Chemical Characterization of Silver Nanoparticle Compounds using Red Algae (*Fucus vesiculosus*) in Freeze Dry Methods

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Abstract

The nanoparticle green synthesis method is an alternative method for synthesizing nanoparticles or nanomaterials. The nanoparticle green synthesis method is a synthesis method that forms metal nanoparticles using natural materials such as plants as bioreductor. Red algae contain active ingredients that are the potential as antioxidants and anti-aging. *Fucus vesiculosus* is one of the red algae species with bioactive as an anti-aging and antioxidant compound. This study aims to synthesize, characterize and determine the antioxidant activity of *F. Vesiculosus* nanoparticles compounds. Characterization for nanoparticle compounds is Scanning Electron Microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Particle Size Analyzer (PSA). The antioxidant activity test used the DPPH method. The results of the SEM characterization of nanoparticles showed the results of the formation of spherical particle surfaces known as a spherical formation. SEM-EDX shows several constituent elements of silver nanoparticles *F. vesiculosus*, namely C, O, Na, Mg, Cl, and K. Characterization silver nanoparticles using PSA measuring the particle size are 74.06 nm at 99.5% distribution. Characterization of silver nanoparticles using XRD showed crystalline character. The FTIR pattern shows the functional groups of alkenes, aromatic rings, ethers, amines/amides, alkenes, and alkanes. The antioxidant test results showed that the inhibition concentration for ascorbic acid was 1.13 ppm, silver nanoparticles *F. vesiculosus* 177.6 ppm, *F. vesiculosus* crude extract 1335 ppm, and respectively, ascorbic acid > silver NP > crude extract *F. vesiculosus*. The test results show that the antioxidant activity of the nanoparticle compounds increased from the very weak into the strong category.

Keywords: antioxidant activity, *Fucus vesiculosus,* Nanoparticle.

INTRODUCTION

Nanoparticles are particles that have a size of 1–100 nm. Nanoparticle compounds are useful in various industries, such as the pharmaceutical industry in terms of drug design and drug delivery, the beauty industry, the agricultural industry for fertilization and growth regulators, electronics, defense, fermentation technology, the food and food processing industry, and the chemical industry [1,2]. Nano-technology is highly developed because it has many advantages, such as smaller particle size having distinctive properties compared to larger particle size and flexibility combined with other technologies so that it can be developed for various purposes. The importance of nanoparticle compounds is based on the reasons for their potential therapeutic effects. This compound also has fewer side effects than other synthetic compounds.

Several studies show that the conversion of nanoparticle compounds can be carried out by various techniques, such as polymer nanoparticles, solvent evaporation, salting-out, dialysis, supercritical fluid technology, micro-emulsion, mini-emulsion, surfactant-free emulsion, and interfacial polymerization [3]. The entire method requires a complex operation. This study demonstrates a new possibility in the simpler conversion of nanoparticle compounds with much greater economic potential. Physical, chemical, and biological methods can synthesize silver nanoparticles. Though physical and chemical methods produce pure particles, these methods are expensive and not environmentally friendly. So that the biological method was chosen using an extract plant as a bio reductor. This method is an environmentally friendly nanoparticle synthesis because it can minimize the use of hazardous inorganic materials that contain waste [4].

The green synthesis nanoparticle method is a synthesis method that forms metal nanoparticles using natural materials derived from organisms (plants and microorganisms). One of the nanoparticles that can be synthesized using the green synthesis method is the silver nanoparticles. This study synthesized silver nanoparticles using extract *F. Vesiculosus* by reacting the extract and silver nitrate ($AgNO_3$) 0.05 M (1:2) solution through a stirring process.

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Free radicals will damage molecules whose electrons are attracted by these free radicals, causing cell damage, impaired cell function, and even cell death [5]. Antioxidants have a role in controlling the reactivity of these free radicals. Antioxidants inhibit oxidation reactions due to free radicals, which neutralize free radicals [6]. As an active ingredient, antioxidants are used to protect the skin from damage due to oxidation so that they can prevent premature aging [7]. Antioxidants protect cells from free radical damage by donating one free electron to free radicals or accepting an unstable electron so that it becomes stable, stops chain reactions, and prevents damage to lipids, proteins, and DNA [8].

Algae has become a biological material that contains active ingredients that are the potential as antioxidants and anti-aging. *Fucus vesiculosus* is one of the red algae species with bioactive as an anti-aging and antioxidant compound. The potential antioxidant and anti-aging of red algae proof and utilized for the pharmaceutical beauty industry. Algae of type *F. Vesiculosus* have botanical activity as an antioxidant and anti-aging with a working mechanism of stimulating collagen production [9].

Three processes are presented in this paper for the simpler and faster conversion of nanoparticle compounds. First, the synthesis process of nanoparticle compounds uses the green synthesis method to convert macro compounds into micro and nano compounds. Second, the process of characterizing nanoparticle compounds with their constituent elements, morphological characters, degree of crystallinity, and particle size. Third, testing the antioxidant activity of the *F. vesiculosus* nanoparticle compound. Characterization of silver nanoparticles based on the character of the constituent elements, morphological characters, particle size, and the pattern of the functional group.

MATERIAL AND METHOD

Material

The material used is a red algae type *F. vesiculosus* obtained from the UK in a dry preparation with a moisture content of 15%, aquadest. The tools used are the MN12A Freeze dryer, Fourier Transform Infrared Spectroscopy (FTIR) Resolution; 4 [$1.cm^{-1}$], Apodization; Shimadzu Happ-Genze Type: IR Prestige 21l, X-ray Diffraction (XRD): Panalytical Type: X'Pert PRO), Malvern Zetasizer Nano Particle Analyzer. SEM/SEM-EDX: FEI, Type: Inspect-S50.

The working procedure of silver nanoparticle compounds using the green synthesis method

The working procedure used in the conversion of nanoparticle compounds uses the modified green synthesis freeze drying method. The raw material of dried algae showed in paper bag (Fig. 1). The initial algae preparation is dried with a maximum water content of 10%, then the algae is mashed with a blender and sieved with a 100 mesh sieve showed in bottle (Fig. 1). The result of the sieve is a powder with a size of 100 mesh which is then macerated in a water solvent.

The working procedure of silver nanoparticle compounds was conducted with several modifications, namely 40 mL of *F. vesiculosus* algal filtrate mixed with 40 mL of 1 mM $AgNO_3$ solution (1:1 ratio) and incubated in the dark by heating at 60°C. After two hours, there was a color change in the solution from white to pale yellow. It indicated that AgNPs had been synthesized. Then the solution that changed color was centrifuged at 4000 RCF for 30 minutes at 20°C. Then, the collected pellets were transferred to an oven to be dried at 45°C for 24 hours [10].

Figure 1. The bagged sample and powder of *F. vesiculosus*

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Characterization of Nanoparticle Compounds using a Scanning Electron Microscope (SEM)

The nanoparticles were placed on the stub using double-sided tape. The powder is conditioned to be electrically conductive with a tuft of a thin layer of platinum and a current strength of 30 mA. The photo was taken at 10 kV electron voltage with the desired magnification [11].

Characterization of functional groups using Fourier Transform Infrared Spectroscopy (FTIR)

A total of 2 mg of powdered NP sample was mixed with 100 mg of KBr. The powder mixture was dried in a vacuum freeze dryer for one day. Next, the powder mixture was subjected to infrared light at a wavelength of 400-4000cm-1 using 100 scans on a Spectrum One Spectrometer (Perkin Elmer, Norwalk, CT, USA) [12].

Characterization of the degree of crystallinity using X-ray Diffraction (XRD)

A total of 200 mg of the sample was imprinted on a 2 x 2.5 cm print made of aluminum. The crystallinity level was determined using XRD with a source wavelength of 1.5406 Å [13].

Particle Size Analyzer (PSA) Characterization

The particle size test was carried out using a digital microscope and PSA testing. Samples were taken using a spatula, dissolved in aquadest with a ratio of 1 mg in 10 mL of aquadest, and then vortexed. The solution is then inserted into a tube with a maximum solution height of 15 mm. Then the sample is measured in diameter distribution using the Malvern Zetasizer Nano Particle Analyzer [14].

Antioxidant Activity Test

The antioxidant activity using the DPPH method, based on the free radical scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH), was determined according to the method with modification. Ascorbic acid act as a positive control. We prepared *F. Vesiculosus* extract samples with concentration series including 50, 100, 150, and 200 ppm and ascorbic acid samples with concentrations of 2, 4, 6, 8, and 10 ppm. Then 500 L of each sample was taken and put into a tube to be reacted. After that, 500 L of DPPH solution was added to each sample taken from the stock solution. Each sample was three replicated. It was then incubated in a dark place at room temperature for 30 minutes. Then we read absorbance using a UV-Vis spectrophotometer with a wavelength of 517 nm and read three times for each sample. Ascorbic acid is used as a standard solution [15]. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm, and the percent inhibitory activity was calculated using the following formula:

$$Scavenging\ activity\ (\%) = \frac{(A\ control - A\ Sample)}{A\ control\ \times 100}$$

Description:
A control = Absorbance DPPH as control antioxidant
A Sample = Absorbance sample

The antioxidant activity test was carried out with two repetitions. The results of the IC50 value calculation were obtained using the program Graph Pad prism8 software, Regression, for analyzing dose-response data.

Data analysis

Data analysis used descriptive qualitative on several parameters of observations of the character of nanoparticle compounds. The analysis sample used three replicates. The sample's standard deviation or standard deviation is used in calculating the *zi* value on the normality test.

RESULTS AND DSICUSSION

Synthesis of Silver Nanoparticle *F. vesiculosus*

Silver nanoparticles were prepared by adding a pure extract of red algae to 1 mM $AgNO_3$ solution. The formation of silver nanoparticles was initially confirmed visually. The mixture of $AgNO_3$ solution with pure extract changed color from light green to brownish yellow after 24 hours of incubation in the dark at 50˚C. The color change occurs due to the excitation experienced by free electrons in the solution. The brown discoloration rate in the solution indicates a good amount and distribution of silver nanoparticles.

The color change occurs due to the excitation of free electrons in the solution. The color change rate of brown in solution indicates the amount and distribution of silver nanoparticles. Silver nanoparticle formation, described with a color change, indicates an ion reduction process of silver into silver nanoparticles [16].

The characterization of nanoparticle compounds was used to characterize that the processed extract had turned into a nanoparticle compound. Some measurements that can be used to measure the formation of nanoparticles compounds are Scanning Electron Microscope (SEM-EDX), X-ray Diffraction (XRD), Particle Size Analyzer (PSA), and Fourier Transform Infrared Spectroscopy (FTIR).

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Scanning Electron Microscope (SEM-EDX)

In developing nanoparticles, the characteristics of using a microscope with SEM are essential to analyze. SEM characterization aims to analyze the surface morphology of the nanoparticles. SEM analysis aims to form an image by shooting a beam of high-energy electrons through the sample, usually with an energy of 1-20 keV.

The results of the SEM characterization of nanoparticles showed the results of the formation of spherical particle surfaces known as a spherical formation. SEM-EDX shows several constituent elements of silver nanoparticles *F. vesiculosus*: C, O, Na, Mg, Cl, and K. The resulting image SEM of the silver nanoparticles showed in Figure 2. The resulting image results are influenced by the elements that make up the resulting nanoparticles. Generally, the constituent metal elements with a more significant atomic number will produce a lighter white color than those with a low atomic number.

In Figure 2a, the SEM results at x 5 µm magnification show spherical particles that are evenly distributed. Figure 2b shows the results of SEM observations at a magnification of x 200 µm showing a closer picture of the shape of the particles. Visually the particle shape has rather sharp corners. Figure 2c shows the magnification at x 40 µm. The shape of the particles is more clearly visible in the form of angular plates with various sizes of around 2.2 - 3.2 µm.

SEM analysis of *Gelidium amansii* - Ag NPs showed morphological uniformity in the distribution of 5 Ag NPs on the surface. The results confirmed the presence of spherical Ag NPs with sizes ranging from 27-54 nm. Biosynthesis of Ag NPs with hydrodynamic diameters between 20-95 nm using seaweed extracts [4,17].

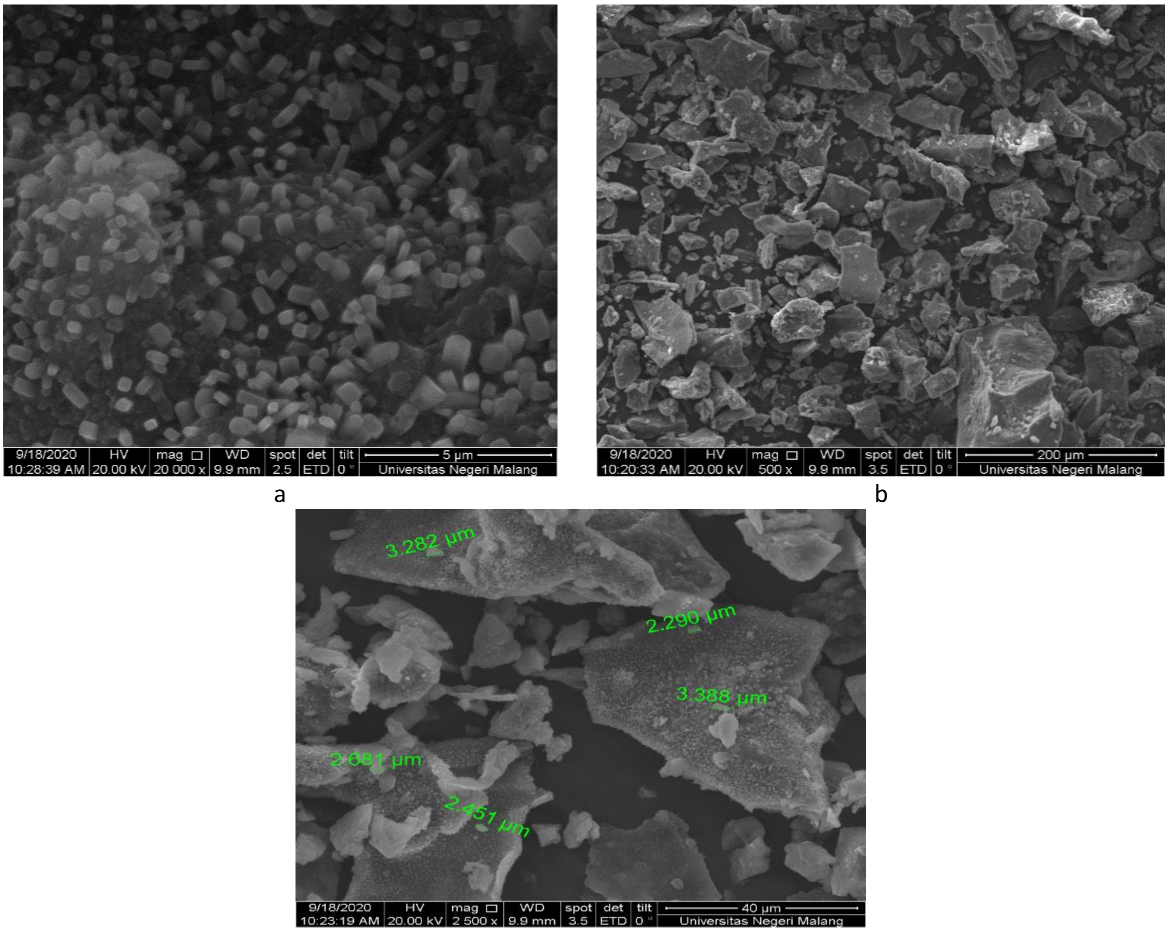

Figure 2. Scanning Electron Microscope (SEM-EDX) Nanoparticles of *F. vesiculosus*
a) x 5 µm, b). x 200 µm, c). x 40 µm

X-ray Diffraction (XRD)

XRD (X-ray diffraction) analysis is performed to analyze and detect the crystal structure, lattice parameters, and atomic positions. X-Ray Diffraction is one of the analytical methods to identify the crystalline phase in the material by determining lattice structure parameters and obtaining particle size.

The results of the characterization of nanoparticle samples using XRD showed crystalline properties. It can be seen from the results of the XRD analysis of the nanoparticles, which produce sharp spectra peaks. This crystalline nature indicates that the constituent particles of a molecule are arranged in an orderly manner. So, this shows the characteristics of a nanoparticle compound (Fig. 3).

Characterization of silver nanoparticles with XRD to support proved that the synthesized nanoparticles are pure silver nanoparticles. XRD results of silver nanoparticles are shown in Figure 3. Figure 3 shows the XRD diffraction pattern produced by silver nanoparticles synthesized using algae extract after Ag+ is reduced to Ag. The resulting diffractogram shows a fairly sharp peak that proves silver nanoparticles have formed. This matter is indicated by the 2θ value of the silver nanoparticles respectively, 2q on: 28.26°; 32.49°; 41.57°; 45.52°; 57.65°, and 66.45° which is close to the silver diffractogram data, the standard is 38.11; 44.30, 64.44 and 77.40.

The most widely used material characterization method is X-ray diffraction (XRD) spectroscopy. XRD is beneficial for studying nanomaterials' crystal structure, chemical composition, and physical properties. This technique is used to identify the crystalline phase in the material by determining the lattice structure parameters and to obtain the particle size of the nanocrystals, crystal structure, chemical composition, and physical properties of nanomaterials [18,19,20].

Particle Size Analyzer (PSA)

This particle size analysis uses different measurement principles and covers a size range from 0.3 nm to > 3 mm. The particle size distribution shows several distributions, namely peak 1 measuring 827.1 nm at 0.5% and peak two measurings 74.06 nm at 99.5% (Fig. 4). It shows that the majority of the tested samples show the size of the nanoparticles.

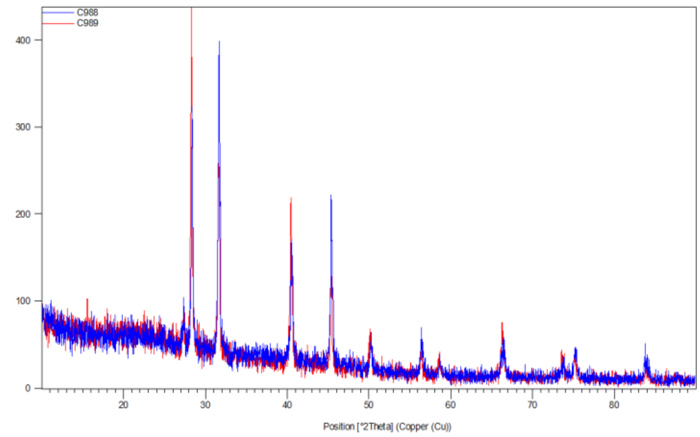

Figure 3. Profile X-ray Diffraction (XRD) nanoparticle *Fucus vesiculosus*

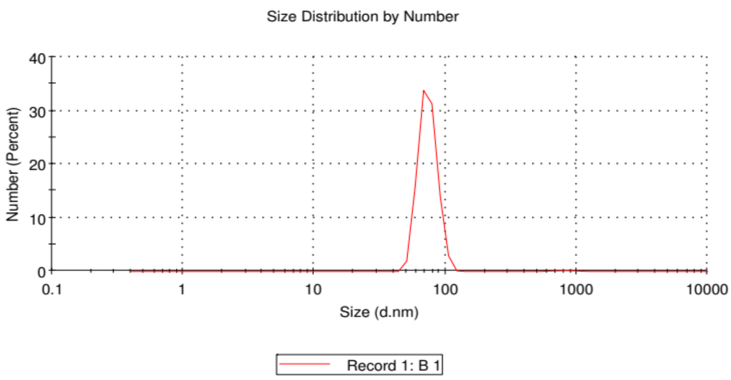

Figure 4. Profile Particle Size Analyzer (PSA) Nanoparticle of *F. vesiculosus*

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The characterization results using PSA showed that overall the average diameter of the silver nanoparticles that had been successfully synthesized was 45.7 nm. The results of this characterization support the results obtained using a UV spectrophotometer. The resulting nano-scale size proves that plant extract has the potential as a reducing agent in the synthesis of nanoparticles [21].

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR technique is used to monitor changes in membrane surface chemistry. Moreover, by knowing the specific functional groups that have changed the surface behavior of alkanes, FTIR can explain the different physicochemical properties that significantly change alkane performance. In addition, the FTIR technique can also be used to monitor the stability and resistance of specific alkanes to their performance. The results of the functional group profiles of the compounds are presented in Figure 5. The FTIR pattern shows the functional groups of alkenes, aromatic rings, ethers, amines/amides, alkenes, and alkanes.

The characterization of $AgNO_3$ showed an absorption band at 3043 cm^{-1} related to the O-H and N-H groups. The absorption band 2559 cm^{-1} is related to the C-H group of alkanes. The absorption bands of 2344 cm^{-1} and 1787 cm^{-1} are related to the C=O groups of esters and aldehydes. The absorption band of 1623 cm^{-1} is related to the C=C group of alkenes. The absorption band of 1287 cm^{-1} is related to the C-O-C group of the ether. The absorption band of 1046 cm^{-1} is related to the C-O functional group of alcohol. The absorption bands of 819 cm^{-1}, 798 cm^{-1}, and 573 cm^{-1} were related to the aromatic C-H group.

The results of the IR spectrum of silver nanoparticles of *F. vesiculosus* showed an absorption band of 3265 cm^{-1} associated with the O-H and N-H groups. The absorption bands 2919 cm^{-1} and 2844 cm^{-1} are related to the C-H group of alkanes (red color line). The absorption band of 1610 cm^{-1} is related to the C=C group of alkenes. The absorption bands of 1538 cm^{-1} and 1425 cm^{-1} are related to the aromatic C-NO2 group. The absorption band of 1231 cm^{-1} is related to the C-O-C group associated with the ether group. The absorption band of 1155 cm-1 is related to the C-N group of the amine. The absorption band of 1024 cm^{-1} is related to the C-O group of alcohol. The absorption band of 822 cm^{-1} is related to the aromatic C-H group.

The results of the IR spectrum on the extract of *F. vesiculosus* showed an absorption band of 3336 cm^{-1} related to the O-H and N-H groups. The absorption bands 2919 cm^{-1} and 2844 cm^{-1} are related to the C-H group of alkanes. The absorption band of 1610 cm^{-1} is related to the C=C group of alkenes. The absorption bands of 1538 cm^{-1} and 1420 cm^{-1} are related to the aromatic C-NO2 group. The absorption band of 1239 cm^{-1} is related to the C-O-C group associated with the ether group. The absorption bands of 1075 cm^{-1} and 1016 cm^{-1} are related to the C-O group of alcohol. The absorption band of 822 cm^{-1} is related to the aromatic C-H group.

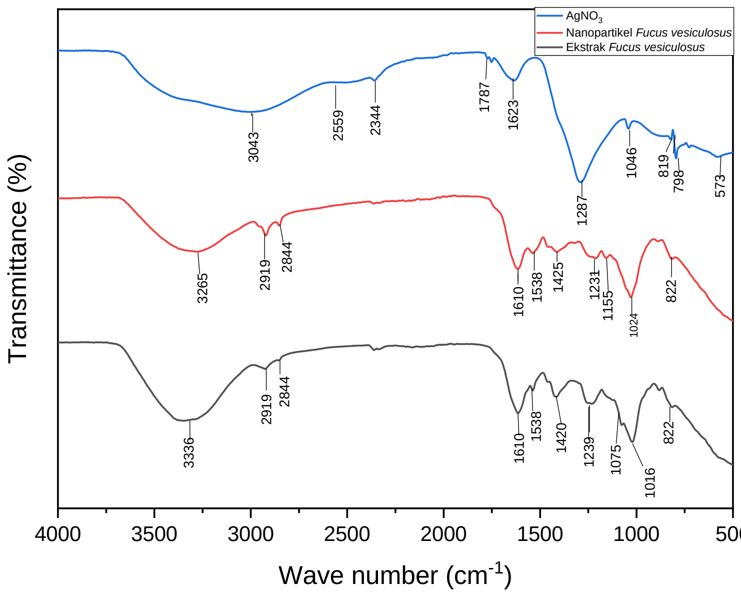

Figure 5. Spectrum and peak value FTIR (A) $AgNO_3$, (B) silver NP *F. vesiculosus,* (C) extract of *F. vesiculosus*

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The absorption band of 1046 cm^{-1} on AgNP *F. vesiculosus* shows the bond between Ag metal and the O-H hydroxyl group. It confirms that the absorption band indicates the synthesis of silver nanoparticles. The O-H, N-H, C=C, and C-H functional groups in the extract of *F. vesiculosus* indicate flavonoid compounds that act as reductants of silver ions in the synthesis of nanoparticles. In addition, in the silver nanoparticles of *F. vesiculosus* there is a C-N amine functional group that acts as a stabilizer for silver nanoparticles. The results of the FTIR spectrum on the *C. roxburghii* extract confirmed the presence of N-H, O-H, C=C, and C-H groups, indicating that the plant extracts contained hydroxyl and amine groups. Both groups indicate the presence of flavonoid compounds. Flavonoid compounds act as reducing agents, which reduce Ag^{+} to Ag^{0}, and amino groups as stabilizers in the green synthesis of AgNP [16].

Antioxidant Activity of *F. vesiculosus* Nanoparticle Compounds

The antioxidant activity test used the DPPH method. Antioxidant activity is known from the absorption value of several sample concentrations after reacting with a DPPH free radical solution. The lower the IC50 value means, the higher the antioxidant activity as a free radical scavenger.

The results of the antioxidant activity test are presented in Figure 6. This test uses ascorbic acid as a control. The test results showed that the inhibition concentration for ascorbic acid was 1.13 ppm, *F. vesiculosus* powder 1335 ppm, and silver nanoparticles *F. vesiculosus* 177.6 ppm. The test results show that the antioxidant activity of the nanoparticle compounds increased from the very weak category into the strong category. Antioxidant activity based on IC50 value (Molyneux, (2004), < 50 ppm in the very strong category, 50-100 ppm strong, 100-150 ppm medium, and 150-200 ppm in the weak category. The biosynthesis of silver nanoparticles from *Blighia sapida* leaf extract has high total phenols and flavonoids exhibiting antioxidant activity through DPPH radical scavenging activity [22]. The study confirms that antioxidant activity ascorbic acid > AgNP > extract/powder *F. vesiculosus*. AgNPs have an antioxidant activity of 29.55%, while ascorbic acid has an antioxidant activity of 24.28%. AgNPs have greater antioxidant activity compared to vitamin C [23].

Compound nanoparticles are very fine units with dimensions measured in nanometers (nm; 1 nm = 10^{-9} meters). The nanoparticles are submicroscopic in size and therefore have unique material characteristics. The resulting nanoparticles can be used for practical applications in various fields, including medicine, engineering, catalysis, and environmental improvement.

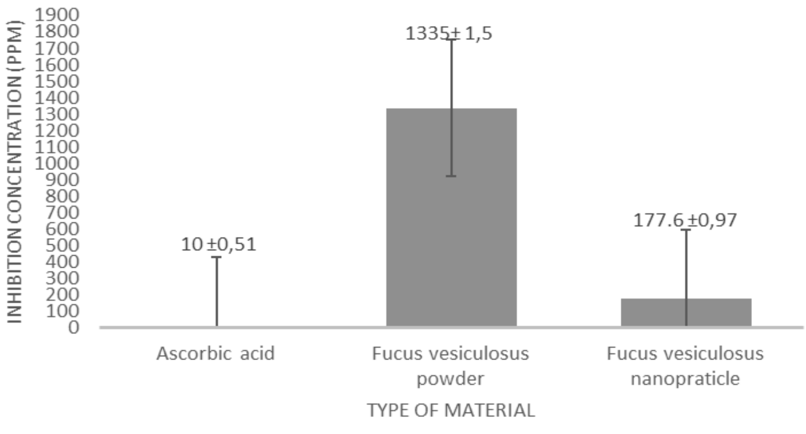

Figure 6. Inhibition concentration (%) of *F. vesiculosus* in antioxidant activity test using DPPH methods

Some of the characteristics that indicate the characteristics of the observed nanoparticles are the results of observations using the Scanning Electron Microscope (SEM-EDX), X-ray Diffraction Diffraction (XRD), Particle Size Analyzer (PSA), and Fourier Transform Infrared Spectroscopy (FTIR). Research for the characterization of nanoparticle compounds using similar parameters [19,24].

The results of this study are the initial stages of testing red algae nanoparticles which are useful as antioxidants and anti-aging. Red algae *F. vesiculosus* is a type of red algae with active anti-aging ingredients. *Fucus* extract has botanical activity as an anti-aging agent by stimulating collagen production. This research is a research for synthesizing nanoparticle compounds using a simple method. In this study, the characteristics of nanoparticle compounds were identified through several characteristics, namely morphology, degree of crystallinity, particle size distribution, and compound functional groups.

Future studies need to test nanoparticle compounds through in vitro tests, anti-collagenation enzyme activity, and collagen activity. In the in vitro MTT (3- (4, 5-dimethylthiazolyl-2) -2, 5-diphenyltetrazolium bromide anti-aging activity assay. Testing of anti-aging activity and differentiation of adiposity cell cultures. In vivo anti-aging testing is a clinical study in humans. Clinical studies in humans include testing differences in wrinkle area, length, depth, and dermatology (visual score). The final product of this study is an anti-aging compound used as an ingredient in nanoparticle-based cosmetic formulations (moisturizers, night creams, and lotions). The results of this research in the future will be useful for cosmetic ingredients that are useful as anti-aging.

CONCLUSION

Fucus vesiculosus extract as a bioreductor to convert silver metal into silver nanoparticle compounds. Morphological characteristics indicate that the surface of the particles is like a ball, known as a spherical formation. The results of the characterization of nanoparticle samples using XRD showed crystalline properties. The particle size distribution shows a particle size of 74.06 nm of 99.5%. The antioxidant test results showed that the inhibition concentration for ascorbic acid was 1.13 ppm, silver nanoparticles *F. Vesiculosus* 177.6 ppm *F. Vesiculosus* crude extract 1335 ppm, respectively ascorbic acid > silverNP > crude extract *F. vesiculosus*. The test results show that the antioxidant activity of the silver nanoparticle compounds increased from the very weak category into the strong category.

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