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SINGLE-STEP GREEN SYNTHESIS OF GOLD NANOPARTICLES (GNPS) MEDIATED *Rhizopohra stylosa* LEAF EXTRACT AND ANTIBACTERIAL EFFECT

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ABSTRACT

This study investigated the single-step synthesis and antibacterial activity of gold nanoparticles (GNPs), produced using leaves aqueous extract of Mangrove *Rhizophora stylosa.* The reaction of the extract with chloroauric acid caused a reduction of auric chloride and the formation of GNPs in 60 minutes at room temperature. The size, shape, and elemental composition of the nanoparticles (NP) were studied using a transmission electron microscope (TEM) with an average particle size of 27 nm with the dominant shape being spherical and partly hexagonal, and trigonal. X-Ray Diffraction (XRD) analysis showed gold crystals with 4 diffraction peaks with PCC crystalline nature. UV-vis spectroscopy shows the SPR peaks found in the wavelength range of 530-546 nm. Furthermore, the NP biological capping agents identified by Fourier-transform infrared spectroscopy (FT-IR) were carbonyl, amine, and polyphenol. Leaf extract serves as an effective bio-reducing agent for the synthesis of GNPs. The results showed that the GNPs synthesized using *Rhizophora stylosa* mangrove aqueous extract had the potential for antibacterial activity.

Keywords: Single Step Synthesis, *Rhizopohra stylosa*, Gold Nanoparticles, Antibacterial.

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INTRODUCTION

Currently, the application of gold nanoparticles as a diagnostic method in the biomedical sector is highly developed because of the unique properties of nanomaterials including specific surface area, high surface activity, good biocompatible properties, and suitability for manipulation at the molecular level. Currently, the nanomaterials commonly used for biomedical replication are graphite, carbon nanotubes, quantum dots, magnetic nanoparticles, and metallic nanoparticles.[1] One of them is the use of gold in the form of nanoparticles which has many advantages, namely that it can be easily synthesized with various sizes and shapes. Gold nanoparticles are prepared in general in the form of colloid nanoparticles (NP), this approach involves reducing agents, stabilizers, and metal precursors.[2] The results of the latest literature survey show that metal NP synthesis through the green synthesis method is one of the best and safer methods that can benefit human well-being.[3] Gold nanoparticles have a biomedical effect, one of which is anti-antibacterial, but they have been produced chemically with the addition of other reducing agents[4] like *cetyltrimethylammonium 4-vinyl benzoate* as a reducing and capping agent.[5] In recent years researchers have successfully explored various types of ethnobotanical plants that are very important for NP production, one of which is mangroves.[6] Riau Archipelago, Indonesia is covered with mangrove forests that stretch along the coastline with various species. For a long time, mangroves have been known as traditional medicinal plants for coastal communities such as diarrhea, anti-inflammatory, antiviral drugs, and even for consumption.[7] Mangroves contain as many bioactive compounds as are scattered throughout the plant. Crude extracts of most mangrove species have been reported to show inhibitory activity against microorganisms and cancer cells, however, the bioactive compounds responsible for most of these activities have not been isolated.[8] In this study, gold nanoparticles were synthesized using single-step green synthesis, namely gold metal precursor added with *Rhizophora stylosa* mangrove leaf extract and single-step water solvent. The ability of mangroves to help reduction because they have components of

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hydroxyl, carbonyl, and amine compounds as active components in leaf extract.[9] *Rhizophora stylosa* leaf extract was used as a reducing agent and capping agent. $HAuCL_4$ concentration $3H_2O$ as a precursor was made constant by varying the amount of extract added to investigate the effect of the extract concentration on the properties of GNPs. Furthermore, this research was carried out by testing the antibacterial activity of gold nanoparticles. This is the first and novel report on the biosynthesis of gold nanoparticles using aqueous leaf extract of RS as a reducing and reducing agent.

EXPERIMENTAL

Materials

$HAuCl_4$ is prepared by dissolving 99.99% Au metal with 8 mL aqua regia to produce HAuCL4. $3H_2O$. The solution was diluted in a 100 mL volumetric flask with distilled water (DDW). The concentration of the gold solution used is 1mM. Healthy and fresh *Rhizophora stylosa* leaves are taken from mangrove forests along the coast of the Riau Islands. Clean leaves cut into small pieces and dried. *Rhizophora stylosa* extract was made by adding 10 grams of fine leaf powder and dissolving it in 100 ml of distilled water then heating it at a temperature of 65 ± 5 ºC for 1 hour. Furthermore, the extract was cooled and stored at 4ºC for further use.

Synthesis of Colloidal Gold Nanoparticle

The amount of the extract ratios, 0,5 mL, 1.5 mL, and 2.5 mL of the extract symbolized by GNPs-1, GNPs-2, and GNPs-3, respectively. The mixture of the extract and gold metal ion precursors with a total volume of 50 ml was stirred constantly at a speed of 500 rpm. The colloid nanoparticle mixture was observed at various time interval ranges. Powder samples for characterization tests were produced using the RS-GNPs colloid precipitation method.

The Characterization of Silver Nanoparticles

UV-Vis Spectroscopy was used to study Surface Plasmon Resonance (SPR) reduction of Au ions to Au nanoparticles and monitor the stability of the nanoparticles at certain time intervals. A small amount of sample aliquot was dissolved with distilled water, and maximum nanoparticle absorbance was monitored with a UV-Vis Shimandu-1800 spectrophotometer in the wavelength range of 300-800 nm. FT-IR spectroscopic analysis is used to identify possible bioactive compounds in nanoparticles and is responsible for the reduction and stability of the nanoparticles. FT-IR spectra of GNPs were recorded with the Parkin Elmer system Frontier S/ N 96772. The scanning wavelength of infrared was 4000-400 cm-1 at a resolution of 4 cm-1 and an interval of 1.0 cm-1. The shape and size of the synthesized nanoparticles were identified by TEM JEOL JEM 1400. X-ray diffraction (Philips X-pert powder PAN Analytical) analysis of GNPs was measured by h, k, and l values. The diffraction pattern is produced with 30kV, 30mA in Cu Kv radiation (λ = 1.5406 Å). The mean particle L is measured by the debye-Scherrer equation.

Antibacterial Activity Test

Antibacterial activity was tested by the good diffusion method. The synthesized GNPs colloid was observed for its antibacterial activity against pathogenic bacterial strains such as *Staphylococcus aureus* (S.A) and *Escherichia coli* (E.C). Nutrient agar is used to cultivate bacteria. Bacterial culture was taken as much as 100 µl inoculum and spread on nutrient agar (NA) media. The bacterial suspension was wiped on the nutrient agar (NA) plate using a sterile cotton swab. 50µL GNPs-2 and GNPS-3 were added to the well. Amoxicillin and DDW water were used as controls. The plates were incubated at 37° C for 24 hours. A positive test result is assessed when a zone of inhibition is observed around the well after incubation. All tests were performed in duplicate, and the results displayed are mean values.

RESULTS AND DISCUSSION

Ultraviolet-Visible Spectroscopy

GNPs with a diameter between 1 and 100 nm exhibit a unique optical phenomenon, known as surface plasmon resonance (SPR), which is the result of the collective oscillation of construction electrons on a metal surface in the presence of electromagnetic radiation. The color of the reaction mixture after adding

Rhizophora stylosa leaf extract to a trihydrate solution of Gold (III) chloride (1 mM) changes within 10 minutes from pale yellow to red wine.

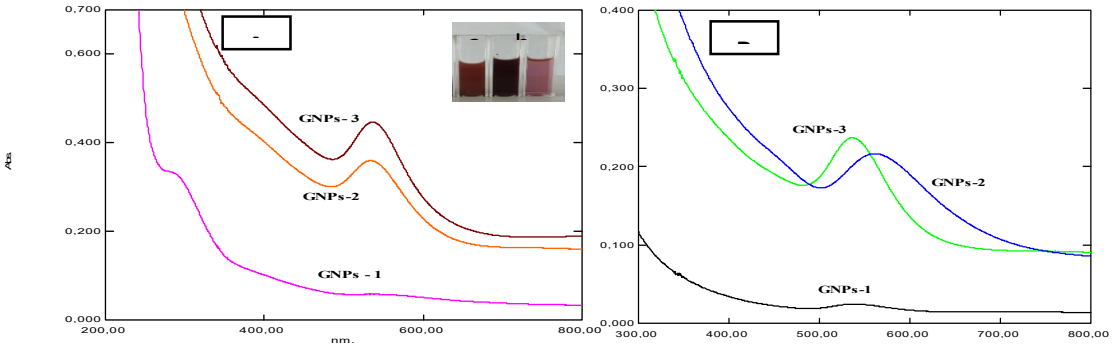


Fig.-1: A. Maximum Wavelength after 6 hours of Reaction (inset: change in color of gold solution for 0.5 hours) B. Stability of Gold Nanoparticles for 168 Hours of Reaction

The effect of adding the extract on the properties of the nanoparticles was investigated at a fixed $HAuCl_4.3H_2O$ concentration (1mM) to investigate the properties of the nanoparticles produced. After mixing the extract with aqueous $HAuCl_4.3H_2O$, the color of the solution quickly changed. The color change process is related to the stages of the nanoparticle formation process. The first step is the reduction of $AuCl^{4-}$ ions to Au atoms followed by nucleation and formation of small nanoclusters. The small nanocluster will be arranged and form a network into nanowires which will later form spherical particle fragments. During synthesis, the color will change progressively from pale yellow ($AuCl4^-$), to colorless (Au atoms) and to red wine or ruby red (GNPs) which shows the reduction of gold ions to gold nanoparticles.[9,10] According to Mie's theory, the color change in gold nanoparticles is caused by the excitation of surface plasmon vibrations[10] which also affects the size of the nanoparticles. In this study, the maximum absorption peaks for GNPs were observed at 535-546 nm in UV-Vis spectroscopy for all variations of extract additions, which are presented in Fig.-1A. Then there was a significant increase in absorbance according to the amount of extract added. The results of the study are similar to the research conducted by Filip GA, et.L 2019[11] using *Cornelian cherry* fruit extract with a maximum peak of 530 nm. The amount of mangrove leaf extract varies from 0.5 to 2.5 mL. For a low amount of extract (0.5 mL), the SPR GNPs-1 band showed low intensity and broad peaks, indicating a partial reduction of Au^{+3} ions. After increasing the amount of extract (1.5 mL), the SPR band of GNPs- 2 shifts to a longer wavelength (redshift), while when the extract is greater than 2.5 mL, there is a shift in wavelength to blue shift (GNPS-3). Observation of the stability of gold nanoparticles was carried out using a UV-Vis spectrophotometer based on the time function, as shown in Fig.-1B. A total of 2.5 mL of extract (GNPS-3) was found to be the optimal amount for reducing Au^{+3} ions. On the other hand, the addition of a smaller amount of mangrove leaf extract (0.5mL) caused the loss of the SPR Au NPs band, indicating agglomeration. This may be due to the more capping molecules in the nanoparticle synthesis, and the more stable the metal nanoparticles will be and maintain their size.[12] Nanoparticle size and morphology are closely related to the reduction and capping of the ability of plant extracts. In this case, the addition of 2.5 ml extract in the formation of gold nanoparticles, is the optimal composition to obtain GNPs with good size and stability.

Fourier Transforms Infrared Spectroscopy (FTIR)

FTIR is used to investigate biomolecules that act as reducing and capping. The FT-IR spectrum as shown in Fig.-2A from RS-GNPs shows a wide and strong absorption band at the peak of 3291.74 cm-1 which is the peak of the OH group (O – H stretching, H-bonded of alcohols, phenols, and N– H stretching of primary, secondary amines, amides of protein). The vibrational C-O bonds of alcohols and phenols were observed at the 1367.02 cm-1 peaks. Vibration (C-O-C) was observed at the peak of 1053.74. The peak of 1618.23 shows the peak of C = O. All bands seen in the FT-IR spectrum support the data that positive flavonoids, triterpenoids, steroids, and tannins contained in *Rhizophora stylosa* leaf extract act as capping and are responsible for the efficient stabilization of colloidal nanoparticles.[9] Flavonoids are polyphenolic compounds that are widely distributed in plants. Flavonoids are the main source of nutraceutical and

herbal medicine in plant biological activity.[13] From peak of FTIR showed 3291.74 cm^{-1} amide, 2146 cm^{-1} alkyne, 1618.23 cm^{-1} alkenyl, 1367.02 cm^{-1}`alkane, 1052.74 cm^{-1}, 873.96 cm^{-1}, and 640.62 cm^{-1} is an alkene group. FTIR measurements show that amides, amines, carboxylic acids, bases, aliphatic compounds, alkenyls, alkanes, and alkenes are potentially involved in the reduction of metal ions and their stabilization. Gold NPs can bind to proteins through free amine groups or carboxylate groups in proteins.[14]

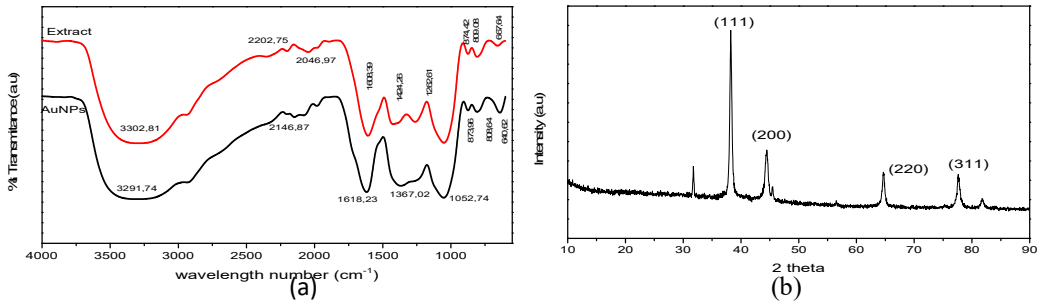


Fig.-2: (a) FTIR Spectrum of RS-GNPs (b) XRD RS-GNPs

Mangrove *Rhizophora stylosa* extract has been proven to contain rich secondary metabolites including polyphenols, alkaloids, and tannins[15–17] which exhibit antibacterial, and antioxidant properties. Other studies have also shown the involvement of amides, carboxylic acids, aliphatic amines, and amino acids present in the Leaf Extract of *Ziziphus zizyphus*[18] and gold nanoparticles in seaweed *Turbinaria ornata*[19] rich in flavonoids and phenolic.

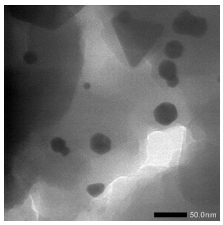
X-ray Diffraction (XRD)

XRD characterization aims to obtain information on the degree of crystallinity (determination of the crystal-amorphous structure) of a sample. Figure-2B shows the peaks of the nanoparticle diffraction pattern, which is shown at a value of 2θ 38.2332; 44.5694; 56.4770; 64.6776; and 77.576 with FWHM values of 0.3582, 0.5117; 0.4093; 0.3582; 0.5117 with a miller index of {111}, {200}, and {202} and {311} Miller index is a crystal lattice plane {hkl} representing the crystal system of material. The crystalline system of the gold nanoparticles is cubic. These data have been compared with standard Au metal data, and it is found that the peaks indicate the presence of gold nanoparticles with ICSD 611625. It is also evident from the spectra that 3 spheres (200), (220), and (311) are small compared to spheres (111). This shows that the synthesized nanoparticles are highly plane-oriented (111).

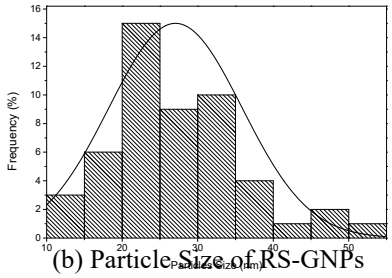
TEM

The morphology, shape, and size of GNPs synthesized using *Rhizophora stylosa* leaf extract are shown in Fig.-3a, 1 mM tetrachloroauric acid with RS leaf extract. The RS-GNPs from the TEM images are observed to be spherical and partly hexagonal and trigonal, and Fig.-3b shows the particle size distribution in the 11-54 nm range with an average particle size of 27 nm. The spherical shape of GNPs is a form that is very widely used in the structure of gold nanoparticles in drug delivery applications.[1] Other studies also explain that the spherical form is more toxic than other forms.[20] The extract mechanism behind the formation of NPs of different sizes and shapes is not fully understood. As previously reported, the formation of polydisperse properties of GNPs is possibly caused by the involvement of various reducing agents present in plant extracts. Similar to previous studies, the synthesis of GNPs using *Indigofera tinctoria* plant extract[21] produces spherical, triangular, and hexagonal particle shapes with an average particle size of 11-54 nm. Similar forms of gold nanoparticles were also found in research using the extract of *Uncaria gambir* Roxb.[22]

The transition from particle form to large particle distribution is due to thermodynamic processes. Due to the decrease in surface free energy, the size and morphology of the nanoparticles were strongly related to the reduction process and the capping ability of plant extracts. The ratio of the reducing agent is essential. If the reducing agent is in excess, the aggregate particles will easily form due to the high reaction rate and the inability of the capping ligands.[9]



(a) TEM Imege RS-GNPs



(b) Particle Size of RS-GNPs

Fig.-3: TEM Image of RS-GNPs: Antibacterial Effect

Table-1: Inhibition Zone of RS-GNPs

Bacteria	Inhibition Zone (mm)			
	(+) Control	(-) Control	GNPs-2	GNP_S-3
Staphylococcus aureus	4.1	-	4.1	4.6
Escherichia coli	4	-	5.3	5.2

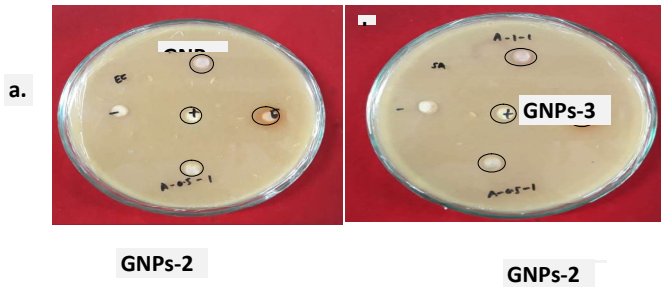


Fig.-4: Antibacterial Activity of RS-GNPs a). *Escherichia coli* b). *Staphylococcus aureus*

The results showed that the difference in the concentration of the addition of the extract did not show a significant difference in the bacterial inhibition zone. Many studies have found that gam-positive (*Staphylococcus aureus*) bacteria are more resistant to the mechanism of action of nanoparticles. Gram-negative like *Escherichia coli,* bacterial cells are covered by a layer of lipopolysaccharides (1-3 µm) thick and peptidoglycan ~ 8nm, this helps regulate the entry of ions released by nanoparticles into cells.[23-27] Similar research was also found in research by Aljabali A, *et al.* 2018.[18]

CONCLUSION

In this work, we describe a single-step, simple, fast, and reproducible method for friendly GNPs synthesis without the need for expensive, reducing agents. The gold ion was chemically reduced to NP with *Rhizophora stylosa* leaf extract, which is rich in phenolic compounds which have been identified through various characterization techniques. Simple incubation of the leaf extract with aqueous gold ions at room temperature produced nanoparticles with an average size of 27nm, indicating that the plant extract acts as a strong reducing agent. The synthesized NPs have excellent stability and biocompatibility. The results of this study emphasize that GNPs produced with *Rhizophora stylosa* leaf extract have the potential to be produced on a large scale for commercial use.

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