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Research Article

Composite of Polylactic Acid/Chitosan/Ag-Hydroxyapatite Synthesized Using Turmeric Leaves Extract-Mediated Silver Nanoparticle and Snail Shell as Antibacterial Material

Rahmania Audita[1], Khoirunisa[1], Bambang Hernawan Nugroho[2], Habibi Hidayat[1], Is Fatimah[1]*

[1] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Islam Indonesia, Kampus Terpadu UII, Jl. Kaliurang Km 14, Sleman, Yogyakarta, Indonesia
[2] Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Islam Indonesia, Kampus Terpadu UII, Jl. Kaliurang Km 14, Sleman, Yogyakarta, Indonesia.

* *Corresponding author: isfatimah@uii.ac.id*

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Abstract: The development of an antibacterial composite of polylactic acid/chitosan/silver nanoparticle-doped hydroxyapatite has been synthesized. The composite was prepared using the silver nanoparticles (AgNPs) green synthesized by using turmeric (Curcuma longa Linn) leaves extract-mediated AgNPs and snail shell as biogenic calcium for hydroxyapatite synthesis. The precipitation method of hydroxyapatite by the doping of AgNPs was the first step, followed by composting with polylactic acid and chitosan as the polymer binder. Physicochemical characterization of the material was studied by using XRD, SEM, and FTIR analyses, and the antibacterial catalytic performance was examined against *Escherichia coli* (E. coli). The results showed that the synthesized AgNPs are within the <100 nm range in size and not significantly influence the crystallinity of the Ag/HAp. The composite materials maintained the antibacterial activity against E. coli.

Keywords: Silver nanoparticles, Hydroxyapatite, Snail shell, Curcuma longa Linn extract

Introduction

The development of biomaterials for medical application highlighted the use of hydroxyapatite (HAp) in abundant demand[1]. Hydroxyapatite (HAp) is a compound formed from the elements calcium and phosphorus with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ or $Ca_5(PO4)_3.OH$. The use of hydroxyapatite in the biomedical field is very wide, including as a material for *repair* or *replacement* of bone and tooth tissue[2]. HAp can be synthesized using biogenic calcium, namely calcium oxide derived from the calcination of animal materials by reaction with ammonium phosphate or phosphoric acid to produce HAp. Snail shell waste (*Achatina fulica*) has the potential to be developed as a source of Calcium Oxide (CaO). Waste snail shell has a mineral content of calcium carbonate ($CaCO_3$) is high. $CaCO_3$ can be decomposed into CaO on heating to high temperatures [3]. In addition, related with the antibacterial activity of biomaterial, some dopants to the HAp structure were utilized, and silver nanoparticles is the most popular dopant due to its high activity and biocompatibility. The development of Ag-doped HAP (Ag-HAp) by applying green chemistry method such as the plant extract-mediated bioreduction was much selected [4].

Silver nanoparticles (AgNPs) have been shown to be most effective due to their good antimicrobial properties against bacteria, viruses, and other eukaryotic microorganisms. Silver nanoparticles are more effective because of their high surface area to volume ratio so that most of the silver nanoparticles are in direct contact with their environment [5]. In the ionic form, silver is a strong antibacterial agent and is toxic to cells. Several studies used a chemical reduction method with the silver metal precursor used is

$AgNO_3$ to produce AgNPs because it is relatively simple, easy, and effective. In the reduction process using the method, it is *green synthesis* which is safe and not dangerous, so that it is environmentally friendly. The principle is to utilize the content of plant secondary metabolites as reducing agents. Bioreductors can be obtained from natural ingredients containing terpenoids, flavonoids and tannins which have antioxidant activity that can reduce silver [6]. Several studies have investigated the physiochemical characteristics of turmeric leaf extract (*Curcuma longa Linn*) to show its functional effects such as antioxidant activity, these effects are mainly derived from curcumin, total phenolic compounds, and flavonoids in turmeric leaves [7]. Using this reason, turmeric leaves can be used as a bioreductant agent in metal nanoparticles and metal oxides.

The combination of silver-doped HAp with biocompatible polymers gives possibility to be more adaptive in the tissue engineering. Polylactic acid and chitosan were reported as potential polymer for several development of biomaterials [8]. Such printed scaffold biomaterials were successfully produced by the combination of both polymers with better mechanical properties and stability [9][10].

Based on these backgrounds, in this study, the synthesis of polylactic acid/chitosan/Ag- HAp Synthesized Using Turmeric Leaves Extract-Mediated Silver Nanoparticle and Snail Shell as Antibacterial Material was conducted. The physicochemical properties of the prepared composites were characterized by UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), particle size analyzer (PSA), and antibacterial activity was evaluated.

Materials and Methods

Materials

fresh *Curcuma longa Linn leaves* and *Achatina fulica* shell were collected from traditional market in Sleman District, Yogyakarta Province, Indonesia. The aqueous leaf extract (henceforth called as CLE) was prepared by grinding 40 g of the fresh leaves followed by maceration in 100 mL of water for an hour, followed by filtration. The biogenic CaO from snail shells was obtained by calcining the crushed snail at 1000 ◦C for 4 h. Chemicals used in this research consist of pro analyst-grade of silver nitrate (Merck), ammonium diphosphate (Sigma-Aldrich), acetic acid, chitosan (Sigma-Aldrich) and polylactic acid (Sigma-Aldrich).

Method

Synthesis of AgNPs

The AgNPs were synthesized using CLE as a bioreductor, using the method reported in previous work[4]. About 9 mL of $AgNO_3$ 10^{-3} M with 1 mL of CLE, followed by microwave heating for 15 min. The reduction mechanism was monitored by UV–Visible spectrophotometry and particle size distribution identification.

Synthesis of Ag-HAp

The silver-doped hydroxyapatites were synthesized from CaO derived from snail shells and $Ca(NO_3)\cdot 2H_2O$ by setting the atomic ratio of Ag/[Ag +Ca] at 0.2 and [Ca+Ag]/P at 1.67. The AgNPs and calcium source were dissolved in deionized water to obtain 250 mL [Ca + Ag]- containing solution and stirred at room temperature. Into the stirred solution, the $(NH_4)2HPO_4$ solution was added slowly until the [Ca + Ag]/P atomic ratio of 1.67 was obtained. The mixture was kept in an autoclave at 110 ◦C overnight. The resulting slurry was then dried in an electric oven at 80 ◦C before sintering at 900 ◦C for 1 h. The silver-doped hydroxyapatites were encoded as Ag/HA for the composite obtained from snail shells and $Ca(NO_3)\cdot 2H_2O$, respectively. As comparisons, HA without silver doping were also synthesized.

Synthesis of PLA/Chitosan/Ag-HAp

PLA/Chitosan/Ag-HAp was synthesized by mixing PLA, chitosan gel and Ag-HAP in the weight ratio of 1: 1: 1. The chitosan gel was prepared by diluted chitosan flakes in 2% of acetic acid.

Antibacterial Activity Test

Antibacterial activity of Ag/HAp was tested for *Escherichia coli* (ATCC 11303). A nutrient medium was prepared by suspending nutrient agar in distilled water and autoclaving before use. The tested bacterial was cultivated in nutrient broth medium by the incubation at 37 °C for 24 h. The bacterial culture was evenly spread throughout a Petri plate, and a 6-sterile filter disc was loaded with 0.02 g of Ag/HAp powder followed by incubation.

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Result and Discussion

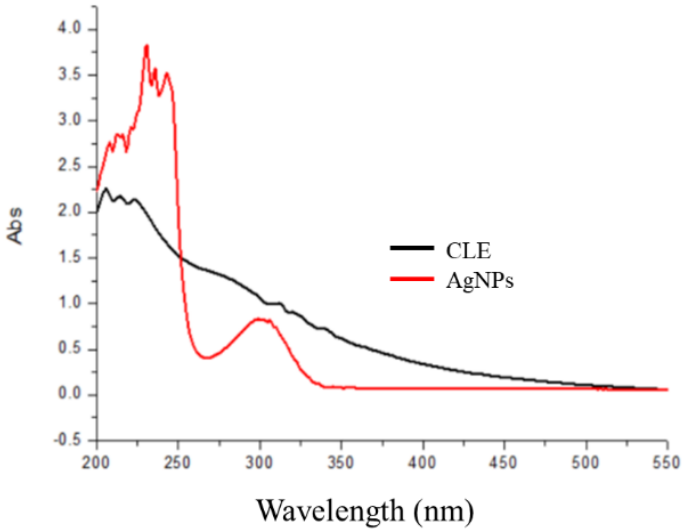

Figure 1. Compared UV-Visible spectra of CLE and Ag NPs

Figure 1 shows the comparison of the CLE and AgNPs. The UV-Vis spectrum of CLE shows representing the aromatic structures from the secondary metabolite content in the extract, together with peak around 216-243 nm and around 302-357 nm indicates the presence of 2 types of flavonoids, namely flavones and flavonols [6]. In addition, the synthesized AgNPs depicts a maximum wavelength at 302 nm indicates the surface plasmon resonance of the metal nanoparticles [11]–[14].

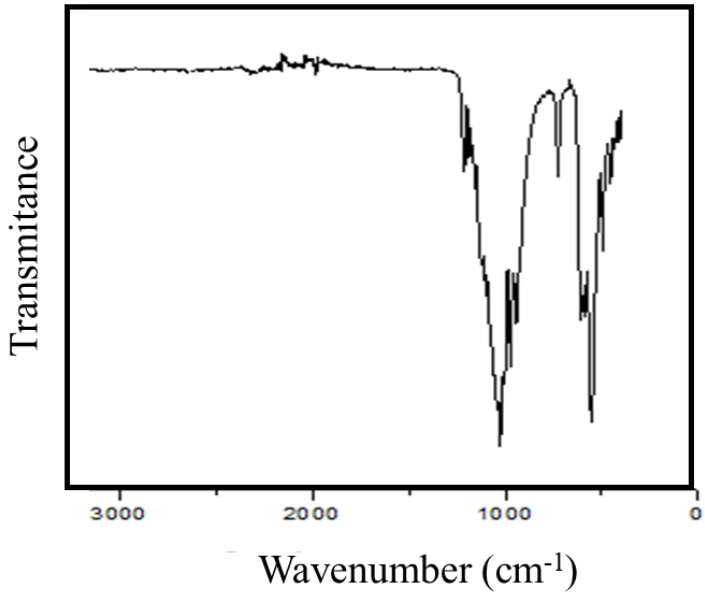

Figure 2. FTIR spectra of AgNPs

The FTIR spectrum presented in Fig. 2. show of AgNPs derived from CLE and $AgNO_3$ showed that the absorbance peaks appeared at 1211, 1188, 1156, and 1100 cm^{-1} indicating the presence of C=O and CN groups. The active compounds of flavonols were also identified at the peaks of 1026 and 972 cm^{-1} [8]. The presence of an amine group in extract *Curcuma longa Linn* indicates that it is [7] involved in the reduction of silver ions to AgNPs because this plant acts as a bioreductor agent and it became the capping agent of the nanoparticles.

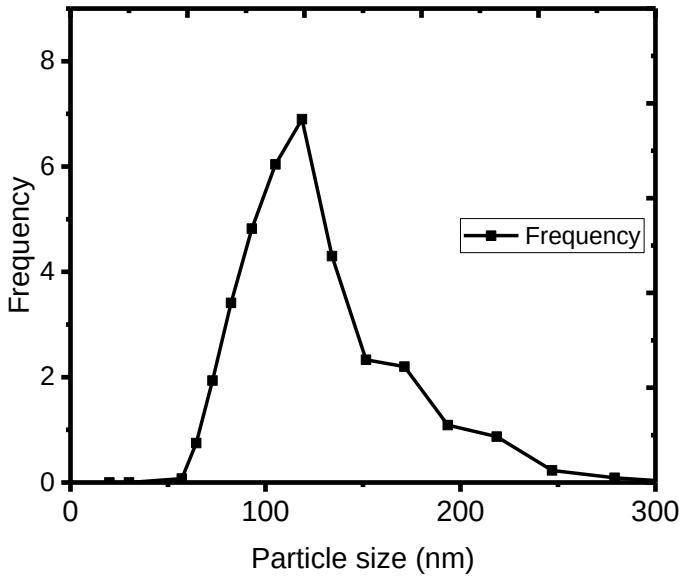

Figure 3. Particle size distribution of Ag NPs

The particle size distribution presented in Fig. 3. shows an uneven distribution because the peak increase and peak decrease are not the same. The highest peak is seen at 153 nm with a peak range of 50-1000 nm, indicating that the AgNPs obtained are still nanoparticles because they are still found in the <100 nm range.

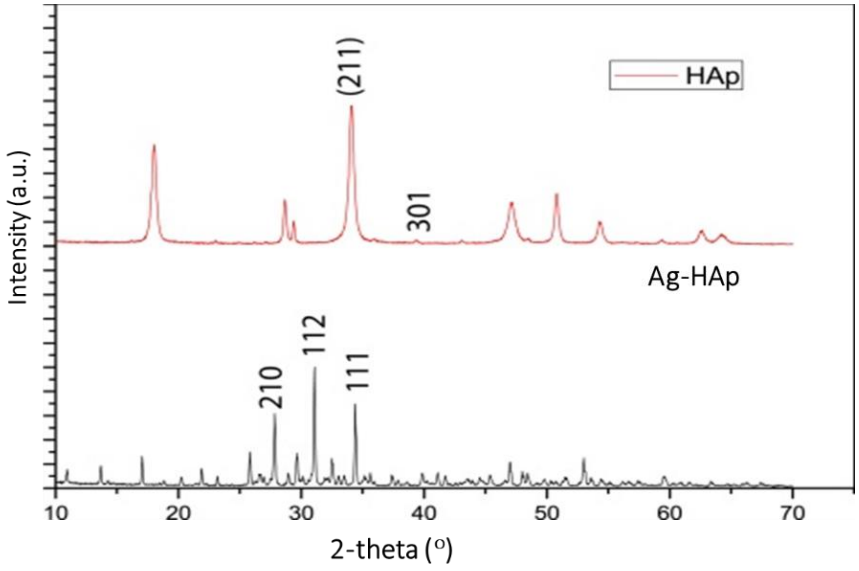

Figure 4. XRD pattern of HAp and Ag-HAp

The XRD patterns of the samples presented in Fig. 4 show peaks characteristic of HAp at 2θ values of about 31.7o and 32.8o representing the reflection planes (211) and (301) respectively; these are consistent with JCPDS card No. 09-432 [9]. And the characteristics of the Hap-Ag peaks these are consistent with JCPDS file no. 01-087-0717 indicate the presence of additional Ag which represents the reflection plane (210), (112), and (111) respectively [10]

Further, the SEM profile confirms the morphology of HAp and Ag/HAp have an open porous structure [Fig. 5]. In Hap-Ag have higher porosity. The results of the EDX analysis showed the presence of Ag at the 6.7% which indicated the success of Ag-HAp formation. In addition, there are peaks for the calcium, phosphorus, and oxygen (in hydroxyapatite crystals) atom [11]

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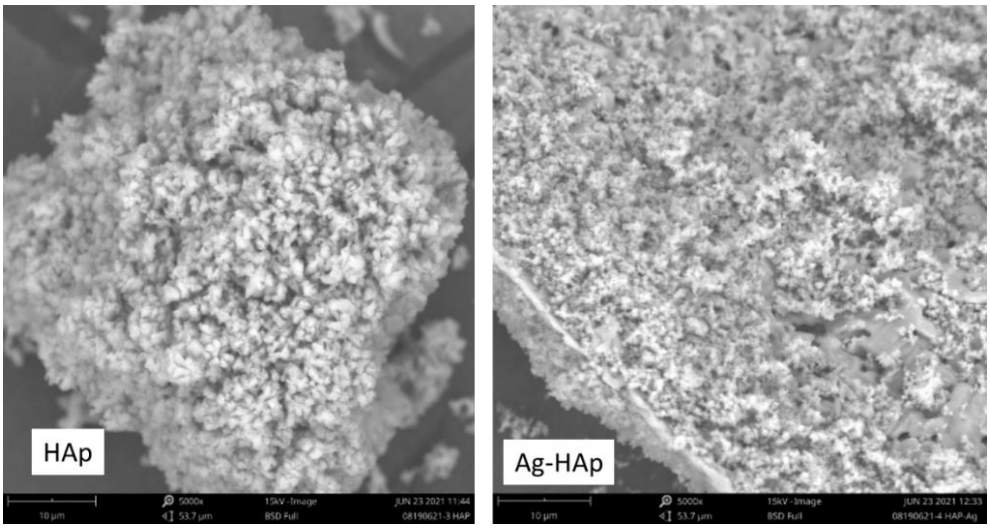

Figure 5. SEM image of HAp and Ag-HAp

The physical appearance of the prepared PLA/chitosan/Ag-HAP composite can be seen in Figure 6. From the images, it is seen a change of the morphology and color of the material, and moreover, a compact mixture of the component was identified.

Figure 6. Physical appearance of Ag-HAp and PLA/Chitosan/Ag-HAp

Figure 7 exhibits the FTIR spectra of the composite. The presence of polymer chains is identified by absorption peaks at around 500-1000 as indication of C-H from the backbond of PLA and chitosan. The characteristic peak at 1747 cm^{-1} and 1760 cm-1 are due to the stretching of carbonyl groups in ester bonds, which was formed by ring-opening polymerization (ROP) of lactide on the hydroxyl groups of HAp. Similar pattern was reported in the synthesis of **Poly(Lactic Acid)** Composites Containing Poly(Glycolic Acid) Fiber and Hydroxyapatite Particles[20]- [22].

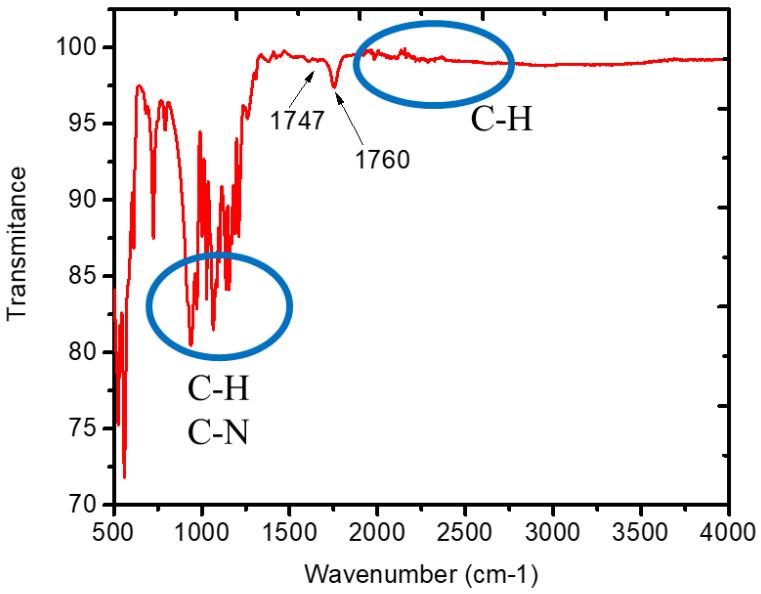

Figure 7. FTIR spectrum of PLA/Chitosan/Ag-HAp

Antibacterial activity

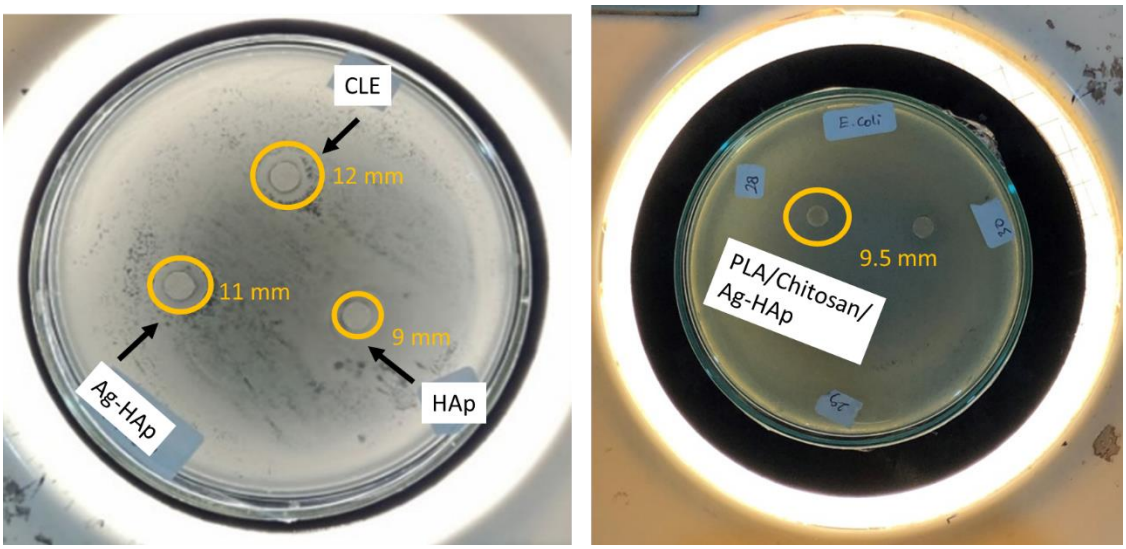

Figure 8. Images from the antibacterial test against *E. coli*

The antibacterial activity test was carried out by measuring the inhibition zone in antibacterial activity test of *Curcuma longa* Linn extract, HAp, Ag-HAp and PLA/Chitosan/Ag-HAp at the incubation time of 24 using gram-negative bacteria (*E. coli*) are 12, 9, 11, and 9.5 mm, respectively. The inhibition zone, as presented in Figure 8, suggest that the Ag-HAp and the PLA/Chitosan/Ag-HAp are considered good enough for further antibacterial applications.

Conclusion

The composite of PLA/Chitosan/Ag-HAp was successfully synthesized using Curcuma longa linn leaves extract for the green synthesis of AgNPs and snail shell as CaO source for HAp. The composite

showed the nanoparticles dispersed in the composite giving influence for the homogeneous performance as well as the antibacterial activity. The nanocomposites exhibit remarkable antibacterial activity against E. coli provide a reference for designing and developing novel antibacterial materials for various applications, such as wound dressings, antibacterial surfaces, and biofilms.

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