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Research Article

Flavonoids and Antioxidant Activities of Silver Nanoparticles of Extract *Galaxaura rugosa*

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ABSTRACT
The algae Galaxaura rugosa contains phenolic compounds, flavonoids, β-carotene, and galactane sulfate, which are natural antioxidants. The development of antioxidants derived from red algae into silver nanoparticles (AgNPs) can improve their effectiveness in preventing reactive oxygen species (ROS). The study aims to synthesize silver nanoparticles using G. rugosa algae and to evaluate the total flavonoid content and antioxidant activity of the resulting silver nanoparticles. The synthesis of silver nanoparticles utilized G. rugosa extract as a bioreductor. Characterization of the silver nanoparticle was conducted using Particle Size Analysis (PSA) and UV-Vis Spectrophotometer. The total flavonoid and antioxidant activities of silver nanoparticles using UV- VIS spectrophotometry. The total flavonoid content and antioxidant activity of the silver nanoparticles were also analyzed using a UV-Vis spectrophotometer. Antioxidant activity was tested using the DPPH method. The results showed that the synthesis of silver nanoparticles using G. rugosa extract resulted in a color change of the solution from green to yellow. UV- VIS spectrophotometry measurements revealed absorption within the 570-580 nm wave range, with a maximum peak at 406 nm. The size distribution of the synthesized nanoparticles, as measured by PSA, averaged 11 nm. The total flavonoid content of G. rugosa silver nanoparticles was 36.21±0.65 mgQE/g, higher than that of the extract alone (32,12±0,79 mgQE/g). The antioxidant activity (IC50) of the G. rugosa silver nanoparticles was 26.658 ±1.44 ppm, classified as very strong, compared to the extract alone, which had an IC50 value of 46,128 ± 1.6, categorized as strong.

Keywords: *Antioxidant; Flavonoid; Galaxaura rugose; Silver nanoparticle.*

Introduction

The main antioxidant components of red algae are predominantly phenolic compounds, β-carotene, and galactane sulfate [1]. These compounds enhance the performance of enzymes involved in scavenging free radicals, such as superoxide dismutase and catalase. *Galaxaura rugosa* is a species of red algae commonly found in Indonesian waters. Its antioxidant activity has been demonstrated, with the ethanol fraction exhibiting an IC50 value of 27.8 ppm [2].

Flavonoids are among the most common polyphenolic compounds found in biological extract, including red algae. These compounds have been proven to actively stabilize free radical atoms through

electron conjugation [3]. The presence of flavonoids or polyphenols in extracts or secondary metabolites correlates positively with their ability to combat free radicals or ROS [4].

Antioxidants are substances capable of inhibiting oxidative reactions. In the human body, antioxidants are essential for preventing oxidative stress, which occurs when there is an imbalance between ROS levels and antioxidants. ROS are free radical molecules harmful to the body and can originate from both internal and external sources [5], [6], [7].

Most antioxidant products are currently available in the form of extracts or simplicia. Recent advancements indicate that reducing particle sizes can enhance the effectiveness of these extracts as a therapeutic agent [8]. Nanotechnology focuses on the development of nanoparticles, which are materials with dimensions smaller than 100 nm in at least one aspect [9]. Transforming antioxidant extract into silver nanoparticles can significantly increase their DPPH inhibitory activity, from 31% to 67%. These nanoparticles have various applications, such as antibacterial agent in the health industry, cosmetics, food preservation, textile coatings, and environmental uses [10].

The synthesis of silver nanoparticles through the "biological method" or "green synthetic" utilizes polysaccharides from living organisms such as plants, algae, and bacteria. This method is cost-efficient, biocompatible, and environmentally non-toxic. Additionally, it mitigates the potential toxicity of silver nanoparticles [11], [12].

Flavonoids are among the most common polyphenolic compounds found in the extracts of living organisms, including red algae. These compounds have been proven to actively stabilize free radical atoms through electron conjugation. Flavonoids are capable of contributing hydrogen atoms via a single-electron transfer mechanism, which gives them high antioxidant activity [3]. The flavonoid or polyphenolic compounds present in the secondary metabolites of living organisms exhibit a positive relationship with their ability to address free radical or ROS-related problems [4].

Antioxidants are substances that inhibit oxidation reactions in the body and play a critical role in preventing oxidative stress. Oxidative stress occurs when there is an imbalance between ROS levels and antioxidants in the body. While natural antioxidants like superoxide dismutase (SOD) help regulate ROS, certain conditions result in ROS levels exceeding the body's antioxidant capacity. In such cases, exogenous antioxidants, synthesized outside the body, are required. Polyphenolic compounds are widely recognized for their strong antioxidant properties [13], [14].

The objective of this study was to characterize the formation of silver nanoparticles synthesized using the red algae *G. rugosa,* to measure the total flavonoid content embedded in silver nanoparticles derived from *G. rugosa,* and to evaluate the antioxidant activity of the synthesized silver nanoparticle.

Materials and Methods

Materials

The materials used in this study include *G. rugosa* red algae obtained from the Jembrana coast of Bali, aquadest, filter paper, AgNO3, 2,2-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, ethanol p.a (pro analysis), sodium acetate, AlCl3, and quercetin.

Methods

Sample Preparation and Extraction

The preparation and extraction process involved several modifications. Samples were dried in an oven method at a temperature of 50-60℃ for 2 days and then ground using a blender. The ground samples were extracted using a maceration method at a ratio of 1:3, where 200 grams of algae powder was dissolved in 600 ml of

p.a. ethanol. The mixture was incubated overnight at room temperature while being occasionally stirred using a shaker. Afterward, the solution was filtered, and the filtrate was evaporated using a rotary vacuum evaporator, resulting in a crude or concentrated extract of *Galaxaura rugosa* [15].

Synthesis of Silver Nanoparticles Using Galaxaura rugosa Extract

Algae powder (1 gram) was mixed with 10 ml of 1 mM AgNO3 solution in a ratio of 1:10. The mixture was stirred using a magnetic stirrer and hotplate at 28°C for 4 hours at a speed of 400 rpm in the dark, covered with aluminum foil. The formation of silver nanoparticles was indicated by a color change in the solution from yellowish green to brownish yellow or reddish brown. The solution was then centrifuged at 4000 rpm for 30 minutes at 20°C. The resulting pellets or deposits were dried in an incubator at 45°C for 24 hours. The dried nanoparticle powder was ground to fine consistency using a mortar [10].

Nanoparticle Characterization Using UV-Vis Spectrophotometry

The characterization of nanoparticle compounds was performed using a UV-Vis spectrophotometer *(Thermo Fisher Scientific)*. The sizes of silver colloid particles were calculated using the following equation [16]:

$$d = \ln\left(\frac{\lambda spr - \lambda 0}{L1}\right) / L2 \quad \text{...(1)}$$

Where:

d	: Diameter of silver colloid particles	L1	: 6,53
λspr	: wavelengths at maximum absorption of silver colloids	L2	: 0,0216
λ0	: 512		

The silver nanoparticle morphology characterization using PSA (Particle Size Analyzer)

The morphological characterization of nanoparticles was conducted using a Particle Size Analyzer (PSA). For this test, 1 mg of silver nanoparticle powder synthesized from *G. rugosa* was dissolved in 10 ml of distilled water. The solution was analyzed using a PSA, which provided results including particle size, zeta potential, and particle distribution (eq.1).

The silver nanoparticle morphology characterization using SEM (Scanning Electron Microscope)

For scanning Electron Microscope (SEM) analysis, a stub was coated with nanoparticles using double-sided tape. During the conditioning process, the powder was made electrically conductive by applying a thin platinum layer with a current intensity of 30 mA. Images were captured using an electron voltage of 10 kV and an appropriate magnification. The SEM specifications used were Merk FEI, Type: Inspect-S50.

Total Flavonoid Content Using UV-Vis Spectrophotometry

The total flavonoid content was determined by measuring absorption using a UV-Vis spectrophotometer at the maximum quercetin wavelength within the 400-800 nm range. The total flavonoid levels were expressed as mg quercetin equivalent per gram of extract and calculated using a linear regression equation (eq.2). The formula is as follows [3].

Antioxidant Activity Test Using the DPPH Method

The DPPH testing method was conducted with several modifications [5]. Antioxidant activity tests were performed on three samples: test sample I (silver nanoparticles), test sample II (pure extracts

of red algae *Galaxaura rugosa*), and test sample III (ascorbic acid/vitamin C) as the positive control.

For each test, 3 ml of the sample solution was added to a reaction tube, followed by the addition of 1 ml of 0.1 mM DPPH. The mixture was incubated in a dark room for 30 minutes until the solution changed color. The treatment was repeated 3 times. Absorbance measurements of the sample were carried out at the wavelengths determined during the initial setup.

$$Y = ax + b \qquad X = \frac{y-b}{a} \longrightarrow \text{KTF (mgQF/g extract)} = \frac{V(ml) x X\left(\frac{mg}{mL}\right) x FP}{\text{g Ekstrak}} \qquad (2)$$

Where:

Y : Absorbance

X : Extracts concentration in quercetin rows (μ/mL)

FP : Dilution factor

The antioxidant activity of the samples was determined by calculating the IC50 value, which represents the concentration of the solution capable of reducing 50% of the DPPH compound (eq.3). The same procedure was applied to the positive control (ascorbic acid). The percentage of inhibition was calculated using the following formula:

$$\%\ \text{Inhibition} = \frac{\text{Abs DPPH} - (\text{Abs sample} - \text{Abs control})}{\text{Abs DPPH}} \times 100 \qquad (3)$$

Results and Discussion

The synthesis of silver nanoparticles was initially confirmed visually. A mixture of AgNO3 solution with pure *G. rugosa* extract underwent a color change from pale green to brown-yellow after being incubated for 24 hours in a dark state at a temperature of 50℃ This color change occurs due to the excitation of free electrons in the solution. (Figure 1).

The sustained brown color of the solution indicates a high concentration and even distribution of silver nanoparticles. The color change signifies the reduction process of silver ion to silver nanoparticles. The Red Green Blue (RGB) color intensity of the solution showed changes: +3 in red, -36 in green, and -20 in blue. A color shift was observed from green (540-560 nm) to yellow (570-580 nm) range [12], [17], [18]. Silver nanoparticles exhibit the ability to scatter and absorb light at specific wavelengths due to the excision of charge density resonance between the conductor and insulator, a phenomenon known as Surface Plasmon Resonance (SPR). SPR is an optical property used to observe interaction between metal surfaces (e.g. silver and gold) using plasmon waves [8], [19].

The reduction of silver ions to silver nanoparticles using *G. rugosa* extract was analyzed using UV-vis spectroscopic photometry. The absorption spectrum revealed a peak at 406 nm, indicating the formation of silver nanoparticles. In the UV-Vis spectrum, AgNP showed an λSPR peak at 406 nm (Figure 2). This peak reflects the presence of chromophoric substances in the algal extract, which contributed to the absorption at visible wavelengths.

The particle size of silver nanoparticles synthesized using algae extract was measured, with diameter ranging from 23.73 to 93.61 nm. This confirms that the nanoparticles fall within the defined range of 1-100 nm. The absorption peak between 400-450 nm corresponds to the SPR of silver. The synthesized nanoparticles were further purified via centrifugation at 8000 rpm, and the resulting pellets were dried to obtain silver nanoparticles in powder form [16].

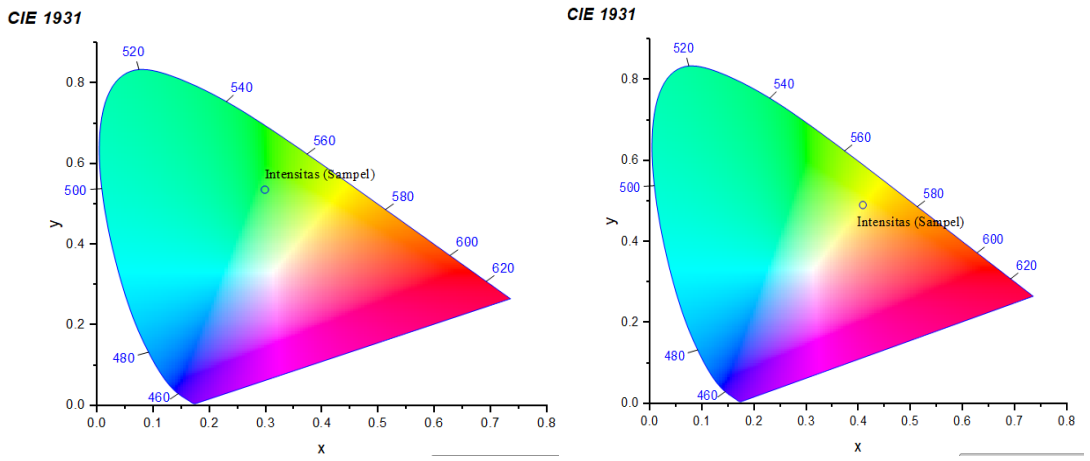

Figure 1. Color spectrum biosynthesis of silver nanoparticle (AgNPs) *G. rugose* a). (a) Before incubation (RGB values: R=80, G=115 B=51) (b). After incubation (RGB values: R=83, G=79 B=31).

G. rugosa contains sulfate polysaccharides with anionic disaccharides, which serve as a powerful reducing agent for silver compounds [20]. The stabilization of polysaccharides relies on multiple binding points along the polystyrene chain, which can "trap" metal nanoparticles and provide protection against chemical aggregation and modification. Polyphenolic compounds act as reducers of Ag+ to Ag0 through single-electron donation by hydroxyl and carboxyl groups, contributing to nanoparticle stability. An increased content of phenolic compounds enhances the reduction process and prevents nanoparticle agglomerating during or after synthesis [21].

The red pigment in *G. rugosa* also plays a crucial role in stabilizing silver compounds. The phycoerythrin pigment present in red algae not only reduces silver ions but also acts as a capping agent in the biosynthesis of silver nanoparticles. Phycoerythrin, tested using the DPPH method, demonstrated potential as an antioxidant by slowing down or inhibiting oxidation, thereby preventing free radical attacks. The pigmented portions of red algae, rich in flavonoid compounds, further contribute to this antioxidant activity [22], [23].

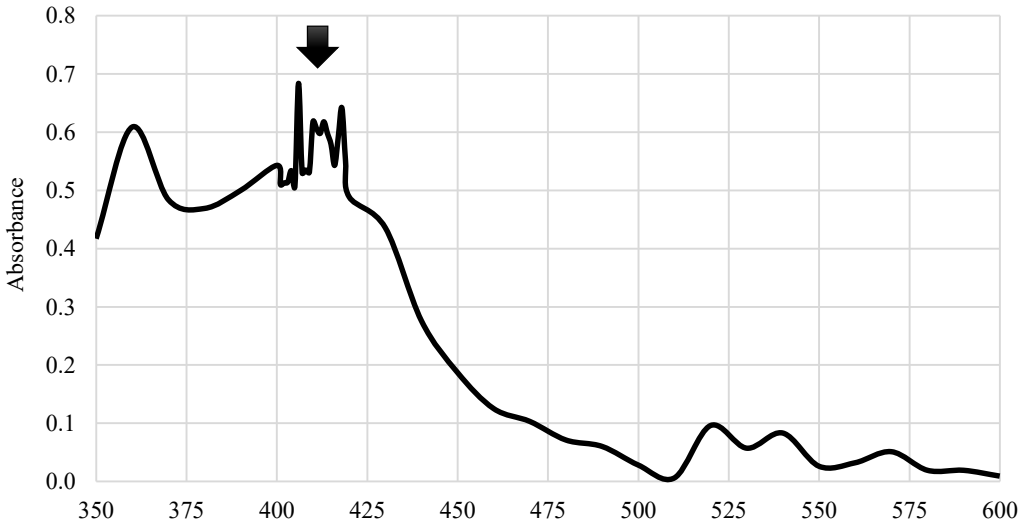

Figure 2. Maximum wavelength of silver nanoparticles from *G. rugosa* using UV-vis spectrophotometry.

The size distribution of the nanoparticles formed shows an average particle size of 11 nm. The silver nanoparticles tested had an MV (mean volume diameter) of 27.22 nm, which represents the average size of larger particles heavily influenced by rough particles. The MN (mean number diameter) of the silver nanoparticles was 10.67 nm, calculated based on the volume distribution of smaller particles, indicating the average size of the population. The MA (mean surface area diameter) of the silver nanoparticles was 11.54 nm, which is smaller than the MV value due to the presence of rough particles. The MA value reflects the particle surface area and confirms the consistency of the nanoparticle size [24], [25], [26].

The size of *G. rugosa* silver nanoparticles ranged between 10-100 nm (Figure 3). The shape and size of silver nanoparticles are influenced by factors such as pH, temperature, silver nitrate concentration, the method applied, and the type of reducer or capping agent used [27], [28], [29].

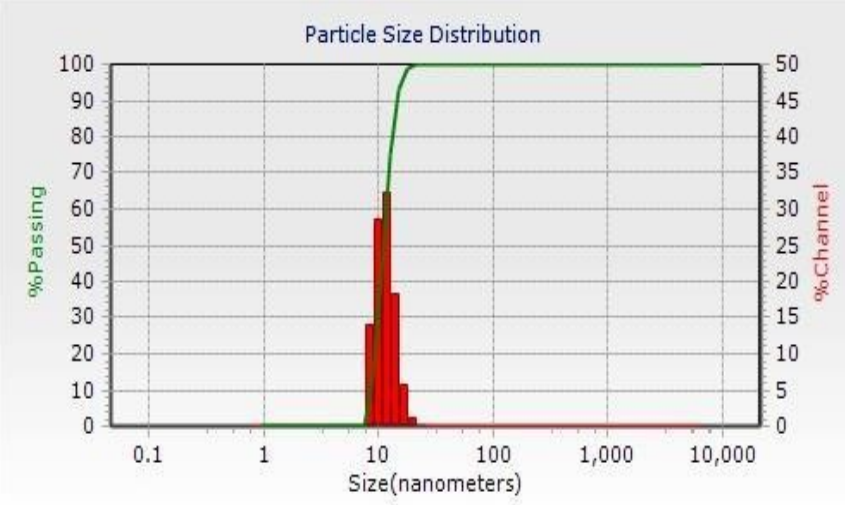

Figure 3. Particle Size Distribution of Silver nanoparticles from *G. rugose.*

Characterization of the nanoparticles using *Scanning Electron Microscopy* (SEM) revealed the formation of spherical surfaces, indicating a globular structure. Observations made at 1000x magnification (Figure 4) provide detailed insights into the particle morphology. The nanoparticles exhibited somewhat angular corners, forming shapes resembling angular plates with a size range of approximately 100 µm.

The *G. rugosa* specimens act as reducers and capping agents in silver nanoparticle synthesis. Plant-based silver nanoparticle synthesis has advantages in stabilizing nanoparticles because plant-derived biomolecules play dual roles: as reducing agents and capping agents. Phenolic and bioactive compounds in plants can reduce silver ions (Ag+) to silver atoms (Ag0) and simultaneously stabilize the nanoparticles, preventing agglomeration. Additionally, nanoparticle stability may be influenced by the presence of enzymes or proteins in the extract. Flavonoids and phenolic compounds are particularly effective as reducing and capping agents. The enol groups found in flavonoids and phenols release electrons by breaking the O-H bands, which are then used to reduce Ag+ to Ag0 [12], [30], [31].

The stability of nanoparticles can be evaluated using zeta potential measurements. The zeta potential value of *G. rugosa* silver nanoparticles was found to be +200mV, placing it in the category of

excellent stability. Zeta potential is a technique used to determine the surface charge of nanoparticles. Nanoparticles with zeta potential values between 0 to ±5 mV are prone to coagulation, while values between ±10 to ±30 mV indicate instability. Moderate stability is observed with values of ±31 to ±40 mV, good stability with values of ± 41 to 60, and excellent stability with values greater than ±60 [11], [32].

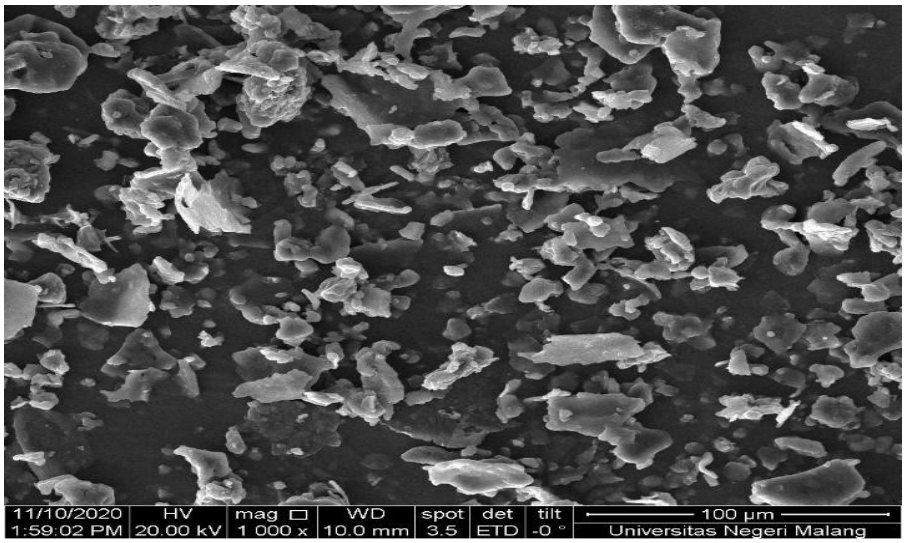

Figure 4. Scanning Electron Microscope (SEM-EDX) image of silver nanoparticle from *G. rugosa* (1000 x Magnification).

The silver nanoparticles tested exhibited a spherical or round shape, which has several advantages. The shape of nanoparticles influences cellular absorption, drug delivery speed, and targeting specificity. Round nanoparticles are especially favourable for drug delivery systems because they enable preferential interactions with specific proteins [33].

The Polydispersion Index (PDI) of *G. rugosa* silver nanoparticles was 0.135, indicating that the particles belong to the monodisperse category. This suggests a high degree of homogeneity within the sample. A PDI value of less than 0.3 confirms monodispersity, which is associated with better zeta potential value and more stable nanoparticles, as well as homogeneous morphology [26], [34], [35].

The determination of total flavonoid levels was conducted by measuring the absorption of pure *G. rugosa* extract and *G. rugosa* silver nanoparticles. Both samples were tested at a constant concentration of 1000 ppm after 30 minutes of incubation. The results of the total flavonoid levels are summarized in Table 1.

The total flavonoid test revealed that the absorption of samples within the samples within the spectrophotometric range was between 0.225 - 0.284. The total flavonoid content of *G. rugosa* silver nanoparticles was higher, measuring 36.21 mg QE/g extract, compared to 32.12 mg QE/g extract for the pure *G. rugosa* extract. The increased flavonoid content in the silver nanoparticles is attributed to the use of extract-based reducing agents during synthesis.

Flavonoids occur in various forms such as isoflavones, flavonols, flavones, and flavanones, each with diverse functions, including antioxidants, anti-inflammatory, antibacterial, and antifungal activities. Flavonoids can be further classified into subgroups such as flavones, flavonols, flavanones, flavonols (catechins), anthocyanins, and chalcones. As a secondary metabolite, flavonoids act as antioxidants by neutralizing free radicals

through the donation of a hydrogen atom, stabilizing oxidizing compounds. The mechanisms by which flavonoids counteract free radicals can be divided into three categories: (1) slowing the formation of formation of Reactive Oxygen Species (ROS), (2) neutralizing ROS, and (3) protecting cells through antioxidants regulation [3], [4], [36], [37].

Table 1. Total Flavonoid Content of Silver Nanoparticles *G. rugosa* (mgQE/g extract)

Sample	Replicated	Sample absorbance (1000 ppm)	Total Flavonoid (mgQE/g extract)	Average of Total Flavonoid (mgQE/g extract)
Extract of *G. rugosa*	1	0.242	32.935	32.12 ± 0.79
	2	0.233	32.093	
	3	0.225	31.346	
Silver nanoparticle *G. rugosa*	1	0.270	35.551	36.21 ± 0. 65
	2	0,284	36.860	
	3	0.277	36.206	

The mechanism of silver nanoparticles synthesis involves flavonoid and phenolic compounds acting as reducing agents. These compounds donate electrons or hydrogen atoms, reducing Ag+ to Ag0 via the keto groups in the flavonoid backbone [10], [38]. Biosynthesis using natural extracts enhances the total flavonoid levels of silver nanoparticles compared to pure extract. Flavonoids serve as capping agents on nanoparticles surfaces, with the diffusion of red algae extract into nanoscale particles potentially increasing flavonoids content [37].

Antioxidant Activity Using the DPPH Method

The antioxidant activity of *G. rugosa* extract and silver nanoparticles was assessed using the DPPH (2,2 diphenil-1-picrilhidrazil) method (Table 2). This method is widely used for its efficiency in analyzing numerous samples and detecting active components at low concentrations [5].

The maximum DPPH-free radical inhibitory activity of the extract was 80.83%, whereas in silver nanoparticles *G. rugosa* silver it was 90.13%. Silver nanoparticles using *G. rugosa* showed 10.03% higher inhibiting activity compared to *G. rugosa* extract. The maximum inhibition percentage of silver nanoparticles compared with the standard ascorbate is 90.88% (16 µ/ml).

The silver nanoparticles of *G. rugosa* demonstrated a smaller IC50 value (26,685 ppm) compared to the exact value of (46.12 ppm), indicating higher antioxidant activity. The IC50 of ascorbic acid (1,321 ppm) served as a benchmark. Silver nanoparticles of *G. rugosa* are effective in inhibiting a total of 50% of the free radicals of DPPH at a minimum concentration of 26.685 ppm. The antioxidant activity of *G. rugosa* silvers is classified as very strong based on IC50 values. The IC50 rating category is considered very strong when the value is ≤ 50; strong when 50 ≤ 100; while when 100 ≤ 150; weak when 150 ≤ 200; and when ≥ 200 is categorized as very weak [13], [39], [40].

Silver nanoparticles using *G. rugosa* as a bioreductant with the method of "green synthesis" can increase antioxidant activity at IC50 values. Silver nanocouples make use of bioactive compounds of red algae capable of increasing antioxidant activity and inhibition of free radicals DPPH, exceeding the activity produced by pure extracts/carcasses of red algae [29], [41]. Nanoparticle mediation using red algae has increased antioxidant activity by

50% and 67%, respectively. The use of algae extract is matched in the mediation of silver nanoparticle manufacturing [42], [43].

The increased antioxidant activity of silver nanoparticles of *G. rugosa* is presumed by the smaller particle size. The smaller the size of the particle obtained results in an increase in the surface area of absorption produced, so it is thought to be able to improve the effectiveness of performance as a drug. The increase in antioxidant activity is strongly influenced by phytochemical compounds that form capping agents on the surface of particles [13], [43].

Table 2. The Antioxidant Activity of Silver Nanoparticles *G. rugosa*

Sample	Concentration (ppm)	Percentage inhibition (%)	IC_{50}	Categorize
Extract of *G. rugosa*	50	54.72 ± 1.12	46.12 ± 1.6	Very strong
	75	58.85 ± 0.33		
	100	64.07 ± 0.71		
	125	71.47 ± 0.35		
	150	80.83 ± 1.93		
Silver nanoparticle *G. rugosa*	50	63.24 ± 0.90	26.65 ±1.44	Very strong
	75	77.46 ± 0.38		
	100	79.98 ± 0.16		
	125	86.54 ± 0.62		
	150	90.13 ± 0.28		
Ascorbic acid	1	42.99 ± 0.55	1.32 ± 0,3	Very strong
	2	56.33 ± 0.29		
	4	74.82 ± 0.21		
	8	81.74 ± 0.63		
	16	90.88 ± 0.50		

Conclusions

The synthesis of silver nanoparticles using *G. rugosa* resulted in a color change to yellow in the 570-580 nm wavelength range, with a maximum absorption wavelength of 406 nm. The total flavonoid content of silver nanoparticles *(*36.21 ± 0.65 mgQE/g) was higher than that of pure extract, indicating an enhancement in biological activity. Silver nanoparticles exhibited smaller IC50 value (26,685 ppm) than pure extract (6,128 ppm), classifying their antioxidant activity as very strong.

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Conflict of Interest

We have no conflicts of interest to disclose. All authors declare that they have no conflicts of interest.

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