flavonoids [29,30], gallotannin [31], glycosides, mangiferin [32], saponins [29], and phenolic compounds [29,33,34], while alkaloids, terpenoids, triterpenes, and phenolic compounds were reported in star apple leaves [35-37]. Furthermore, previous studies reported the use of mango leaf extract for the synthesis of gold nanoparticles [38,39] and silver nanoparticles [15,40] but few studies reported the use of star apple leaf extracts for metal nanoparticle synthesis.

The combination of silver nano/microparticles (SN/M) and chitosan as SN/M-chitosan composite (SN/M-C) as antimicrobial textile finishing could be superior to chitosan or SN/M alone because of their possible synergistic antimicrobial effect and controlled release of SN/M. Thus, the present study aims to apply SN/M-C to natural fiber polyester blend fabric to impart antibacterial property.

In this work, the SN/M were synthesized using leaf extracts of mango (*M. indica*) and star apple (*C. cainito*) and the SN/M formed in this process were characterized and were applied in pineapple (*Ananas comosus*)-polyester fabric. The SN/M-C finished fabric could be used for possible biomedical and textile applications such as wound dressing, packaging material, and other industrial applications.

## 2. EXPERIMENTAL

### 2.1. Plant Material

The leaves of mango (*M. indica* L.) and star apple (*C. cainito* L.) were collected in Quezon City, Philippines.

### 2.2. Chemicals Used

Chitosan, silver nitrate, ethanol, acetic acid, citric acid and sodium lauryl sulfate were used as supplied.

### 2.3. Fabric Used

Pineapple (*A. comosus* Linn.) polyester (80:20) fabric was purchased from the Fiber Industry Development Authority, Quezon City, Philippines.

### 2.4. Preparation of Leaf Extract

Mango and star apple leaves were washed with tap water and distilled water, and were air-dried. The leaves were ground separately using an electrical blender, transferred in a clean bottle, and set aside for further use. About 15 g of the powdered leaves were placed in separate Erlenmeyer flask and were soaked in 100 ml of 70% (v/v) ethanol. The flasks were stored in a cabinet for 24 h. The mixture was filtered and the resulting filtrate was used as reducing agent for silver ion ( $\text{Ag}^+$ ).

### 2.5. Biosynthesis of SN/M

20 ml leaf extract was slowly added to silver nitrate solution in separate flasks with continuous stirring at room temperature. In the present study, SN/M1, SN/M2, SN/M3, and SN/M4 refers to SN/M produced using mango leaf extracts and 1 mM silver nitrate, mango extract, and 5 mM silver nitrate; star apple extract and 1 mM silver nitrate, and star apple extract, and 5 mM silver nitrate, respectively. The mixtures were stored in dark condition and were observed for color change. After 24 h, a dark brown and precipitate were observed indicating the formation of silver nanoparticles.

### 2.6. Characterization of SN/M

The surface morphology and size of SN/M were determined using JEOL JSM-5310 scanning electron microscope (SEM) and elemental composition was characterized by energy dispersive X-ray (EDX) at Solid State Physics Laboratory, De La Salle University, Manila.

### 2.7. Preparation of SN/M Chitosan Finishing

Approximately, 2.00 g of chitosan was dissolved in 200 ml of 2% (v/v) acetic acid with constant stirring. The SN/M (SN/M1, SN/M2, SN/M3, and SN/M4) prepared using mango and star apple extracts were mixed with chitosan solution with continuous stirring. The prepared SN/M-C was used as fabric finishing.

### 2.8. Application of SN/M-C to Fabric

Citric acid was used as a cross-linker to anchor the chitosan to the cellulose backbone of the pineapple-polyester fabric. The esterification of chitosan with cellulose and citric acid occurs mainly on the hydroxyl group of the carbon-6 of chitosan [41].

The fabric was cut into 30 mm × 30 mm, washed with neutral soap and air dried. The desized fabrics were immersed in 1% citric acid for about 5 min, padded manually to remove the excess solution, air dried, and cured at 140°C for 3 min. Afterward, the fabrics were immersed in 2 g L<sup>-1</sup> of sodium lauryl sulfate for about 5 min to remove the unbound particles, rinsed with distilled water to remove the soap solution, and air dried [42]. This procedure was repeated 3 times. Thereafter, the fabrics were immersed in chitosan

and SN/M-C for 15 min, and the fabrics were padded manually using a wooden roller. After padding, the treated fabrics were air dried and cured at 140°C for 3 min. Afterward, the treated fabrics were immersed in 2 g L<sup>-1</sup> sodium lauryl sulfate for 5 min to remove the unbound SN/M-C, rinsed with distilled water to remove the soap solution, and air dried [42].

### 2.9. Antibacterial Assay of Finished Fabric

Modified parallel streak assay [43] was used to evaluate the antibacterial property of the SN/M-C finished fabrics. The fabrics were submitted to the Natural Science Research Institute, University of the Philippines, Diliman, Quezon City for antibacterial assay. The test organisms used were *Escherichia coli* UPCC 1195 and *Staphylococcus aureus* UPCC 1143. Bacterial growth was observed underneath and around the cloth after incubation.

### 2.10. Effect of Washing on Antibacterial Activity

The finished fabrics were soaked in neutral soap at 40°C for 30 min, followed by rinsing with distilled water and air drying. This procedure was repeated 5 times. After five washing, the fabrics were evaluated for antibacterial assay according to American Association of Textile Chemists and Colorists [43].

## 3. RESULTS AND DISCUSSION

### 3.1. Biosynthesis of SN/M

It was observed that the color of the mixture of plant extract and silver nitrate changed to reddish-brown, and precipitates were also observed after 24 h indicative that silver ions (Ag<sup>+</sup>) were already reduced to metallic silver (Ag<sup>0</sup>). It was also observed that many small black precipitates were formed in the mixture of mango extract and silver nitrate. Several researchers associate the color change as a strong indication of the formation of silver nanoparticles [6,16,17,19,44,45]. This property known as surface plasmon resonance is a consequence of their nanometer size and at this dimension the electron cloud oscillates on the particle surface and absorbs electromagnetic radiation at a particular energy [45].

In the present study, qualitative phytochemical test of mango leaf extracts showed presence of flavonoid while tannins were detected in both mango and star apple leaf extracts. Phytochemical constituents such as organic acids, polyphenolic compounds, polysaccharides, proteins, and other biomolecules are potential reducing agents for silver ions. For instance, phenolic compounds such as flavonoids and tannins are capable of reducing Ag<sup>+</sup> to Ag<sup>0</sup> through hydrogen abstraction of the free hydroxyl groups of phenolic groups [6] which subsequently undergo oxidation to quinone forms. Consequently, the reduction reaction promotes nucleation and growth of silver nanoparticles. In this study, we postulated

that the phenolic compounds and alcohol groups in biomolecules present in the extracts of mango and star apple may be involved in the reduction of silver ions and stabilization of SN/M.

### 3.2. Morphological Characteristics

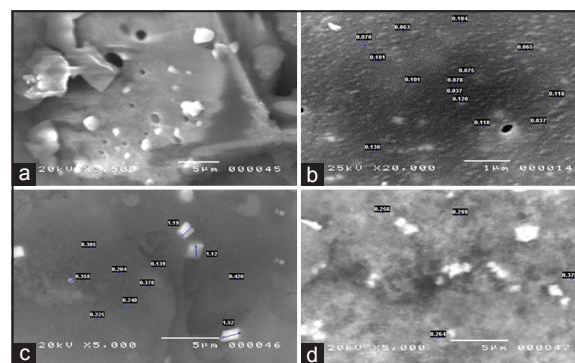
The surface morphology of biosynthesized SN/M was visualized using SEM (Figure 1). It was observed that the size of SN/M2 was smaller and well-dispersed than SN/M1. On the other hand, SN/M3 and SN/M4 obtained using star apple leaf extract were generally bigger crystals.

### 3.3. EDX Analysis

EDX provides valuable information about the metallic nature of SN/M and the presence of other elements. An absorption peak at approximately 3 keV typical for the absorption of metallic silver nanocrystals due to surface plasmon resonance was observed in the EDX profile of all SN/M (Figure 2). In addition, oxygen was also detected in all samples which could suggest that the SN/M are capped with oxygen containing compounds, such as phenolic groups, flavonoids, proteins, and carbohydrates, or the possible presence of silver oxide. Furthermore, it could be possible that once the silver ions were reduced to nano/microparticles the biomolecules present in the plant extract adhere to the surface of SN/Ms.

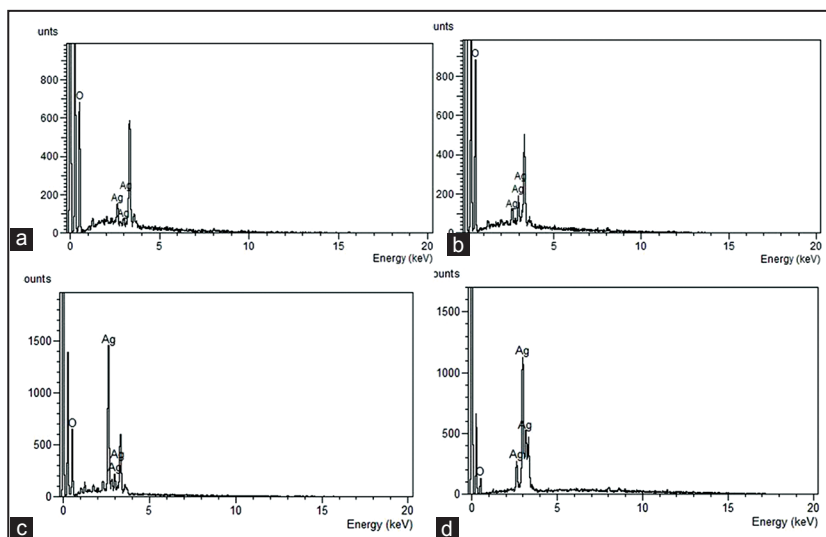
### 3.4. Antibacterial Activity of SN/M-C Finished Fabrics

The antibacterial property of the SN/M-C finished fabrics against *E. coli* and *S. aureus* were evaluated by the parallel streak method by observing and measuring the bacterial growth underneath and around the cloth (Figure 3). The result of bioassay showed that the bacterial growth of *E. coli* was visible along the edges of the untreated fabric (control), chitosan treated fabric, and fabric treated with SN/M2-C (mango) and SN/M4-C (star apple) suggesting that the SN/M-C treated fabrics were not bactericidal to *E. coli*. The same observation was also noted in chitosan treated

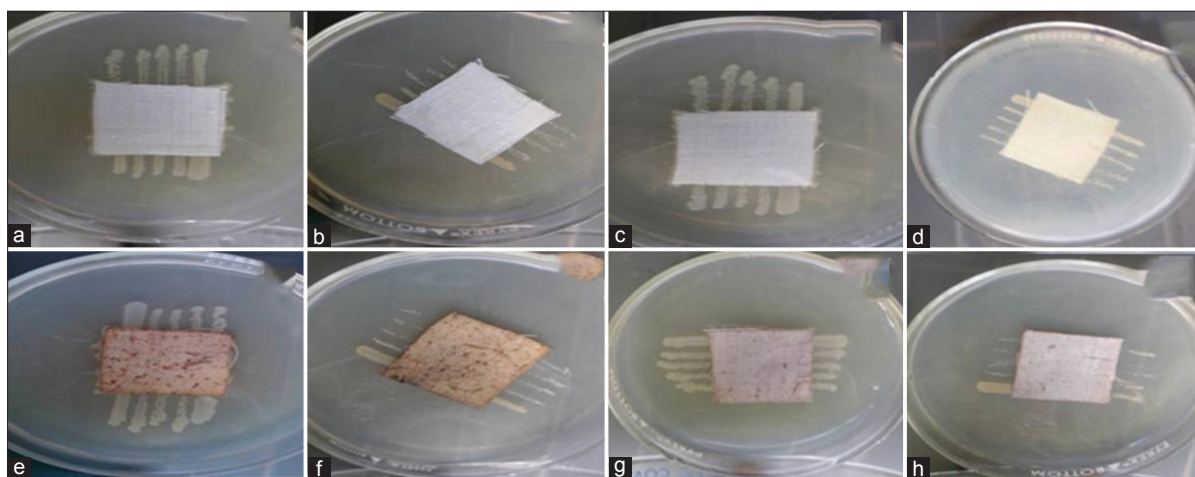


**Figure 1:** Scanning electron microscopy image of: (a) Silver nano/microparticles (SN/M1), (b) SN/M2, (c) SN/M3, and (d) SN/M4.





**Figure 2:** Energy dispersive X-ray spectra of: (a) Silver nano/microparticles (SN/M1), (b) SM/M2, (c) SM/N3, and (d) SN/M4.



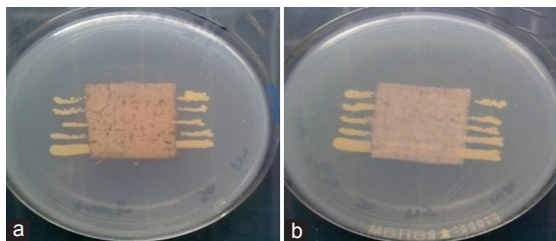
**Figure 3:** Parallel streak assay of: (a) Untreated fabric against *Escherichia coli*, (b) untreated fabric against *Staphylococcus aureus*, (c) chitosan finished fabric against *E. coli*, (d) chitosan finished fabric against *S. aureus*, (e) silver nano/microparticles chitosan composite (SN/M2-C) finished fabric against *E. coli*, (f) SN/M2-C finished fabric against *S. aureus*, (g) SN/M4-C against *E. coli*, and (h) SN/M4-C against *S. aureus*.

fabric, SN/M2-C, and SN/M4-C finished fabric against *S. aureus*. In the case of fabrics treated with SN/M2-C and SN/M4-C, no growth of *S. aureus* underneath the cloth was observed indicating that SN/M prepared using mango and star apple extract exerted antibacterial property to *S. aureus*. The result of this study is consistent with previous studies on the antibacterial activity of silver nanoparticles against *S. aureus* [7,12,16,25]. The differential sensitivity of *E. coli* and *S. aureus* toward SN/M-C could be due to the structural difference of their cell wall. Another possible explanation is that the biomolecules and phytochemicals such as phenolic groups which are capped in SN/M contributed to the antibacterial activity [46].

Chitosan, a biopolymer derived from deacetylation of chitin has been well investigated for its antimicrobial

property, and its antimicrobial activity works well in acidic medium or in low pH condition [28]. Several mechanisms have been postulated for its antimicrobial action, and the most acceptable mechanism is based on the electrostatic interaction between the positively charged protonated amino groups in chitosan and electronegative charges on the surface of the bacterial cell wall causing disruption of the bacterial cell membrane, leakage of intracellular components, and eventually cell death.

The antibacterial property of silver nanoparticles could be attributed to their nanocrystalline size which provides large surface area and higher affinity with the bacterial cells [25]. The size-dependent bactericidal activity of silver nanoparticles is enhanced in nanoparticles smaller than 10 nm [22]. Because of



**Figure 4:** Parallel streak assay of: (a) Silver nano/microparticles (SN/M2) finished fabric, and (b) SN/M4 finished fabric against *Staphylococcus aureus*.

the small size and large surface area of nanoparticles, silver nanoparticles could be tightly adsorbed onto the surface of the bacterial cells which increases membrane permeability, leakage of intracellular components, and causes death of bacteria or the nanoparticles could effectively penetrate deep inside the bacteria, deactivates enzymes by binding to the thiol groups in enzymes or denature DNA molecules by intercalating between the purine and pyrimidine base pairing and disrupting H-bonding [21]. The mechanism of antibacterial activity of SN/M-C applied to pineapple-polyester fabric could be possibly due to controlled release of SN/Ms that are dissociated from the chitosan matrix. The cell wall of Gram-negative bacteria such *E. coli* is composed of a thick layer of peptidoglycan, consisting of linear polysaccharide chains cross-linked by short peptides which give rigid structure, thus more difficult to penetrate for silver nanoparticles. On one hand, the cell wall of Gram-positive bacteria such as *S. aureus* has thinner layer of peptidoglycan, thus easier to penetrate.

### 3.5. Effect of Washing on Antibacterial Activity

The retention of antibacterial finishing agents applied to the surface of fabric could be potentially removed when immersed in water and after repeated laundering. Thus, the durability of antibacterial fabric is a major consideration [47]. The washing stability of the SN/M-C finished fabrics after five washing cycles was evaluated against *S. aureus* by parallel streak assay. Result showed that the washed fabrics possess bactericidal effect against *S. aureus* and demonstrated stable affinity and retention of SN/M-C on the surface of fabric (Figure 4).

## 4. CONCLUSION

In this study, SN/M were successfully prepared employing green synthesis approach using mango and star apple leaf extracts and were applied to pineapple-polyester fabric as SN/M-chitosan finishing. SEM images and EDX revealed the formation of SN/M. The result of parallel streak assay showed that fabrics treated with SN/M2-C and SN/M4-C were active against *S. aureus* even after five washing cycles. The possible mechanism for this antibacterial property could be due to sustained release of the SN/M in

chitosan matrix. The present study showed the use of ecofriendly and natural reducing agent for producing SN/M. The application of biosynthesized SN/M as SN/M-C textile finishing in natural fiber polyester blend fabric could offer potential possible applications in various agricultural, biomedical, pharmaceutical, and other textile applications.

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