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Green Synthesis of Copper Nanoparticles Using Red Dragon Fruit (*Hylocereus polyrhizus*) Extract and Its Antibacterial Activity for Liquid Disinfectant

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Abstract

The effort of copper nanoparticle exploration for antibacterial cleaning agents needs to prevent infection or contamination of microorganisms such as bacteria and viruses due to its potential characteristic as an environmentally benign material. The synthesis of copper nanoparticles using 0.02 M $Cu(NO_3)_2$ as a precursor and red dragon fruit extract as a bioreduction agent has been conducted. The extraction process of red dragon fruit was done by the maceration method using methanol as solvent. Copper nanoparticles were prepared through green chemistry with the reduction method. The compound in red dragon fruit extract reduced Cu^{2+} to Cu. The experimental nanoparticle synthesis method was performed with a combination of red dragon fruit extract and $Cu(NO_3)_2$ solution mixed with a volume ratio of bioreductor extract toward Cu2+ precursor variation of 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, and 1:9. The mixtures were stirred using a magnetic stirrer for 30 minutes and then incubated. The samples were characterized using UV-Vis (Ultraviolet-Visible), FTIR (Fourier Transform Infrared), and a digital microscope to observe morphology. The observation continued to assess its antibacterial activity and potency as an active disinfectant. UV-Vis analysis showed that the absorbance value for 30 minutes tended to be stable. Particle size estimation showed the smallest size of 122.12 nm at a volume ratio of 1:9. Functional group analysis using FTIR showed a shift in wavenumber between dragon fruit extract and copper nanoparticle (CuNPs), indicating a functional group interaction. The results of morphological analysis using a digital microscope showed that the particles with fine powder granules were produced with the composition of fruit extract: $Cu(NO_3)_2$ of 1:9. Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* indicated that the composition of Cu-nanoparticles of 1:9 had given an inhibition value.

1. Introduction

At the end of 2019, the world was shocked by the incidence of severe infections until the WHO named it the novel coronavirus (nCoV-19). Since WHO upgraded the global Covid-19 status to azz pandemic, the Indonesian government has made efforts to improve the prevention of the virus attack by issuing Covid-19 Handling Protocols for various sectors. One of the recommendations is disinfection to prevent microorganisms from growing around the environment by providing guidelines for disinfection in public places to prevent transmission in public areas and the surrounding environment [1]. On the other hand, an attempt to explore cleanliness must also be established to prevent viruses or pathogenic bacteria from spreading. Human activities generally affect the environment and interact with an environment full of microorganisms, parasites, and viruses [2].

Microorganisms in the air are a very significant element of pollution. The various colonies of microorganisms in the air depend on their activity in the room, dust, and other impurities that can cause several diseases [3]. By its condition, disinfection diminishes the manifold of the pathogen to a lower hazard level on surfaces needed for microorganisms contamination employing disinfectants [4].

Disinfectants are chemicals or physical effects that are accustomed to prevent infection or contamination of microorganisms such as bacteria and viruses and kill or reduce the number of microorganisms or other germs. It is additionally used to disinfect hands, floors, rooms, equipment, and clothing. Disinfectants are occasionally employed as a technique for sterilization or in the removal of germs. There are two approaches for disinfection: physical and chemical procedures. Many chemicals can function as disinfectants, but are generally grouped into materials containing aldehyde or reducing compounds (-COH), alcohol compounds (-OH), halide compounds (-X), phenolic and halogenated phenolic compounds, quaternary ammonium salts, compounds oxidizers, and biguanide compounds [5]. Chemical disinfection processes have the benefits of fast action and relatively inexpensive costs. However, numerous studies have shown evidence that could be well thought of as a health threat to humans [6].

Recently, several disinfectants have been obtained with the application of nanotechnology. It is understood that the preparation of nanoparticles (NPs) has been established by physical and chemical methods [7], and it also has been broadly categorized as synthetic inorganic and organic nanoparticles such as Au, Ag, Cu, and Al [8]. Although these metal nanoparticles have numerous uses [9], they also have harmful environmental impacts. It is stated that copper compounds have high antibacterial properties, are generally harmless to humans, and have low environmental toxicity [10]. Therefore, plant extracts are a kind of alternative as a bioreduction agent to replace chemical reducing agents because plant extracts contain metabolites [11]. Additionally, producing nanoparticles via a plant-based biological synthesis process is a cost-effective, environmentally friendly method with many potential applications [12].

Metal nanoparticles are one of the most unique and intriguing materials for disinfectant application due to their high contact surface area, stable properties, and broad potential in diverse aspects of catalysts, detectors, optical sensors, and antibacterial agents that clean the air and manage clothing [13]. Several articles have mentioned metal nanoparticles synthesized by plant extract as a reductor. It can potentially replace chemical-reducing agents because the plant extracts contain metabolites and do not always have side products that are harmful to the environment [14, 15, 16, 17].

One of the crop extracts that has a potency for producing copper nanoparticles is dragon fruit flesh extract. It is known that the red dragon fruit contains anthocyanins, which range in concentration from 19.72 mg per 100 g [15, 18], as well as pigments and biochromes, which absorb light in the visible spectrum [19]. In the meantime, anthocyanin molecules have antioxidant and free radical scavenger properties [20] and are also easily oxidized [21]. It is noted that phenol or polyphenol derivatives stabilize free radicals by increasing the electron deficiency of free radicals. It inhibits the chain reaction of free radical formation [22].

It has been published in several previous articles and proven that the copper nanoparticle is produced by using plant extract with numerous most common precursor copper salts, such as cupric acetate [23], copper chloride [24], and copper sulfate [25]. All of these precursors are excellent used for various plant extracts with various applications that have been reported. It was thought that the extract's constituents have a role in the stability of copper nanoparticles in addition to their reducing and capping abilities [26]. Dragon fruit peel extract can be employed as a bioreductor since the use of dragon fruit flesh extract as a reductor in synthesizing copper nanoparticles has not been previously reported.

Herein, the challenge of its natural materials as bioreduction agents can contribute to obtaining the copper nanoparticle for disinfectant ingredients. Therefore, this research was conducted to determine the characteristics of copper (Cu) nanoparticle-red dragon fruit extracts, such as stabilization properties, grain size, chemical functional groups, and morphology. In addition, the antibacterial activity as an active ingredient in disinfectant formulas was assessed.

2. Methodology

2.1. Equipment and Materials

The equipment used was ultraviolet-visible (UV-Vis) spectrophotometry (Spectroquant Pharo 300 M), Particle Size Analyzer (Horiba SZ 100), Fourier Transform Infrared spectrometer (Shimadzu), and digital microscope (Leica Microsystems), oven (Gallenkamp England), autoclave, analytical balance (Mettler Toledo Al204), magnetic stirrer (C-Mag Hs 7), and Laminar Air Flow (Robust). The materials were 2 kg of red dragon fruit, 0.02 M $Cu(NO_3)_2$ (Emsure Merck), methanol (Emsure Merck), ethanol (Emsure Merck), triclosan (Emsure Merck), sodium metasilicate (Emsure Merck), Whatman No. 42 filter paper, nutrient broth, Gram-negative *Escherichia coli*, Gram-positive *Staphylococcus aureus*, distilled water (Water One), streptomycin, tissue paper (Nice), label paper (Fox), plastic wrap (Cling Wrap) and aluminum foil (Klin Pak). All materials purchased were analytical-grade materials without further purification.

2.2. Extraction of Red Dragon Fruit

The bioreducing agent compounds from red flesh dragon fruit were extracted using the extraction method from previous studies [27]. Sample preparation was started by washing 2 kg red dragon fruit using running water and drying it at ambient temperature. Red flesh dragon fruit was separated from the peel, thinly sliced, dried at room temperature, and then dried in an oven at 40℃ for 24 hours. A total of 180 g of flesh red dragon fruit was macerated with 1 L of methanol solvent for three days while stirring two times a day. This mixture was then filtered using a 0.22 μm membrane filter, and the filtrate was evaporated for three hours using a rotary evaporator at 50℃.

2.3. Synthesis and Characterization of Nanoparticles

Synthesis of copper nanoparticles from red flesh dragon fruit via sustainable chemistry was adapted from previous research methods [28]. In this experiment, red flesh dragon fruit extract acted as a bioreduction agent and 0.02 M $Cu(NO_3)_2$ solution as a precursor. Cu^{2+} precursor was reduced with red flesh dragon fruit extract in a 100 mL beaker with a volume ratios of red flesh dragon fruit extract: $Cu(NO_3)_2$ solution of 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, and 1:9. These compositions were assigned as Dragon Fruit-Cu (DF-Cu) 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9 respectively. This mixture was stirred at ambient temperature for 30 minutes. Then each miscellany 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℃. Each DF-Cu were characterized using a UV-Vis spectrophotometer, FTIR, and digital microscope.

Particle size estimation of CuNP was performed by inserting the wavelength and absorbance values of the nanoparticle solution using Equation 1 [29]. The composition ratio of 1:10 was not further prepared because preliminary experimental tends to form an aggregation.

$$d = \frac{\ln\left(\frac{\lambda_{SPR} - \lambda_0}{L_1}\right)}{L_2} \qquad (1)$$

Where, d is particle diameter (nm), λ_{SPR} is maximum absorption wavelength, λ_0 is minimum absorption wavelength that occurs at the front of the SPR, L_1 (6.53) and L_2 (0.0216) are constant values attained from the transmission electron microscope (TEM) vs. UV-Vis fit data for the metal nanoparticle by using fit parameters determined from the value theory for d > 25 nm according to Haiss *et al.* [29].

2.4. Antibacterial Activity Test

The antibacterial activity test was performed by applying the agar well diffusion method from the previous study [7]. The antibacterial activity test began with sterilizing tools and materials using an autoclave at 121℃ for 15 minutes. The antibacterial activity of copper nanoparticles-red dragon fruit extract was evaluated against pure cultures of *Staphylococcus aureus* and *Escherichia coli*, diluted using 900 μL of sterile 0.9% NaCl until the turbidity was equivalent to 0.5 McFarland standard solution. About 100 μL of each bacterial suspension was mixed with 15 mL of nutrient agar liquid in a test tube and homogenized. Liquid media for nutrients was poured into a Petri dish as the bottom layer. After the bottom layer had solidified, the semi-solid medium was poured into a petri dish as the second layer and allowed to solidify. After completely solidifying, the plate was perforated using a cork borer into several wells. Each well was filled with 15 μL of each composition of copper nanoparticles-red dragon fruit extract, red dragon fruit flesh extract, methanol, distilled water as a negative control, and streptomycin as a positive control. Plates were incubated at 37℃ for 24 hours. Then, the antibacterial activity was determined by measuring the diameter of the inhibition zone.

2.5. Disinfectant Formulation

The disinfectant liquid was prepared using the mechanical dispersion method by dispersing all ingredients of copper nanoparticles-red dragon fruit extract, sodium metasilicate, triclosan, and ethanol into the solvent according to their respective roles. The mixture was further stirred until a homogeneous mass of disinfectant was obtained. The disinfectant formulation was adapted from previous research methods [30]. It could be considered there are four formulas of environmentally friendly liquid disinfectant. The disinfectant liquid formula and its composition are offered in Table 1 and assigned as F1, F2, F3, and F4. These formulae were then characterized for their transmittance percentage using a UV-Vis spectrophotometer to evaluate the clarity of each formula and organoleptically tested, including color and odor parameters, to decide the panelists' preference for the prepared disinfectant.

Table 1. Disinfectant formulation of copper nanoparticles-red dragon fruit extract

Materials	Variation of materials				Function of materials
	F1 (ml)	F2 (ml)	F3 (ml)	F4 (ml)	
Copper nanoparticles-red dragon fruit extract	10	15	20	25	active ingredient
Sodium metasilicate	2	2	2	2	builder
Triclosan	2	2	2	2	preservative
Ethanol	86	81	76	71	solvent

3. Results and Discussion

3.1. Extraction of Red Dragon Fruit

Extraction of red dragon fruit by maceration method was done using 180 g of red dragon fruit with 1000 mL of methanol solvent. Methanol solvent was chosen because it is effective for extracting and has the ability to attract polar compounds and some nonpolar compounds [31]. Methanol, as the extraction solvent, is certainly evaporated. Methanol solvent was employed since red dragon fruit contains anthocyanin color pigments in the class of flavonoid derivatives in order to obtain the best extract in this study. It is assumed that the highest possible concentrations of phenolics, flavonoids, alkaloids, and terpenoids may be achieved using methanol due to the solubility of these substances [32]. In this study, a red-brown dragon fruit extract solution was obtained with a yield of 9.27%, as shown in Figure 1.

Figure 1. Extract of red dragon fruit

3.2. Synthesis of Copper Nanoparticles-Red Dragon Fruit Extract

Synthesis of copper nanoparticles can be performed using several methods, such as physical, chemical, and green synthesis [33]. Herein, we applied a green chemistry approach in synthesizing copper nanoparticles using a red dragon fruit extract as a bioreductor. It is widely known that the total phenolic content, total flavonoid substance, and antioxidant activities contained in red dragon fruit extract play a big part in the preparation of nanoparticles due to their excellent oxidant activity [34].

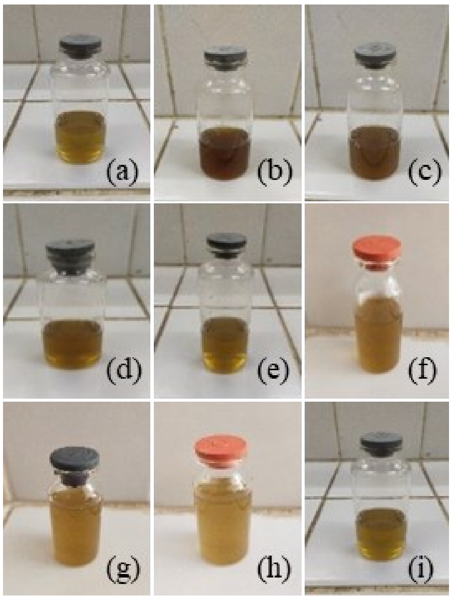

Figure 2. The products of the oxidation-reduction reaction of red dragon fruit extract bioreduction agent with $Cu(NO_3)_2$ precursor (a) DF-Cu 1:1, (b) DF-Cu 1:2, (c) DF-Cu 1:3, (d) DF-Cu 1:4, (e) DF-Cu 1:5, (f) DF-Cu 1:6, (g) DF-Cu 1:7, (h) DF-Cu 1:8, and (i) DF-Cu 1:9

Mixing $Cu(NO_3)_2$ solution with red dragon fruit extract produced a color change. This is in line with the previous study [35] that the color of the mixed solution changed from brown to yellowish brown. The color will gradually change to yellow-green, and a large amount of floating precipitate will develop. The color change occurs by the ions deposition, indicating the successful reduction of Cu particles to CuNP [28]. The color change suggests a reduction process in the mixture solution, which is attributed to anthocyanin found in dragon fruit extract. This compound in dragon fruit extract may be responsible for the reduction of Cu^{2+} to Cu^0. The reduction of Cu^{2+} to Cu^0 is caused by electron donor compounds called reducing agents. The color change of red dragon fruit extract and Cu^{2+} precursor mixture with a ratio of 1:1, 1;2; 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, and 1:9 are shown in Figure 2, and the illustration of copper nanoparticles-red dragon fruit extract process is shown in Figure 3.

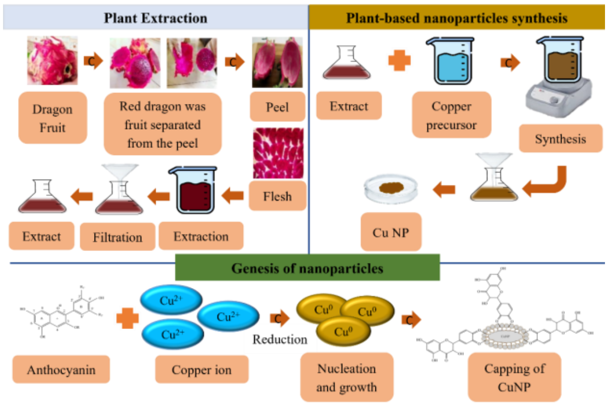

Figure 3. The illustration of CuNP synthesis and genesis process [36]

The synthesized products were separated into the precipitate and filtrate, which were subsequently analyzed using a variety of test parameters, including surface plasmons, peak shift characteristics in the UV-Vis spectra, particle grain size analysis, functional groups, surface morphology, and antibacterial activity.

3.3. SPR (Surface Plasmon Resonance) and Stability Analysis

UV-Vis absorbance spectrum of a transparent colloid solution using a UV-Vis spectrophotometer with a wave range of 200-800 nm. From the UV-Vis spectrum, there is an interaction of organic compounds, namely anthocyanins from red dragon fruit extract, with Cu. The stableness of the complex compound copper nanoparticles-red dragon fruit extract is quite good, as illustrated in Figure 4, using time intervals of 0, 10, 20, and 30 minutes. The interaction with UV-Vis light for 30 minutes did not change significantly. The maximum wavelength of copper nanoparticles-red dragon fruit extract is shown at 400-450 nm [13]. It is assumed that the stability of copper nanoparticles occurs after the reaction. Meanwhile, the peaks are shifted, and new peaks are formed at a wavelength of 400–410 nm, referring to the formation of CuNPs.

The characterization of copper nanoparticles-red dragon fruit extract began with a visual color change based on surface plasmon resonance (SPR). The combined oscillation of the free conduction band electrons in the metal complex, excited by incident UV radiation, is the reason for the absorption of surface plasmons [37]. The formation of the CuNPs absorption peak is caused by the presence of plasmon bands on the surface of colloidal Cu, which suggested that the reduction of Cu^{2+} to Cu^0 in the utilized extract was successful.

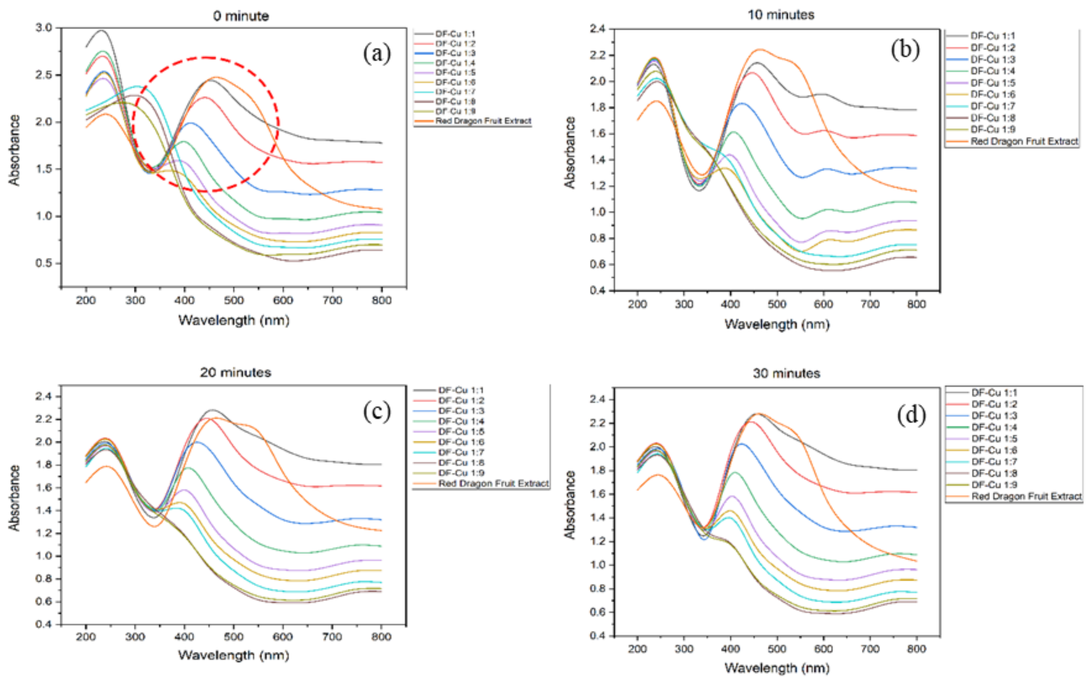

Figure 4. UV-Vis spectrum profile of the synthesized DF-Cu and red dragon fruit extract at (a) 0, (b) 10, (c) 20, and (d) 30 minutes intervals

Metal nanoparticles of each metal gave specific peaks at different absorptions seen using UV-Visible spectrophotometry [37]. As the $Cu(NO_3)_2$ composition increases, the peak shifts towards a lower maximum wavelength. This could mean that the interaction energy between the organic compounds in the extract and Cu increases. This fact agrees with the previously reported copper nanoparticles using *Ageratum houstonianum* Mill leaf extract [24] with a maximum wavelength of 326 nm obtained at a 1:4 ratio, where the reduction of Cu^{2+} ions to Cu^0 was found after 24 hours of incubation.

The SPR phenomenon indicates the formation of nanoparticles and can be used to consider the size of nanoparticles using Equation 1. The UV-Vis spectrum example used to estimate the particle size of all compositions is shown in Figure 5. The estimated particle size range of copper nanoparticles-red dragon fruit extract based on the λ_{max} is shown in Table 2.

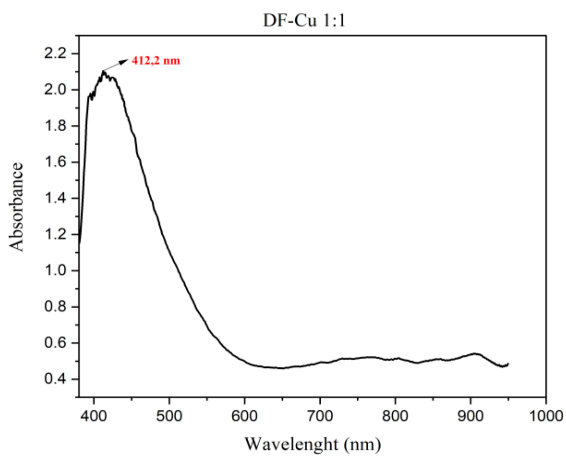

Figure 5. The illustrated example of particle size estimation for copper nanoparticles-red dragon fruit extract at a 1:1 volume ratio

Table 2. Particle size estimation of DF-Cu

Copper nanoparticle	λ_{SPR}	λ_0	L_1	L_2	d (nm)
DF-Cu 1:1	412.2	300	6.53	0.0216	131.66
DF-Cu 1:2	412.2	300	6.53	0.0216	131.66
DF-Cu 1:3	395.2	300	6.53	0.0216	124.05
DF-Cu 1:4	392.1	300	6.53	0.0216	122.52
DF-Cu 1:5	392.1	300	6.53	0.0216	122.52
DF-Cu 1:6	391.3	300	6.53	0.0216	122.12
DF-Cu 1:7	392.1	300	6.53	0.0216	122.52
DF-Cu 1:8	392.1	300	6.53	0.0216	122.52
DF-Cu 1:9	391.3	300	6.53	0.0216	122.12

As shown in Table 2, it can be seen that the synthesized DF-Cus have particle sizes in the range of 122.12 to 131.66 nm. This demonstrates that nanometer-sized nanoparticles were successfully synthesized utilizing red dragon fruit flesh in this study.

3.4. FTIR (Fourier Transform Infrared) Analysis

The results of FTIR analysis (Figure 6) show that the functional group contained in the red dragon fruit extract is the -OH group at 3381.21 cm^{-1}, C-H bonds of alkanes at 2935.66 cm^{-1}, C=O bonds of alkanes at 1722.43cm^{-1}, C-O bonds of alkanes at 1242.16 cm^{-1}, and C-H bonds of alkenes at 775.38 cm^{-1}. The wavenumbers generated from the FTIR analysis match the frequency data [38].

The shortening in the absorption peak at 3381.21 cm^{-1} indicates a reduction process of Cu^{2+} ions into colloidal Cu^0 nanoparticles [13]. The shift in wavenumber between dragon fruit extract and CuNPs suggests an interaction of functional groups between red dragon fruit extract and CuNPs, where the O-H and C=O groups that may be contained in tannin compounds have specific absorption peaks in the order of 3500–3200 cm^{-1} and 1725–1705 cm^{-1}. Aromatic compounds have absorption peaks between 1404 to 1515 cm^{-1} and 1058 cm^{-1} of C-O. This compound plays the best role in reducing metal ions and results in the establishment of CuNPs.

The compounded nanoparticles encircled the metabolites such as terpenoids with alcohol, ketone, aldehyde, and carboxylic acid functional groups. The FTIR analysis results verified that the phenolic moiety had a more excellent proficiency in tying up metals, indicating that the phenol might be derived from metal nanoparticles (i.e., capping Cu nanoparticles) to avert agglomeration. This suggests that molecular biology may allow performing the dual function of forming and stabilizing Cu nanoparticles in an aqueous medium [39].

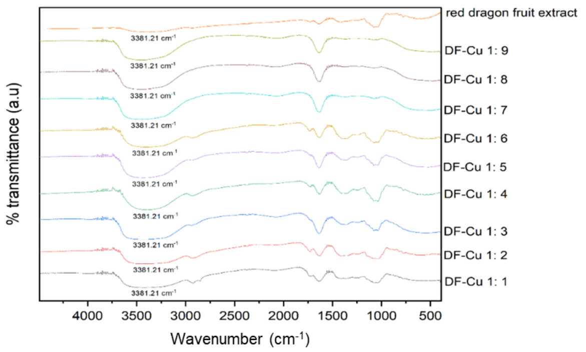

Figure 6. FTIR spectrum of copper nanoparticles-red dragon fruit extract

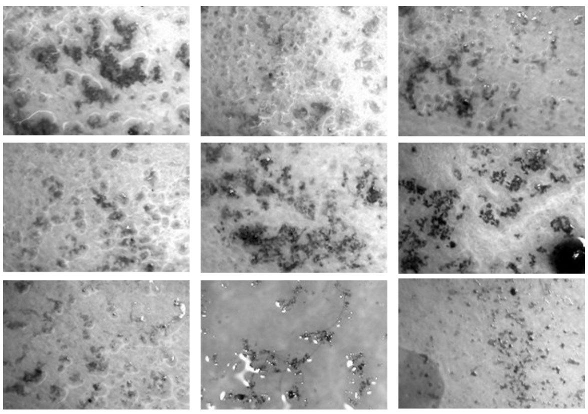

Figure 7. Optical microscope images of copper nanoparticles-red dragon fruit extract with volume ratios of (a) DF-Cu 1:1, (b) DF-Cu 1:2, (c) DF-Cu 1:3, (d) DF-Cu 1:4, (e) DF-Cu 1:5, (f) DF-Cu 1:6, (g) DF-Cu 1:7, (h) DF-Cu 1:8, (i) DF-Cu 1:9

3.5. Morphological Analysis

The analysis showed that the copper nanoparticles exhibit good uniformity, according to the research [40]. The precipitate size of each composition is disclosed by the results of the morphological analysis of nanoparticles using a digital microscope. Figure 7 reveals that the composition of 1:9 exhibits the smallest size at a magnification of 1000. It could occur from the influence of the precursor volume, where the ratio of 1:9 has the most volume of the precursor. The shape of the particles becomes more uniform and random as the concentration of Cu^{2+} precursors increases, showing small copper aggregation.

3.6. Antibacterial Activity

The antibacterial activity of the active ingredient of copper nanoparticles was tested using the agar well diffusion method by measuring the clear zone formed around the wells. The greater the antibacterial activity of an active ingredient, the larger the diameter of the clear zone produced.

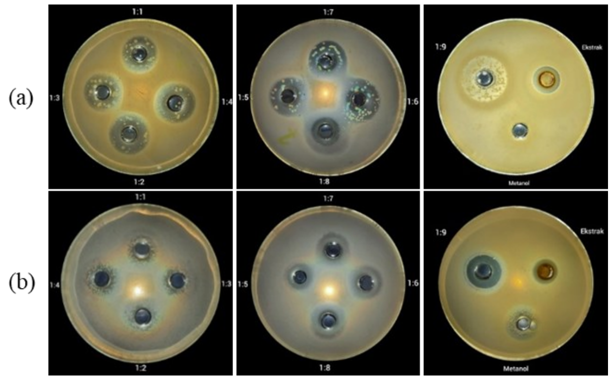

Figure 8. Antibacterial activity test results of (a) *Staphylococcus aureus* (b) *Escherichia coli*

Antibacterial activity test was conducted against *Staphylococcus aureus* and *Escherichia coli* [41]. Antibacterial activity test results against *Staphylococcus aureus* and *Escherichia coli* showed that all composition variations had excellent bacterial growth inhibition appropriate to the inhibitory diameter measurement shown in Table 3. All volume ratios revealed better inhibition against *Staphylococcus aureus* than *Escherichia coli*. This indicates that the copper (Cu) nanoparticles of dragon fruit extract have excellent antibacterial activity for both bacteria, as exhibited in Figure 8. The results revealed that the volume ratio from 1:1 to 1:9 can inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* with the highest inhibition zone diameters of 20.66 mm and 15.66 mm, respectively, obtained with a volume ratio of 1:9.

Table 3. Diameter of inhibition zone of copper nanoparticles-red dragon fruit extract at different volume ratios between 1:1 to 1:9 against *Staphylococcus aureus* and *Escherichia coli*

Copper nanoparticle	Diameter of inhibition zone (mm)	
	Staphylococcus aureus	*Escherichia coli*
DF-Cu 1:1	14.33	12.00
DF-Cu 1:2	16.00	12.33
DF-Cu 1:3	17.00	13.00
DF-Cu 1:4	17.66	13.66
DF-Cu 1:5	18.00	14.00
DF-Cu 1:6	18.66	14.33
DF-Cu 1:7	19.00	14.66
DF-Cu 1:8	20.00	15.00
DF-Cu 1:9	20.66	15.66

It was also explained that previous research on the synthesis of copper nanoparticles had good antibacterial activity against five different bacteria, including *Staphylococcus aureus* (13 mm) and *Escherichia coli* (10 mm) [42]. Copper nanoparticles exhibit antibacterial activity against pathogenic bacteria such as *Escherichia coli* due to their helpful decontamination capabilities against multiple infectious microorganisms that have the potential to be employed as an antibacterial agent [43].

3.7. Disinfectant Formulation

Transmittance and organoleptic of disinfectant were evaluated before the antibacterial activity test. The transmittance test was analyzed using a UV-Vis spectrophotometer. Percent transmittance (%T) is used to measure the clarity of a solution or dispersion system quantitatively. A high transmittance value means that the particle size is getting smaller. Furthermore, the nanoparticle dispersion system physically provides a clear and transparent view [44]. The organoleptic test is a sensory evaluation technique that relies on texture, color, and fragrance ratings to determine whether a product is of a high enough quality to be accepted [45].

Table 4. Test results of % transmittance and organoleptic disinfectant formula

Formula	Transmittance (%)	Clarity	Color	Odor
F1	51.70	Clear	Clear green	typical red dragon fruit extract-ethanol
F2	86.50	Clear	Clear green	typical red dragon fruit extract-ethanol
F3	50.40	Clear	Clear green	typical red dragon fruit extract-ethanol
F4	56.80	Clear	Clear green	typical red dragon fruit extract-ethanol

The results of the transparency test using the % transmittance test show that each formula has a % transmittance of greater than 50% and nearly 100% [46]. This is mentioned that the droplet size of the dispersion obtained by the nanoparticles in all the formulations has a reasonably good clarity dispersion and remained stable against the light. Organoleptic test results were performed on 25 panelists on two test parameters—color and aroma—which showed that each disinfectant formula was clear greenish, with a distinctively ethanol-like red dragon fruit odor, as exhibited in Table 3.

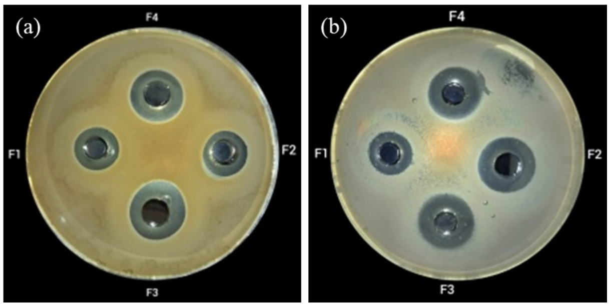

Figure 9. Antibacterial activity test results of (a) *Staphylococcus aureus* (b) *Escherichia coli*

The results of the antibacterial activity test on the disinfectant formulas of F1, F2, F3, and F4 against *Staphylococcus aureus* and *Escherichia coli* showed that all formulas had excellent bacterial growth inhibition, as shown in Table 5. The bacterial inhibition of all formulations against *Staphylococcus aureus* is more effective than *Escherichia coli*. Additionally, for all of the tested bacteria illustrated in Figure 8, the F4 had the greater bacterial inhibitory power. This suggests that the F4 formulation is preferable to the other formulation for inhibiting bacterial growth [47, 48]. Red dragon fruit extract and triclosan in the disinfectant formula work simultaneously in deactivating bacteria.

Table 5. Diameter of bacterial inhibition zone of disinfectant formula against *Staphylococcus aureus* and *Escherichia coli*

Disinfectant formula	Diameter of bacterial inhibition zone (mm)	
	Staphylococcus aureus	*Escherichia coli*
F1	20.33	20.33
F2	20.66	24.00
F3	25.00	24.33
F4	26.00	24.66

Visually, the disinfectant product can be seen in Figure 10. The copper nanoparticles-red dragon fruit extract effectively inhibited the growth of each tested bacteria. This means that the disinfectant formula containing green-synthesized nanoparticles has the potential to be a formula and allows further application after being examined under the Indonesian National Standard (SNI) and earning intellectual property rights.

Figure 10. Four disinfectant products with different volumes of active ingredients F1, F2, F3, and F4

4. Conclusion

Green synthesis of copper nanoparticles has been successfully conducted by mixing red dragon fruit extract and 0.02 M $(CuNO_3)_2$ solution with different DF-Cu volume ratios of 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, and 1:9. Copper nanoparticle synthesis is relatively stable due to the interaction between anthocyanin compounds from red dragon fruit extract and Cu, which can reduce Cu^{2+} ions to Cu^0. Copper nanoparticles with a DF-Cu 1:9 ratio had the smallest size at 122.12 nm. The antibacterial activity test confirmed that the 1:9 volume ratio had the best activity in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli*. The utilization of copper nanoparticles as an active disinfectant has proven beneficial, demonstrating excellent bacteria inhibition, a high transmittance value, and a favorable response from the panelists.

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