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Silver Nanoparticles Biosynthesis Using Mangosteen (*Garcinia Mangostana L.*) Rind Extract For Environmentally Friendly Liquid Disinfectant Active Ingredients

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ABSTRACT

Disinfectant is one of the materials that can be used to inactivate pathogenic microorganisms, but most of the disinfectants used in the community are disinfectants made from synthetic chemicals that are harmful to the environment. The purpose of this study was to synthesize and evaluate the antimicrobial activity of silver (Ag) nanoparticles-mangosteen rind extract as an active ingredient in an environmentally friendly disinfectant formula. The synthesis process of silver nanoparticles was carried out by adding a bioreductant of mangosteen rind extract into a 0.01 M $AgNO_3$ solution precursor with a variation of the precursor : bioreductor volume ratio of 1:1, 1:2, 1:3, 1:4, 1:5, and 1:6, respectively. The results of the analysis with UV-Vis spectroscopy showed that the silver nanoparticles of mangosteen rind extract had good stability. The decrease in the absorption peak in the FTIR spectrum at a wave number of 3390.86 cm^{-1} indicates the contribution of the –OH group in the bioreductant compound in the reduction process of silver nanoparticles. PSA analysis and digital microscopy showed that the diameter of the synthesized silver nanoparticles with a volume ratio of bioreductor : precursor 1:1 was 82.33 nm. The antimicrobial activity test showed that the active ingredient mixture of silver-mangosteen rind extract with a composition of 1:1 had the best activity in deactivating of gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria. The results of this study indicate that the mixture also can be used as active ingredients for environmentally friendly liquid disinfectants.

Keywords: Silver Nanoparticles, *Mangostana L*, Bioreductors, Eco-friendly Disinfectants

INTRODUCTION

Environmental cleanliness is an important factor that affects human health. However, the environment is also a place for the spread of various types of microorganisms, some of which are pathogenic. Disinfectants are one of the important ingredients to inactivate pathogenic microorganisms that are spread especially in public places. However, the disinfectant products used are mostly made of chemical formulas that are harmful to the environment such as alcohols, chlorine, aldehydes, peroxygens, and quaternary ammonium compounds [1]. Among these substances are harmful to the environment and human health. Chlorine and its compound forms have many health hazards. When chlorine compounds dissolved in water as wastewater, it can form toxic solution. Chlorination of wastewater can

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also produce toxic drug-derived disinfection by-products (DBPs). Many DBPs are carcinogenic to humans, and some of them are genotoxic, cytotoxic, and mutagenic. DBPs can be harmful to the flora and fauna of the receiving water body and may have adverse effects on microorganisms and plankton present in these ecosystems [2]. Quaternary ammonium compounds (QACs) are active ingredients in many disinfectants. QACs have an effect on antibiotic resistance, which may include formation of nitrosamine disinfection, proliferation of antibiotic resistance, and impacts on biota in surface waters [3]. Glutaraldehyde, one of aldehyde compounds, is one of the most highly effective, broad spectrum disinfectants, which typically achieve sterilization by denaturing proteins and disrupting nucleic acids. Glutaraldehyde are effective against bacteria, fungi, viruses, mycobacteria, and spores. These chemicals are toxic to humans or animals with contact or inhalation, highly irritating, and are potentially carcinogenic [4]. Therefore, production of environmentally friendly disinfectants is very important to develop. Such disinfectants can be made using antimicrobial active ingredients as well as safe solvents. Antimicrobial active ingredients that are safe for the environment generally come from natural sources and also nanoparticles materials.

Research on nanoparticle materials is developing very rapidly because this material can be widely applied in the environmental, electronics, optical and biomedical fields. One of the most studied examples of materials is silver nanoparticles [5]. Silver nanoparticles have wide application potential as antibacterial and antifungal because of their ability to deactivate of these microorganisms [6], [7]. Most importantly, silver nanoparticles can be used as antimicrobial agents, biomedical device coatings, drug-delivery carriers, diagnostic and optoelectronic platforms, and imaging probes do tue their easily controlled size and shape [8]. In particular, silver nanoparticles can act as an antimicrobial active material, even at low concentrations, because silver nanoparticles have the ability to penetrate bacterial cell walls, change cell membrane structure, and even cause cell death. The capability of these nanoparticles is not only due to their size at the nanoscale, but also due to their large surface area to volume ratio. The greater the ratio of surface area to volume, the faster the overall inactivation reaction to microbial growth takes place. Such characteristics can increase cell membrane permeability, generate reactive oxygen species, and interfere with deoxyribonucleic acid replication by releasing silver ions [9]. Therefore, in addition to its low price and easy manufacture, silver nanoparticles have good catalytic properties in the microbial inactivation process.

The antimicrobial performance of silver nanoparticles can be broadly described as follows: silver nanoparticles can release silver ions (Ag^+) continuously as a microbial killing mechanism. Due to their electrostatic attraction and attachment to sulfur proteins, Ag^+ ions can adhere to cell walls and cytoplasmic membranes. Adhered ions can increase the permeability of the cytoplasmic membrane and affect the removal of bacterial membranes. After absorption of free Ag^+ ions into cells, respiratory enzymes are deactivated and produce reactive oxygen species that interfere with the production of adenosine triphosphate. Reactive oxygen species are the main agents in provoking cell membrane disruption and deoxyribonucleic acid (DNA) modification. Since sulfur and phosphorus are important components of DNA, the interaction of Ag^+ ions with sulfur and phosphorus in DNA can cause problems in DNA replication, cell reproduction, and even result in the cessation of growth of microorganisms. In addition, Ag^+ ions can inhibit protein synthesis by denaturing ribosomes in the cytoplasm. Besides being able to release Ag^+ ions, silver nanoparticles themselves can kill bacteria. Silver nanoparticles can accumulate in the holes formed in the cell wall after accumulating to the cell surface. The accumulation of silver nanoparticles can cause denaturation of cell membranes. Due to their

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nanoscale size, silver nanoparticles also have the ability to penetrate bacterial cell walls and further alter the structure of cell membranes. Denaturation of the cytoplasmic membrane can damage organelles and even result in cell lysis (destruction). In addition, silver nanoparticles can be involved in bacterial signal transduction. Bacterial signal transduction is affected by phosphorylation of protein substrates. Nanoparticles can dephosphorylate tyrosine residues on peptide substrates. Impaired signal transduction can lead to cell apoptosis (death) and cessation of cell division. In addition, silver nanoparticles show lower toxicity to human health while, do not damage the environment, and are easy to manufacture so they do not require handling under special conditions [10]. For this reason, it is believed that silver nanoparticles can be used as antimicrobial active ingredients disinfectant formulas.

The most commonly used synthesis of silver nanoparticles is the chemical reduction method with synthetic chemicals [11]. In this method, some of the reducing agents that can be used are hydrogen peroxide, sodium borohydrate, gallic acid, citric acid, and mixtures of these substances [12]. Sodium borohydrate reduces Ag^+ ions to produce diborane (B_2H_6) and $NaNO_3$ by-products. These two by-products are harmfull to environment. B_2H_6 is a gas that can spontaneously burn or explode in air at normal room temperatures. Diborane is slightly soluble in water, but it will decompose rapidly when in contact with water producing boric acid and hydrogen gas which is very flammable. Diborane is a very dangerous gas that is only used in chemical laboratories by experienced professionals. Diborane is a very toxic, flammable, gas used by chemists to make other compounds. Workers employed in occupations that manufacture or use diborane may be exposed to this compound by breathing in its vapors. Consumption of drinking water with an increased concentration of nitrate may affect the human body in two ways: (i) acutely, most often manifested as methemoglobinemia, nitrates in the digestive system are reduced to nitrites, which then oxidize the hemoglobin iron forming methemoglobin unable to transmit oxygen in the body, resulting in blue skin, and (ii) chronic, manifested by the occurrence of cancer as a result of organism exposure to nitrosamines which produced from reaction of nitrates with amines in the body [13]. Based on the reasons, it is necessary to propose an alternative approach to the synthesis of silver nanoparticles that is safer, while reducing the amount of plant and fruit waste that is harmful to the environment and human health.

Another alternative in the synthesis of silver nanoparticles is to apply a green chemistry approach. In this approach, silver nanoparticles are synthesized through the reduction of Ag^+ ion precursors using plant extract bioreductors [14], [15], [16]. Plant extracts are environmentally friendly because they are non-toxic and provide natural capping agents [15], [17]. Some of the plant extract bioreductants that have been studied are Peruvera [18], carboxymethyl starch [19], *Imperata cylindrica L* extract [6], *Averrhoa bilimbi* fruit extract [20], *Pongamia pinnata* (*L*) pierre extract [21], Moringa oleifera leaves extract [22], *Angelica keiskei* extract [23], and *Pinus desiflora* [24]. All of above extracts can act as bioreductants because they contain phenolic compounds that can be oxidized when reacted with Ag^+ precursors. However, the bioreduction process with some of the extracts still produced large silver particles, even up to 4705 nm [22]. The larger the particle size, the smaller the ratio of the surface area to the volume so that the slower the rate of microbial inactivation by these particles.

Recent research results show that mangosteen rind extract can also be used as a bioreductant in the synthesis of silver nanoparticles because this extract also contains compounds that have pharmacological and antioxidant activities [25]. Mangosteen rind contains tannins and xanthones which contribute to its antibacterial and antioxidant properties

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[26], [27], [28]. It also shown that mangosteen fruit contains xanthones as α-mangostin as much as 1 - 17% [29]. Tannins and xanthones are natural chemical compounds that can be classified as phenolic or polyphenolic compounds [30]. The presence of hydroxyl groups in these compounds allows mangosteen rind extract to be used as a bioreductant in the synthesis of silver nanoparticles [31].

The use of mangosteen rind as a bioreductant for the synthesis of silver nanoparticles has been carried out previously with variations in the concentration of Ag^+ precursors and variations in the amount of mangosteen rind extract. The synthesized silver nanoparticles have been shown to be able to inactivate pathogenic bacteria *Pseudomonas* and *Staphylococcus* [26]. Based on these informations, we suspect that a mixture of silver nanoparticles and mangosteen rind extract can be used as an active ingredient in an environmentally friendly disinfectant formula. Studies on disinfectants with formulas containing the active ingredient of silver nanoparticles-mangosteen fruit extract have not been reported.

This study aims to create an environmentally friendly disinfectant formula with active anti-microbial ingredients mixed with silver nanoparticles and mangosteen rind extract. In this article, we report the results of the synthesis and characterization of silver nanoparticles obtained by reducing Ag^+ precursors with mangosteen rind extract as bioreductant. We also report the antimicrobial performance of a mixture of silver nanoparticles with mangosteen fruit extract dan the antimicrobial performance of the disinfectant formulas with active ingredients of silver nanoparticles and mangosteen rind extract against different test bacteria from previous studies, namely Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* as well as organoleptic tests of the disinfectant formulas using color and odor test parameters. In the manufacture of this disinfectant, we prefer to use ethanol as a solvent rather than methanol, because ethanol is non-toxic and non-volatile so that it meets the requirements as a solvent that is safe for the environment.

EXPERIMENT

Chemicals and instrumentation

The materials and chemicals used in this study are mangosteen fruit (bought at Kendari traditional market), 0.01 M $AgNO_3$ (*Emsure Merck*), ethanol (*Emsure Merck*), triclosan (*Emsure Merck*), sodium metasilicate (*Emsure Merck*), *Whatmann* No. 42 filter paper, nutrient broth, Gram negative *Escherechia coli* bacteria, Gram-positive *Sthapycoccus aureus* bacteria, distilled water (*Water One)*, streptomicyn, tissue paper (*Nice*), label paper (*Fox*), plastic wrap (*Cling Wrap*), and aluminium foil (*Klin Pak*). All chamicals purchased are analytical grade materials without further purification, while all bacteria are self-regenerated in the microbiology laboratory of the department of Chemistry, Halu Oleo UniversityInstrumentation applied for analysis should be written all tools are used during research. They can contain instrumentation specification or operational conditions include brand manufacturer. For example: FTIR spectrophotometer (Shimadzu FTIR QP89500, sample was analyzed using NaCl plate or thin film).

By adapting previeous studies, the analytical tools used in this study are UV-Vis Spectrophotometer (*Spectroquant Pharo* 300 M), *Partikel Size Analyzer* (*Horiba SZ* 100), and *Fourier Transform Infrared spectrometer* (*Shimadzu*), [32], [33], [34], [35]. For the observation of particle morphology, in this study a digital microscope (*Leica Microsystems*) was used

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Procedure reaction

Extraction

Sample preparation was started by washing the mangosteen fruit using running water and drying it at room temperature. The mangosteen rind was separated from the pulp and thinly sliced, dried at room temperature for four days, and further dried in an oven at 50°C for 24 hours. The dried mangosteen rind was crushed using a blender to obtain mangosteen rind powder. Extraction of bioreductant compounds from mangosteen rind was carried out by applying the extraction method modified from previous studies [36]. A total of 250 g of mangosteen rind powder was macerated with 1 L of ethanol solvent for 3 days while stirring 2 times a day. This mixture was then filtered using Whatman No.42 filter paper and the filtered filtrate was evaporated for 3 hours with a rotary evaporator at a temperature of 50°C.

Biosynthesis and Characterization

Green synthesis of silver nanoparticles using a bioreductant of mangosteen rind extract was adapted from previous research methods [33], [35]: A 0.01 N $AgNO_3$ solution (Ag^+ precursor) was reduced with mangosteen rind extract in 100 mL beaker with a volume ratio of mangosteen rind extract:$AgNO_3$ solution of 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6, respectively. This mixture was stirred at room temperature for 30 minutes. Then each mixture was centrifuged at 3500 rpm for 10 minutes. The centrifuged filtrate was put in different vials, while the precipitate was dried in an oven at 40 °C. Silver nanoparticles were characterized using UV.Vis spectrophotometer, FTIR spectrometer, PSA, and digital microscope.

Antimicrobial Activity Test

Antimicrobial activity testing was carried out using the well diffusion method by adapting the method used in previous studies [37]. The first stage is making the base of the test media using nutrient agar solid as follows: 10 mL of nutrient agar is poured into a sterile petri dish and allowed to solidify. The second stage is the preparation of two semi-solid media as follows: 100 µL of each suspension of *Staphylococcus aureus* and *Escherichia coli* bacteria are mixed with 15 mL of nutrient agar liquid in a test tube and homogenized to form a semi-solid medium. These two semi-solid media were then poured into two petri dishes of different nutrient agar media until solidified which was then perforated with four holes as wells. Each well was dripped with a different antibacterial test material, namely a mixture of silver nanoparticles-mangosteen rind extract, mangosteen rind extract, methanol, distilled water as a negative control, and 15 µL of streptomycin as a positive control. The petri dish containing the test compound was put in an incubator for 24 hours at 37ºC. After the incubation period ended, the clear zone formed was measured.

There are four formulas, named as F1, F2, F3, and F4, of environmentally friendly liquid disinfectant prepared by dispersing all ingredients into a solvent and stirring until a homogeneous liquid disinfectant is obtained. The antibacterial performance of the four formulas was tested using a method similar to that performed in the antibacterial assay of silver nanoparticles-mangosteen rind extract mixture. In this study we did not test the antibacterial performance of other formulas because this study is only the initial stage of testing the application of silver nanoparticles as an active ingredient in disinfectants. The four disinfectant formulas consisted of Ag nanoparticles-mangosteen rind extract as the active ingredient, sodium metasilicate as a builder, triclosan as a preservative, and ethanol as a solvent. The total volume of each formula is 100 mL with the volume composition of the active ingredients in F1, F2, F3, and F4 being 10, 15, 20, and 25 mL, respectively, 2 mL of metasilicate, 2 mL of

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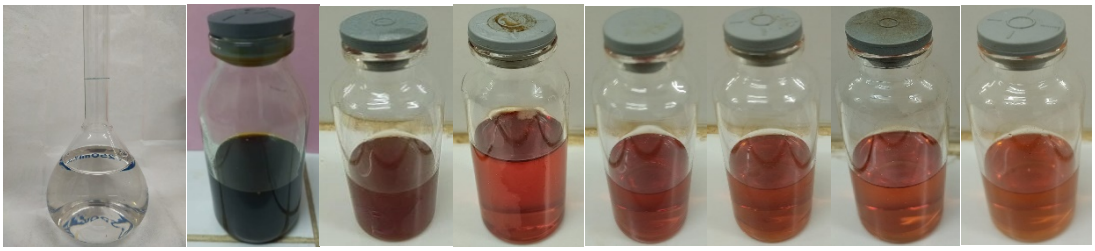
triclosan, and ethanol as much as 86, 81, 76, and 71 mL, respectively. The disinfectant formula was then tested for the percent transmittance using a UV-Vis spectrophotometer to determine the clarity of each formula and organoleptically tested with color and odor parameters to determine the panelists' preference for the disinfectant that had been made.

RESULT AND DISCUSSION

Biosynthesis of Silver Nanoparticles

Silver nanoparticles can be synthesized using chemical reducing agents such as sodium borohydrate and formamide, but these reducing compounds are toxic [38]. Therefore, we applied a green chemistry approach in the synthesis of silver nanoparticles using a bioreductant of mangosteen rind extract. Phenolic compounds contained in mangosteen rind extract play an important role in the synthesis of silver nanoparticles because of their high oxidant activity [39]. In this study, we obtained a dark brown mangosteen rind extract solution with a yield of 5.33% (Figure 1b).



(a) (b) (c) (d) (e) (f) (g) (h)

Figure 1. Reactants and the product of oxidation-reduction reaction of mangosteen rind extract bioreductant with $AgNO_3$ precursor in the synthesis of silver nanoparticles-mangosteen rind extract. (a) 0.01 N $AgNO_3$ solution, (b) mangosteen rind extract, and (c) – (h) reaction products with a volume ratio of bioreductant to precursor 1:1, 1:2, 1:3, 1:4, 1:5, and 1:6, respectively.

This extract solution contains phenolic compunds such as xanthones and tannins which are known to have antioxidant, anti-inflammatory, antibacterial, anti-allergic properties and can fight the development of cancer cells [26], [39]. These results corroborate previous studies showing the presence of these two compounds in mangosteen fruit extract [40], [41], [42]. Mangosteen peel contained high amount of phenolic compounds, such as mangosteen and gartanin, which are be responsible for antioxidant and antimicrobial activities [27], [42]. Chemical structure of tannin and some xanthone derivatives were shown in Figure 2.

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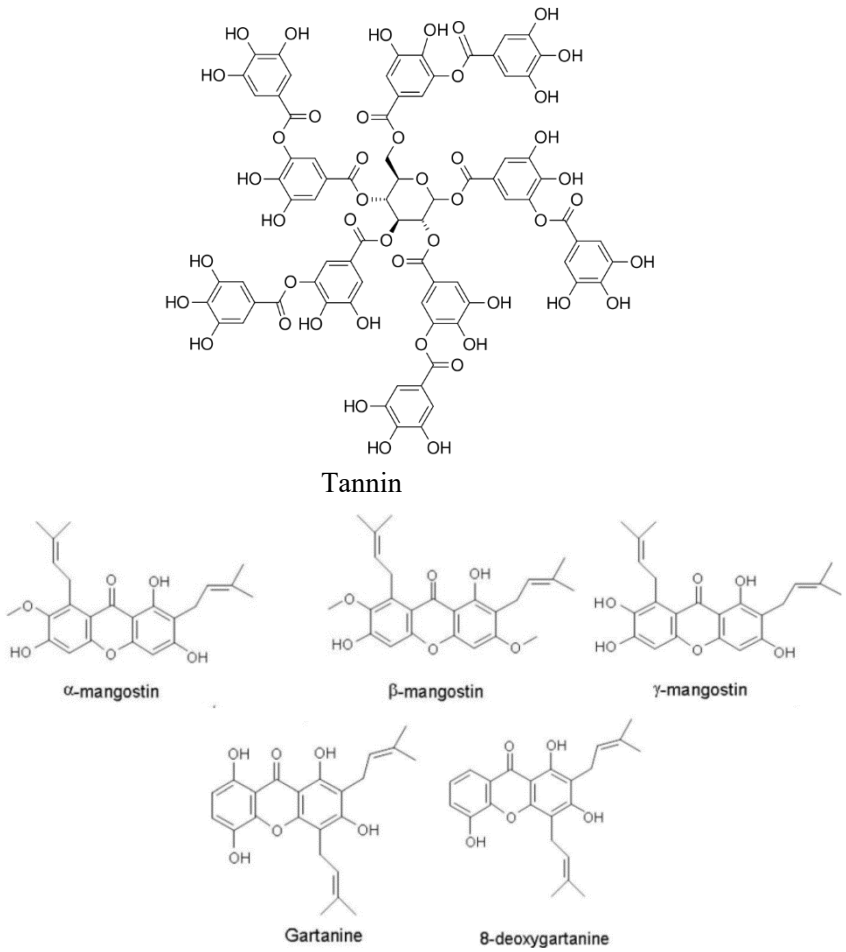

Figure 2. Chemical structure of tannin [43] and some xanthone derivatives [44] isolated from mangosteen fruit.

The redox reaction mechanism between Ag^+ precursor and phenolic compound bioreductors is complicated. However, this mechanism can be described simply as in Figure 3.

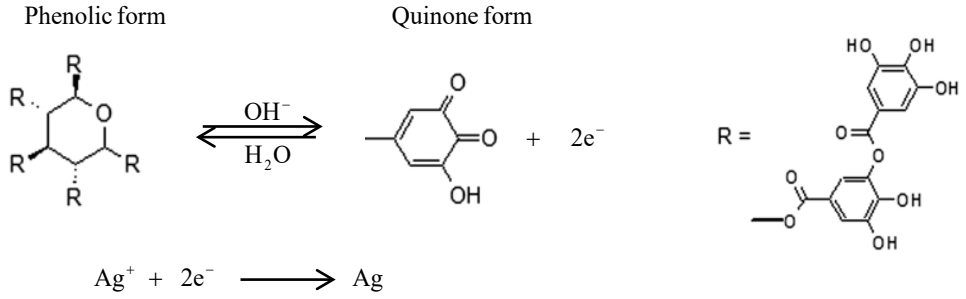

Figure 3. The simply reaction mechanism in the formation of silver nanoparticles using a phenolic compound bioreductant [45].

By adapting the reaction mechanism for the formation of silver nanoparticles from another the previeous study, a biosynthetic reaction mechanism of silver nanoparticles with phenolic

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functional group of phenolic compounds in the mangosteen fruit extract was also proposed as shown in Figure 4 [34] with the forming silver nanoparticles take place through a process as shown in Figure 5.

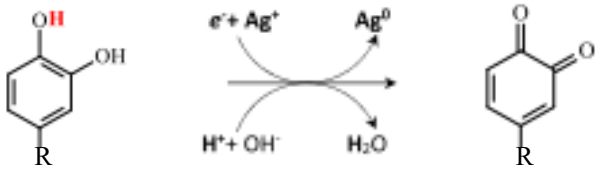

Figure 4. Proposed reaction mechanism for the biosynthesis of silver nanoparticles using bioreductants of phenolic compounds in mangosteen fruit extract [34].

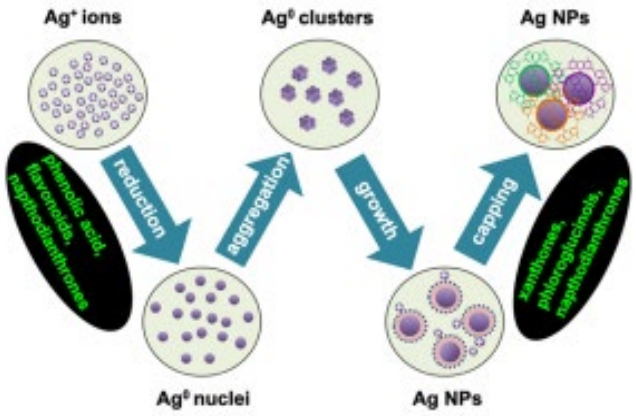

Figure 5. Putative model for green synthesis of Ag-Nps [46].

Silver nanoparticles exhibit yellowish brown colour in water due surface plasmon vibration of the nanoparticles [47]. In the process of synthesizing silver nanoparticles, the color change of the mangosteen rind extract solution after the addition of Ag^+ precursor was an indication that colloidal silver was starting to form. In this study, the reaction of mangosteen rind extract with $AgNO_3$ was accompanied by a change in the color of the mangosteen rind extract solution from dark brown to yellowish after mixing for 1 minute and then to brown after mixing for more than 30 minutes. (Figure 5c). At the same time, the colour of silver nitrate solution (Figure 1a) changed from colourless to brown that indicate the formation of silver particles [48]. This indication is in accordance with the indications shown in the process of synthesizing silver nanoparticles with banana leaf bioreductors. The color change indicates that the reduction reaction of Ag^+ ions by the bioreductant of mangosteen rind extract has occurred [49]. As shown in Figure 3, silver nanoparticles are formed by the following reduction reaction:

$$Ag^+ + e^- \longrightarrow Ag$$

The color difference is related to the difference in the size distribution of the nanoparticles [50]. The appearance of brown color is caused by excitation of surface plasmon resonance (SPR) on silver nanoparticles [17] [51]. The more Ag^+ precursors were added, the clearer the color change of the solution (Fig. 1c – 1h). This fact indicates that the amount of silver formed is increasing [50]. However, we can not guarantee whether these particles are all

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nanometers in size. A mixture of bioreductant extract of mangosteen rind and Ag^+ precursor with a ratio of 1:1, 1;2; 1:3, 1:4, 1:5, and 1:6 yielded the mixture of silver nanoparticle-mangosteen rind extract with yield percentages of 9.40, 9.03, 7.70, 6.88, 2.53, and 1.56%, respectively. This fact is in accordance with the results of previous studies which showed the effect of the number of Ag^+ ions on particle size [31]. The reduced percentage of yield indicates that the number of silver nanoparticles formed is decreasing. The decrease in yield percentage is thought to be related to Ag particles that form aggregates with a larger size which has an effect on reducing the amount of Ag nanoparticles. This aggregation tendency can be observed in the morphological image shown through observations using a digital microscope in Figure 9. Thus, it cannot be concluded that the higher the number of Ag^+ precursors, the higher the Ag nanoparticles formed. This is related to the interaction between particles through the van der Waals interaction force to form particle aggregates with a larger size.

In the observation of the stability of silver nanoparticles, it can be seen that when the silver particles begin to form, there is a shift in the absorbance in the visible region. This fact shows that silver nanoparticle has a large enough surface tension energy, so it is less stable and not capable enough to resist the aggregation process to form larger particles. Large surface tension forces cause the formation of a greater van der Waals interactions making silver nanoparticle size to tend to be larger due to cluster formation [49]. The formation of this cluster causes the particle size to get bigger and form an aggregate of particles. These data indicate that the decrease in yield percentage is not directly proportional to the increase in the number of silver nanoparticles formed. This phenomenon is also shown by observing particle size using a particle size analyzer and can be explained based on surface plasmon resonance (SPR) analysis using UV-Vis spectrophotometer.

SPR Analysis

SPR analysis is an optical sensor analysis that utilizes surface plasmon waves to observe the interaction between the silver metal surface and the bioreductant material. Silver nanoparticles have a maximum absorption wavelength between 400-500 nm [52]. In this study, the synthesized silver nanoparticles had a maximum absorption that varied at a wavelength of 400-450 nm. This information indicates that silver nanoparticles have been formed in the extract from the reduction of Ag^+ ions to Ag by bioreductant compounds in the mangosteen rind extract (Figure 6). The absorbance peaks at consistent wavelengths in the time range of 0, 10, 20, and 30 minutes indicate that the mixture of silver nanoparticles formed has good stability. This stability is related to the presence of excess bioreductant extract and all other secondary metabolites [48], [50].

The shift of the maximum wavelength to a lower wavelength with the increasing number of Ag^+ precursors indicates that the interaction energy of Ag nanoparticles with bioreductant compounds increases. On the other hand, the decrease in absorbance value with increasing the number of Ag^+ precursors indicates that the number of Ag nanoparticles formed is decreasing due to particle aggregation [50] caused by the increasing surface tension energy of the particles [49].

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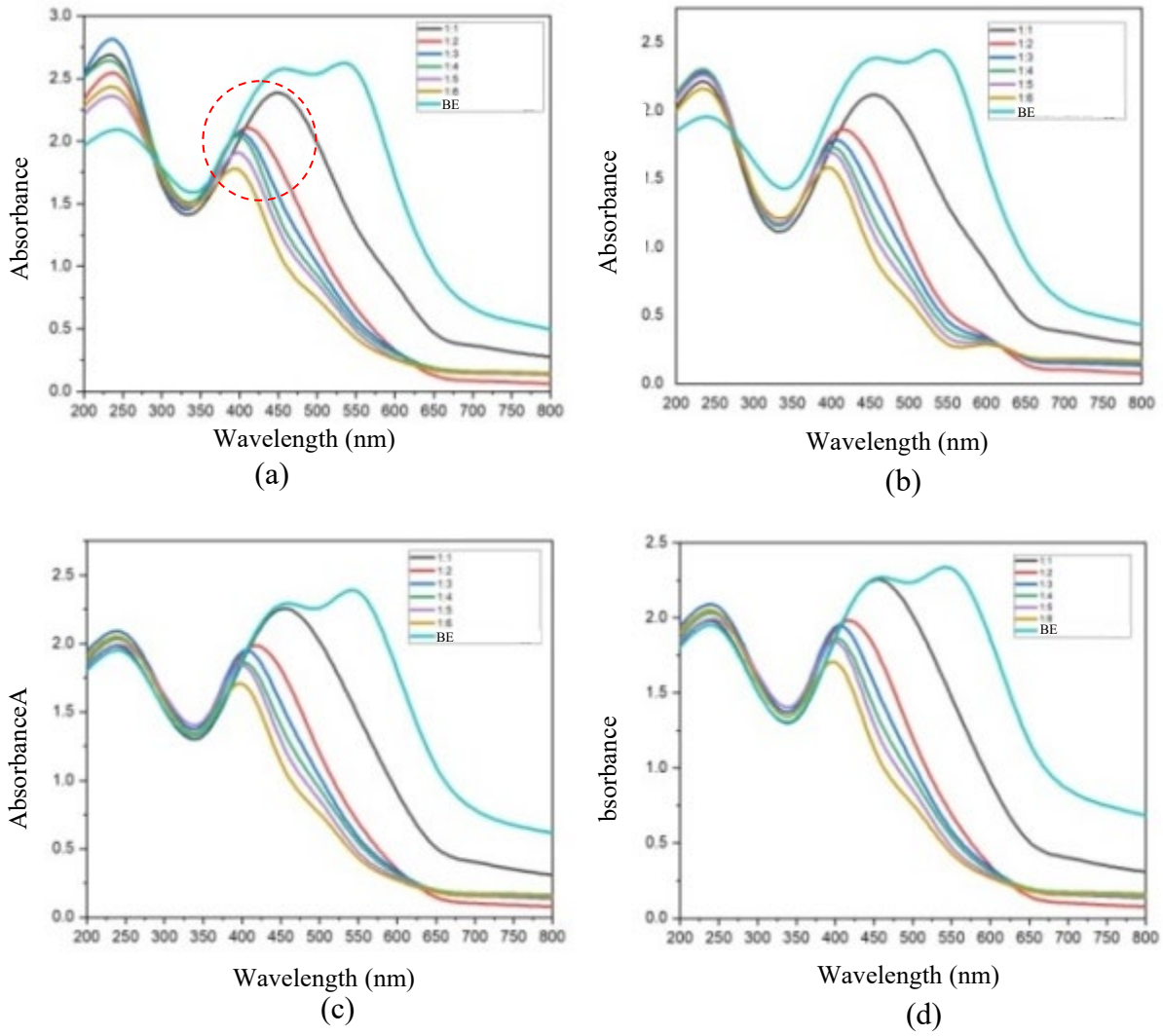

Figure 6. UV-Vis spectrum of silver nanoparticles-mangosteen rind extract at (a) 0 minutes, (b) 10 minutes, (c) 20 minutes and (d) 30 minutes. The spectral peaks in the red dotted circles indicate the maximum wavelengths of Ag nanoparticles. BE = Bioreductor extract

FTIR Analysis

Another indication of the reduction-oxidation reaction of Ag^+ with bioreductors can be observed from the change in the peak of the vibrational spectrum of the hydroxyl group (–OH) in the FTIR spectrum, because this group is oxidized to form other groups such as ketones [31], [46], [52]. The results of the analysis using FTIR showed that the compounds in the mangosteen rind extract contained the –OH functional group at a wave number of 3390.86 cm^{-1}, strain C=C at 1610.56 cm^{-1}, C–H bonds from alkanes at 1448, 54 cm^{-1}, C–O bonds at 1282.66 cm^{-1}, C–H bonds of alkenes at 817.82 cm^{-1}, and C–I strain at 626.87 cm^{-1} as reported in previous studies [6].

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Mangosteen rind extract showed absorption with a strong intensity at a wave number of 3390.86 cm^{-1} which indicated the presence of O–H bond vibrations originating from phenolic compounds. The reduction in the absorption peak of the silver nanoparticle spectrum at this wave number indicates an oxidation process of the –OH group to another group and the reduction of Ag^+ ions to colloidal Ag nanoparticles (Figure 7). However, the Ag^+ ions and the bioreductant compounds that reacted were probably not much so that the change in the spectral peaks was not very clearly visible. This phenomenon indicates that the amount of silver formed, both nano-sized and larger ones, may not be too much.

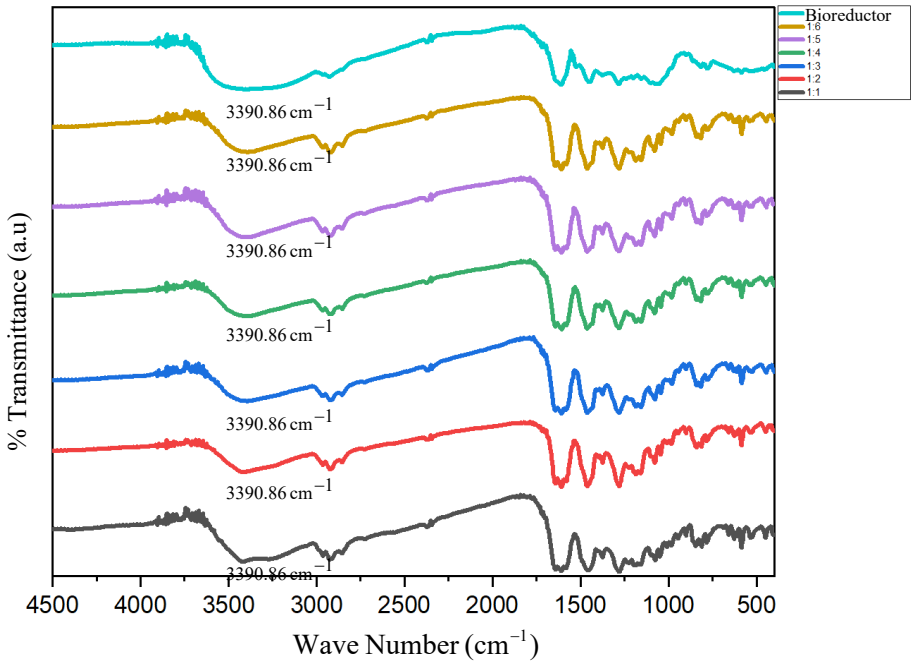

Figure 7. FTIR spectrum of mangosteen rind extract and a mixture of silver nanoparticles-mangosteen rind extract.

Particle Size Analysis

Analysis using a particle size analyzer (PSA) aims to determine the size and distribution of nanoparticles. The results of PSA analysis showed that the particle size of silver obtained from the bioreductor-precursor mixture with a volume ratio of 1:1 and 1:2 were 82.33 nm (Fig. 8a) and 356.2 nm (Fig. 8b), respectively. The larger silver particle size in the synthesis with more Ag^+ precursor is due to the aggregation of Ag particles formed [33]. This particle size is smaller than the size of silver nanoparticles synthesized with a bioreductant of thatch leaf extract with a particle diameter silver nanoparticles of 1160 nm [6], a mangosteen leaf methanol extract with a particle diameter of silver nanoparticles up to 562.49 nm [33], and Moringa oleifera leaves extract with a particle diameter of silver nanoparticles up to 4705 nm [22]. This difference in particle size is related to differences in the content of bioreductant compounds in different plant extracts such as tannins, flavonoids, enzymes, and alkaloids [52], because different compounds have different capping abilities.

The aggregation of nanoparticles affects the changes in their physical and chemical properties, because this aggregation reduces the surface area of the particles. It is possible that the decrease in the surface area of the particles will result in a decrease in the reaction rate and the antimicrobial activity of the particles.

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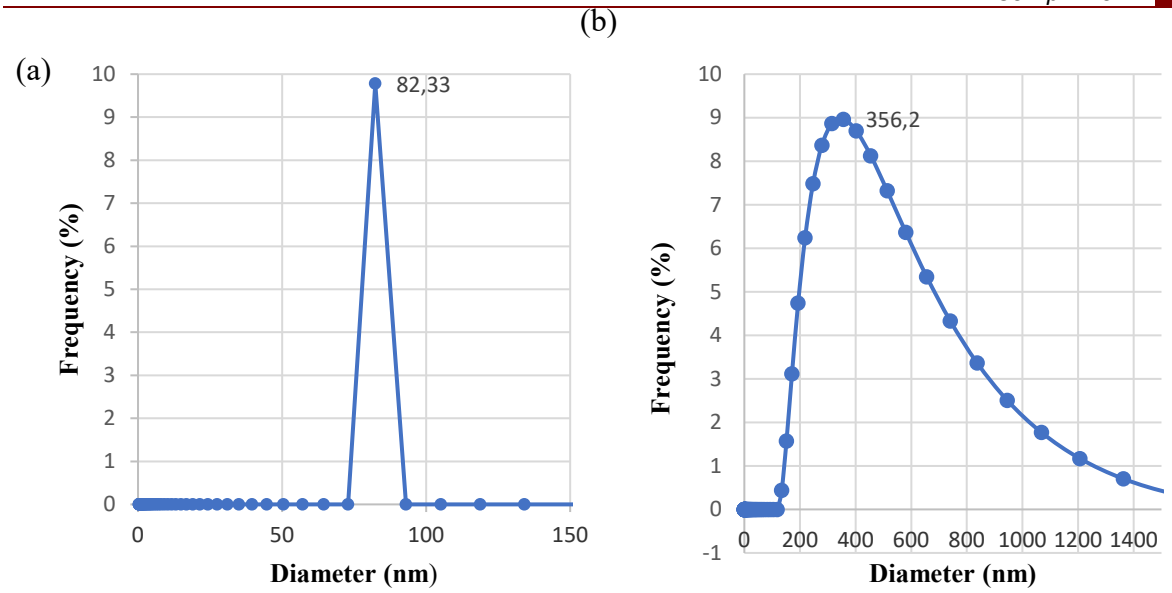

Figure 8. The distribution of silver particle size based on the frequency obtained by mixing the mangosteen rind extract bioreductant and Ag^+ precursor with a volume ratio of (a) 1:1 and (b) 1:2.

Morphological Analysis

Analysis using a digital microscope aims to identify the morphology of the synthesized silver nanoparticles. The results of the analysis showed that the silver nanoparticles had a non-uniform spherical shape and blended together (Figure 9). The more the number of Ag^+ precursors, the more non-uniform and the more random the shape of the particles due to the formation of the resulting silver aggregation. This fact is in agreement with the previously reported silver nanoparticles synthesized using a bay leaf (*Syzigium polyanthum*) bioreductant [53]. The tendency of nanoparticles to aggregate is caused by the effect of Brownian motion and van der Waals forces of the particles in solution. The results of this analysis strengthen the fact that the particle size and distribution of the PSA analysis results.

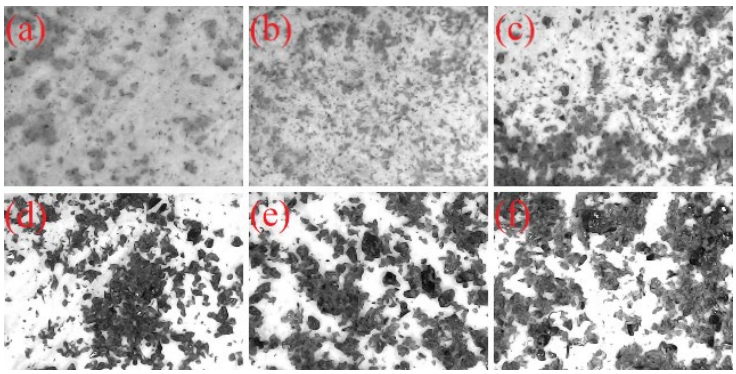

Figure 9. Results of digital microscope analysis with 1000 times magnification for silver nanoparticles obtained by mixing mangosteen rind extract with Ag^+ precursor with volume ratio: (a) 1:1, (b) 1:2, (c) 1:3, (d) 1:4, (e) 1:5, and (f) 1:6.

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Antimicrobial Activity Test

In this study, the antimicrobial activity of the active ingredient of silver nanoparticles was tested by the well method based on the diameter of the clear zone around each formula of the antimicrobial active ingredient and the comparion added to the bacterial growth medium. The greater the antimicrobial activity of an active ingredient, the larger the diameter of the resulting clear zone.

Variations in the diameter of the clear zone on the bacterial growth medium showed that silver nanoparticles had the ability to inhibit bacterial growth (Figure 10 and Table 1). Silver nanoparticles obtained from the Ag^+ reduction process in a bioreductor-precursor mixture with a volume ratio of 1:1 were able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria with the highest clear zone diameters of 12.67 mm and 12.00 mm, respectively. The antibacterial activities of the Ag-NPs against the Gram-positive S. aureus and Gramnegative E. coli were almost identical as shown by previous study [53], but contraditive to the another study [12]. The difference between the results of this study and the results of previous studies could be made possible by differences in the composition of the mixture of Ag^+ precursors and their bioreductant extracts, differences in the bioreductant compounds, and differences in working conditions in testing antibacterial activity which need to be explored in further research. The diameter of the clear zone decreases with the increase in Ag^+ precursors due to reduced silver nanoparticles produced due to silver aggregation to form silver deposits with a larger size. Silver nanoparticles are more effective in inhibiting bacterial growth compared to silver particles with a larger size [54].

In this study, it was also shown that the mangosteen fruit extract itself was also able to inactivate the growth of both types of bacteria. However, the inactivation ability is still lower than the mixture of silver nanoparticles and the extract (Figure 10). The inactivation ability of mangosteen fruit extract against *Escherichia coli* bacteria was greater than its inactivation against *Staphylococcus aureus* bacteria with zone diameters of 13.33 mm and 13.0 mm, respectively (Table 1). This is due to the presence of polyphenol compounds in the mangosteen fruit extract which also act as antimicrobial active compounds such as tannins and xanthones [27] [43], [55], [56].

Based on the observation of the diameter of the clear zone, it turns out that the antimicrobial activity of a mixture of bioreductors and Ag^+ precursors with a composition of 1:1 is still slightly lower than the antimicrobial activity of mangosteen fruit extract. This fact indicates that the concentration of silver nanoparticles in the mixture is still too low. Therefore, it is necessary to conduct further studies to obtain a more accurate concentration of Ag^+ precursors in the biosynthesis of silver nanoparticles. On the other hand, when compared to mangosteen fruit extract, the mixture of mangosteen fruit extract and silver nanoparticles with a composition other than 1:1 showed a lower clear zone diameter. This fact is possible because the silver particles present in the mixture with this composition are larger than 100 nm as aggregate particles.

The manufacture of the disinfectant formulas in this study used triclosan as a preservative. Triclosan (TCS) is a multi-purpose antimicrobial agent used as a common ingredient in everyday household personal care and consumer products such as toothpastes, mouthwash, soaps, under arm deodorants, and liquid dishwashing soap [57]. Therefore, the antibacterial action of this disinfectant formula was also affected by the presence of triclosan. However, in this study, the effect on inactivation of the observed bacterial growth has not been studied separately.

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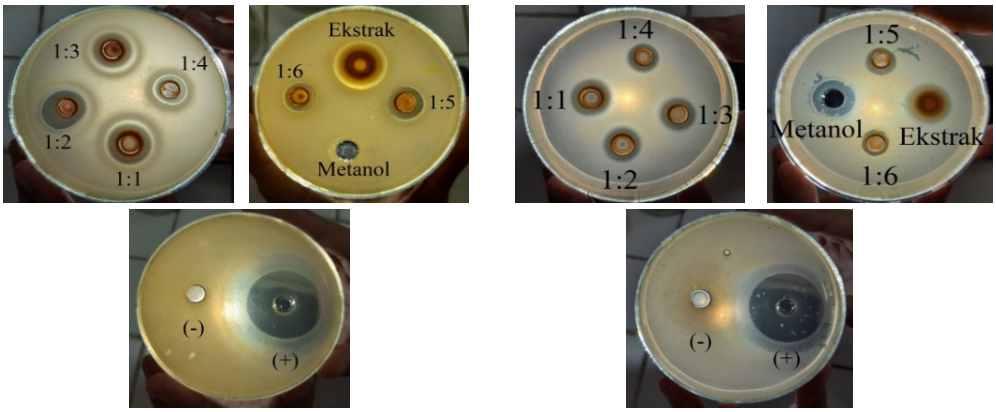
Based in this study, it can be expressed that the results of this study have not shown a significant role of silver nanoparticles themselves in inactivating the observed bacterial growth. This role will be observed through the antibacterial activity test of silver nanoparticles that have been separated from the mixture with mangosteen fruit extract bioreductant. However, the potential trend of silver nanoparticles as an antibacterial material has begun to be observed.



(a) (b)

Figure 10. Inhibitory activity of mangosteen rind extract, methanol, and silver nanoparticles in the bioreductant mixture of mangosteen rind extract-$AgNO_3$ precursor with a volume ratio of 1:1 – 1:6 against pathogenic bacteria (a) *Staphylococcus aureus* and (b) *Escherichia coli*. Label: (-) = distilled water as negative control and (+) = streptomycin as positive control.

Table 1. Diameter of the Clear Zone of Antimicrobial Inhibition

Antimicrobial Ingredients	Clear Zone Diameter (mm)	
	Staphylococcus aureus	***Escherichia coli***
Ag nanoparticles in 1:1	12.67	12.00
Ag nanoparticles in 1:2	10.33	10.00
Ag nanoparticles in 1:3	9.67	9.33
Ag nanoparticles in 1:4	7.67	8.00
Ag nanoparticles in 1:5	7.00	6.67
Ag nanoparticles in 1:6	7.33	5.67
Streptomycin	26.33	27
Distilled water	-	-
Mangosteen rind extract	13.00	13.33
Methanol	-	-

Disinfectant Formulation

Disinfectants with formulas F1, F2, F3, and F4 which were made in this study contain antibacterial active ingredients derived from a mixture of mangosteen fruit extract and Ag^+ precursors of 1:1 rasio by volume with the increasing volume from F1 to F4. The antimicrobial activity test showed that the four disinfectant formulations had excellent antibacterial activity in inhibiting bacterial growth (Figure 11). Disinfectants F3 and F4 had the same activity in inhibiting the growth of *Staphylococcus aureus* bacteria with a clear zone of inhibition diameter

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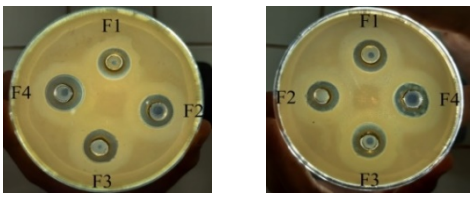
of 22.67 mm (Table 2). This table also shows that the greater the number of active ingredients, the larger the diameter of the clear zone of inhibition of *Escherichia coli* growth. In addition, Table 2 shows that *Staphylococcus aureus* bacteria haves a little higher resistance to disinfectants than *Escherichia coli* bacteria.



(a) (b)

Figure 11. Antimicrobial Test Results of Disinfectant Formula against Bacteria (a) *Staphylococcus aureus* and (b) *Escherichia coli*

Table 2. Diameter of the Clear Zone of Antimicrobial Inhibition in Disinfectants

Disinfectant Formula	Clear Zone Diameter (mm)	
	Staphylococcus aureus	***Escherichia coli***
F1	20.00	18.33
F2	20.67	19.67
F3	22.67	21.00
F4	22.67	22.00

Clarity test was determined to evaluate the presence of particulate mattervisually [58]. In this research, the clarity and organoleptic test of the disinfectant formula was also carried out. The test parameters in the test organoleptic are color and odor [59]. Visually, it can be seen in Figure 12 that these four disinfectant formulas do not contain particulate deposits. The level of clarity is evaluated based on the percent transmittance using UV-Vis spectrophtometer. The four disinfectant formulas have different transmittance values (Table 3). In this study, the disinfectant F4 looked the clearest compared to the other three formulas. As shown in Figure 12, the clearer the appearance of the disinfectant, the higher the percent transmitance.

Figure 12. Four Disinfectant products with different volumes of active ingredients. F1, F2, F3, and F4 respectively contain active ingredients from a mixture of silver nanoparticles with mangosteen fruit extract of 10, 15, 20, and 25 mL.

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The transmittance value that is close to 100% indicates that the droplet size of the dispersion produced by the nanoparticles has reached the nanometer size, which can be seen visually from the clarity of the system formed [60]. Based on this, it can be expressed that the four disinfectant formulas have good clarity. However, there is no specific standard that becomes a reference to express a good level of clarity of a disinfectant formula. Most importantly, this formula does not contain harsh particulates that interfere with the comfort and health of the skin.

Organoleptic testing of the disinfectant formula by 25 panelists on the color and smell showed that all the disinfectant formulas had a yellowish brown color with a distinctive odor of mangosteen peel extract-ethanol (Table 3). This level of clarity, color and odor is the hallmark of the disinfectants with these formulas. With these parameters, we believe that this disinfectant formula is not harmful to the environment and human health because these formulas does not contain volatile and harmful chemicals

Table 3. Test Results of % Transmittance and Organoleptic Disinfectant Formula

Formula	Transmittance (%)	Clarity	Color	Odor
F1	60.4%	Clear	yellowish brown	typical mangosteen rind extract-ethanol
F2	71.5%	Clear	yellowish brown	typical mangosteen rind extract-ethanol
F3	75.7%	Clear	yellowish brown	typical mangosteen rind extract-ethanol
F4	91.2%	Clear	yellowish brown	typical mangosteen rind extract-ethanol

CONCLUSION

Silver nanoparticles can be synthesized using a green chemistry approach by mixing a bioreductant of mangosteen rind extract with 0.01 M $AgNO_3$ with volume ratio variations of 1:1. Silver nanoparticles obtained have a fairly good stability. Mixing bioreductors with precursors in a ratio of 1:1 resulted in silver nanoparticles with the smallest diameter of 82.33 nm and non-uniform spherical shape. These silver nanoparticles have the best activity in deactivating the growth of gram positive *Staphylococcus aureus* and gram negative *Escherichia coli* bacteria and can be used as an active ingredient in environmentally friendly liquid disinfectants. However, the antibacterial performance of this disinfectant was not only due to the presence of silver nanoparticles, but also to the presence of triclosan and mangosteen fruit extract. Therefore, it is necessary to conduct further research on the biosynthesis of silver nanoparticles by adjusting the necessary conditions such as pH, mixing time, and others.

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