Preparation of silver nanoparticle by green synthesis and application to leather in enhancements of antimicrobial activity of leather

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Abstract. The objectives of this study were to prepare nanoparticle by using extract of Tectona grandis L. leaf and to deposit nanoparticle on goat leather via ex situ method, and also to characterize the antimicrobial activity of modified goat leather. The stages in this study were preparing of silver nanoparticles via green synthesis method by using extract of teak leaf, characterizing of nanoparticle by using a UV-Vis spectrophotometer and Particle Size Analyzer (PSA), impregnating the nanoparticle on the pickle leather by ex situ method, and analysing the antibacterial and antifungal activities of modified leather by the diffusion method. The silver nanoparticles were greenish brown in colour, having absorption at the wavelengths of 449 nm which indicator the formation of silver nanoparticles. The silver nanoparticles have an average particle size of 109.9 nm. The results of antimicrobial tests against the bacteria Staphylococcus epidermidis DNCC 6018, Escherichia coli FNCC 0047, and the fungus Candida albicans ANCC 0048 showed that all variations of the leather have different abilities in inhibiting the growth of the bacteria and the fungi. The pickle leathers after modification with nanoparticle Ag showed the highest antibacterial activities against Staphylococcus epidermidis DNCC 6018 and Escherichia coli FNCC 0047.

1. Introduction

Leather products are the fourth most industrial product contributing to non-oil exports, worth 6.57% in 2019 [1]. The availability of goat leather as one of the raw materials in the manufacture of textile products has also increased with the increasing number of goat population in 2018, amounting to 2.82% [2]. Raw skin has poor resistance, because it is easily damaged by the influence of microorganisms, causing a decrease in leather quality [3].

Besides using preservatives to improve leather quality, many researchers are developing textile products with added value in the form of antimicrobial properties. Microbial growth in textile products can cause colour damage, decrease in mechanical strength, cause stains and cause odour due to sweat. The development of antimicrobial textiles is mostly done through engineering of metal particles and metal oxides such as silver, copper, TiO2, ZnO, and MgO [4]. So far silver is the metal most widely applied in the manufacture of antimicrobial materials. Besides having antimicrobial activity, silver is
also environmentally friendly [5]. Microorganisms that can be inhibited by silver nanoparticles include Staphylococcus aureus [6], Aspergillus niger [7], Escherichia coli [8], Candida albicans [8,9], and Staphylococcus epidermidis [10].

However, many of the leading garment leather industries require that their products have applied nanotechnology to produce superior antibacterial and antifungal leather and are more environmentally friendly. In an effort to prepare environmentally friendly nanoparticles, it is necessary to synthesize nanoparticles using natural materials. Silver nanoparticles can be synthesized by biosynthetic method, which is used as a plant extract to reduce silver ions to nano-sized. The compounds involved in the biosynthesis of these nanoparticles are secondary metabolites which are present in plants. One of the plants that can be used for biosynthesis is teak leaves. Teak leaf extract has flavonoid content in the form of anthocyanin which can play a role in reducing Ag⁺ ions to Ag⁰ [11]. This study was useful in improving the antimicrobial properties of pikel goat skin.

Antimicrobial activity testing is carried out by the diffusion method, which measures the clear zone that is around the leather sample that was previously placed on the microbial culture in a petridish. The larger diameter of the clear zone measured shows that more microbes are damaged and dead [12]. This study aims to prepare silver nanoparticle by green synthesis method, modify goat leather by using silver nanoparticle, and determine the antimicrobial activity of modified goat leather against Staphylococcus epidermidis DNCC 6018, Escherichia coli FNCC 0047, and the fungus Candida albicans ANCC 0048.

2. Method

The tools used in this study were the UV-Vis spectrophotometer (Shimadzu UV-Vis 2400 Pc series, Japan), Particle Size Analyser (Microtrac S3500, China), Laminar Air Flow (Shimadzu SCB-1000A, Japan), analytical digital, hot plates, thermometers, shakers, glassware, autoclaves, ose wires, and callipers. While the material used in the study were solution of AgNO₃ 1 mM, distilled water, teak leaves (Tectona grandis L), goat leather, silane compounds (MethylTriMethoxySilane/MTMS), solution of starch of 0.05%, microorganisms of S. epidermidis, E. coli, and Candida albicans.

2.1. Preparing extract of teak leaves

Teak leaf extract was prepared by boiling teak leaves with the distilled water in a ratio (1: 5) at 100°C for ± 15 minutes. The biosynthesis of silver nanoparticles was then carried out by reducing 65 mL of 1 mM AgNO₃ solution with 10 mL of teak leaf extract and allowed to stand for 2 hours. Then added 0.05% of starch solution as a stabilizer while shaker the solution for 2 hours.

2.2. Characterizing of silver nanoparticles

The silver nanoparticles obtained were characterized using a UV-Vis spectrophotometer at a wavelength of 200-500 nm and using PSA to determine the particle size distribution.

2.3. Depositing of silver nanoparticle on the leather

Modification of goat leather was carried out using silver nanoparticles and MTMS. There were four variations of modifications on goat leather, namely P-NAg, P-NAgM, P-MNAg and P-M. Application nanoparticle silver on goat leather was conducted by using a shaker for 24 hours then dried the goat leather in the open air.

2.4. Testing of antimicrobial activities of modified leather

Antimicrobial activity tests were carried out by the diffusion method and the results of the measurement of the clear zone are then analysed using the ANOVA test. There are three types of tests, namely test of the effect of leather treatment, test of the effect of incubation time, and test of interactions between variations of sample treatment and incubation time on antimicrobial activity of leather.
3. Result and Discussion

The produced silver nanoparticles by green synthesis method are greenish brown (Figure 1) which have absorption at the wavelengths of 421.6 nm and 449 nm which indicated the formation of silver nanoparticles. The result of PSA showed that the distribution of silver nanoparticle size ranged from 79.1 nm to 155.9 nm with an average size of 109.9 nm (Figure 2). The presence of nanoparticles above 100 nm showed that the silver nanoparticles agglomerated. This can occur because silver nanoparticles were formed more so that collisions between particles were more frequent and caused agglomeration [5].

The producing of Ag nanoparticles will cause the changing of colours that were derived from oxidized organic materials [13]. The more concentrated the colour produced the more organic materials are oxidized and the more Ag+ changed to Ag⁰, the more silver nanoparticles has resulted. The producing of nanoparticles with colour changes that occur from colourless to dark yellow until brown [14,15].

![Figure 1. The spectra of UV-Vis of silver nitrate solution and nanoparticle of silver.](image1)

![Figure 2. The Particle size distribution of silver nanoparticle.](image2)

Goat leather deposited with silver nanoparticles and MTMS have different colours which indicate that silver nanoparticles and MTMS have coated on the goat leather (Figure 3).
The Figure 4 shows mechanism of the formation of Ag nanoparticles via the reduction oxidation reaction by phenolic acid, flavonoid, alcohols, terpenoids, and polyphenols [16,17]. The reaction that occurred was an autocatalytic reaction that was a chemical reaction in which there were products or reactants that could be produced as catalysts. It could be seen that the reduction reaction occurred between polyphenols (component of plant) and Ag+ where Ag+ become reactants which were also involved as catalysts.

The results of inhibitory zone measurements in goat leather samples are shown in Figure 5, 6, and 7. Based on Figure 5, 6, and 7 shows that all variations of modified goat leather have activity in bacteria and fungi inhibition. For bacterial inhibitory activity, pickle goat leather after modification with addition of silver nanoparticle has the greatest inhibitory activity as much as 10.07 mm against S. epidermidis (Figure 5) and 10.29 mm against E. coli (Figure 6). The silver nanoparticles have the ability to inhibit the growth of Escherichia coli and Staphylococcus epidermidis bacteria [18,19]. S. epidermidis as gram-positive bacteria has several layers of peptidoglycan which combine to form a thick and stiff structure. While E. coli is a gram-negative bacterium that has a thin layer of peptidoglycan and a protected outer membrane. Based on the inhibitory activity of fungi, the leather with the greatest in an antifungal activity was pickle leather after modification with silver nanoparticle and MTMS as much as 6.74 mm (Figure 7). However, the diameter of inhibition zone of leather against fungi was the lower than against bacteria. The difference is only in the structure of protein molecules owned by bacteria and fungi. Bacteria have a simpler structure than fungi.

![Figure 3. Goat leather after modification with silver nanoparticles and MTMS](image)

![Figure 4. The scheme of synthetic of silver nanoparticle by using plant extracts [17].](image)
Figure 5. The inhibition zone of leather before and after modification against *S. epidermidis*

The main mechanism of silver nanoparticles as antimicrobial agents consists of several stages. Initially, silver nanoparticles will stick to the cell surface which is negatively charged so that it changes the physical and chemical properties of the cell membrane and cell walls attached, such as interfering with the activity of electron transport, respiration, cell permeability and disrupting other important functions [8,12,16]. Furthermore, silver nanoparticles will go deeper into the cell so that silver nanoparticles can interact with DNA, proteins and the inside of cells that contain phosphorus and sulphur. The last process is cell death caused by interactions between phosphorus and sulphur with silver nanoparticles which can deactivate proteins in cells and decrease membrane permeability and change the structure of DNA in cells [9].

Figure 6. The inhibition zone of pickle leather before and after modification against *E. coli*
Figure 7. The inhibition zone of pickle leather before and after modification against *C. albicans*

The results of the ANOVA test of antimicrobial goat leather before and after modification are shown in Table 1. The significance value (p value) for the three microorganisms is less than 0.05. This shows that the three factors provide a significant difference in inhibiting the activity of microorganism growth. In line with this result, the incubation time and the type of fabric, and also the time and type of fabric simultaneously effected the antibacterial activity [20].

| Microorganism | Source | Treatment (P) | Time (T) | (P) x (T) | Conclusion |
|---------------|--------|---------------|----------|-----------|------------|
|               |        | Significant   |           |           |            |
| *S. epidermidis* | 0.000  | 0.000         | 0.000    |           | Significant |
| *E. coli*     | 0.000  | 0.000         | 0.000    |           | Significant |
| *C. albicans* | 0.000  | 0.000         | 0.002    |           | Significant |

4. Conclusion
The silver nanoparticles had absorption at the wavelengths of 449 nm and an average particle size of 109.9 nm. The results of antimicrobial tests against the bacteria *Staphylococcus epidermidis* DNCC 6018, *Escherichia coli* FNCC 0047, and the fungus *Candida albicans* ANCC 0048 showed that all variations of the leather have different abilities in inhibiting the growth of the bacteria and the fungi. The pickle leathers after modification with nanoparticle Ag showed the highest antibacterial activities against *Staphylococcus epidermidis* DNCC 6018 and *Escherichia coli* FNCC 0047. However, nanoparticle Ag had the highest antifungal activity against *Candida albicans* ANCC 0048. Antifungal activity test showed that goat leather after modification with silver nanoparticles and MTMS had the higher antifungal activity than others leather. The leather treatment and the incubation time effected to antimicrobial activity of leathers simultaneously. For further research, it is necessary to pay attention to the type of treatment and incubation time to produce optimum antibacterial activity of goat leather.

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6. References

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