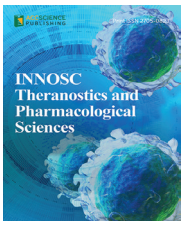
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RESEARCH ARTICLE

# Conjugation of Silver and Gold Nanoparticles for Enhancing Antimicrobial Activity

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**Abstract:**

Silver and gold nanoparticles are promising agents that can enhance the antibacterial activity of conjugated/capped extract of plant compounds. The aim of our study is to synthesize silver nanoparticles from *Gymnema sylvestre* plant extract. In this study, the antimicrobial activity of conjugated silver which was green synthesized and gold nanoparticle that was obtained from a commercial source was evaluated using disk-diffusion method against *Escherichia coli* and *Aspergillus niger*. Transmission electron microscopy, scanning electron microscopy, ultraviolet-visible spectroscopy, and Fourier transform infrared spectroscopy analyses were also carried out to characterize the conjugated silver and gold nanoparticles. The conjugation of silver and gold nanoparticles was performed chemically using 16-mercaptodecanoic acid. Our results revealed that conjugated silver and gold nanoparticle both showing larger inhibition zones against *E. coli* and *A. niger*. The minimum inhibitory concentration (MIC) of the above metallic conjugation was found to be stable with MIC was reported to be 1 mM. When comparing the sample analysis on conjugation of gold and silver nanoparticles against *E. coli* and *A. niger*, the antimicrobial activity recorded was 2.0 ± 0.01 and 2.32 ± 0.04 cm, respectively, with no significance difference was found ($P = 0.85$). In conclusion, the combination of two different nanoparticles is efficient for microbial inhibition and can be useful for studying antimicrobial activities with the combination of different nanomaterials in future.

**Keywords:** Nanomaterial, Pathogen, Inhibition, Surface morphology

## 1. Introduction

Nanotechnology is one of the fastest developing branches of technology which produce the particles with improved characteristics, properties, and mechanism for industrial purposes. The nanoparticle increases the membrane permeability and accumulation of nanoparticles in the bacterial membrane hinders the development of resistance by bacteria [1]. Besides, nanotechnology is one of the important fields in the research dealing with the synthesis, strategy, and structure of nanoparticles ranging from approximately 1 – 100 nm in size [2]. It has been demonstrated that the shape and size of the nanoparticles depend on the physiochemical properties using advanced techniques [3]. Nanotechnology has been used around the world and exhibits uniqueness, such as good conductivity,

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good chemical reaction, and antimicrobial property. Nanotechnology deals with several nanoparticles, which includes gold and silver nanoparticles. Nanoparticles can be potentially produced by microorganism and plant extract [4]. Silver and gold nanoparticles are widely used in the research dealing with biology, physical or chemical methods. Moreover, silver nanoparticles are usually used to control bacterial growth due to its antimicrobial effects [5].

The green synthesis of nanoparticles by plants is a greener approach that interconnects nanotechnology and plant biotechnology. Several plants and plant products are successfully used for efficient and rapid extracellular synthesis of silver and gold nanoparticles [6]. Green synthesis generally refers to the production of materials without involving toxic compounds. The advantages of green synthesis are that they contain hazardous-free material and the chemical usage during the production is not dangerous to the human, plants, and animals. Apart from that, green synthesis method is one of the simplest method that does not involve much cost for the procedure and it is more efficient too [7]. Furthermore, the utilization of biological method, such as using the plant extract to produce a nanoparticle, is an eco-friendly process as they are free from chemicals that cause harmful to the environment.

Silver nanoparticle can be utilized in a variety of applications such as in medical devices, soap, and detergent-making due to its unique antimicrobial properties. Green synthesis has been proven to be the best method for using biological sources and producing the nanoparticles. The use of biological materials, for example, plant extract, bacteria, fungi, and enzymes for the synthesis of silver nanoparticles is suitable for pharmaceutical and other biomass applications as they do not involve the usage of toxic chemicals [1]. Furthermore, it has been reported that silver nanoparticles have the ability to inhibit the growth of bacteria, virus, and other eukaryotic microorganisms. In this context, they are also termed as new-generation antimicrobial agents [8] where they are used not only in consumer products such as cosmetics, textiles, dietary supplement, and food packaging but also used medicinally against multidrug susceptible bacterial strains and for surgical coatings and medical implants [9].

In the early of 20$^{th}$ century, the gold nanoparticle has fascinated the scientific community due to its unique phytochemical and biological properties [10]. Plant extracts are used for metal ions bioreduction to produce nanoparticles. It has been demonstrated that plant metabolites such as sugars, terpenoids, polyphenols, alkaloids, phenolic acids, and proteins play an important role in metal ions reduction into nanoparticles and in supporting their subsequent stability [11].

Conjugation is a process by which two materials are connected. Nowadays, researchers are performing different conjugation methods with silver and gold nanoparticles to assess their antimicrobial activities with other materials. Conjugation with a silver nanoparticle is commonly performed because it is useful for a wide range of biological applications. Several methods that include passive adsorption and covalent bonding are used to produce a stable conjugation. Although gold nanoparticle has its own unique characteristics, such as rich in surface chemistry and low toxicity, it is not often used in the conjugation process due to it is highly costly to produce a final product [12-14]. Taken together, it can be concluded that conjugation method is safe and easy to use when combining with different materials. In our study, we used the extracted compounds from *Gymnema sylvestre* to conjugate on the nanoparticles, as *G. sylvestre* is one of the potential plants that contain antidiabetic and antimicrobial properties. We aimed to further explore the antimicrobial potential of the extract by including stable release of these compounds through conjugating gold and silver nanoparticles [15,16]. This study also complements the earlier studies performed using the extracts of *Ziziphora tenuior* [8] and *Memecylon edule* [9] to attest the importance of compounds from the plant extract.

## 2. Materials and methods

### 2.1. Chemical and equipment

Luria Bertani (LB) agar and LB broth media were purchased from Thermo Scientific (USA). Gold nanoparticle was purchased from Sigma-Aldrich (USA). Disposable petri dishes are from MyMedio (Malaysia). Other reagents used were of analytical grade and all reagents were stored as recommended by the manufacturer. *G. sylvestre* was obtained

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from Institute of Sustainable AgroTechnology Universiti Malaysia Perlis, Perlis. The procedure of the experiment was divided into several parts such as preparation of the plant extract and silver nanoparticles that were capped with plant extraction; preparation of gold nanoparticle that was commercially purchased; the conjugation process between gold and silver nanoparticles, was characterized through Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and ultraviolet-visible spectroscopy (UV-Vis). Agar plate for antimicrobial activity against *Escherichia coli* and *Aspergillus niger* was prepared accordingly. [17]. *A. niger* was subcultured into potato dextrose agar (PDA) and incubated at 30°C for 7 days hours while *E. coli* was subcultured into nutrient agar (NA) and incubated at 30°C for 24 h.

### 2.2. Preparation of plant sample and extraction process

Fresh leaves of *G. sylvestre* were collected and washed thoroughly in running water to remove dust particles and debris. Dust particle and debris were removed using Tween-20. 100 µL of 0.05% Tween-20 was used to wash the plant sample. After washing the plant sample with Tween-20, the sample was rinsed in the beaker filled with distilled water. The sample was then left overnight at 35°C for 24 h. This sample was used for the extraction process. The plant sample was then rinsed, and a hot plate with a magnetic stirrer at 55°C was used for heating the solution with plant sample. The solution was left for 1 h to make the plant extract solution to be cooled. The strainer was then used to filter the bark and leaf extract solution and removed other suspended particles. The filtered solution was then filtered again by using a 0.45 µm microfilter to obtain an uncontaminated bark and leaf extract solution. The bark and leaf extract solution was kept in 4°C for silver nanoparticle synthesis.

### 2.3. Synthesis of silver nanoparticles

Synthesis of silver nanoparticles was performed on completion of the plant extraction processes. About 0.085 g of silver nitrate ($AgNO_3$) was taken and dissolved in 25 ml of distilled water. *G. sylvestre* was capped with the silver nanoparticle. About 0.5 ml of bark and leaf extract was added with 2.5 ml of silver nitrate solution. After that, 4 ml of distilled water was added and was mixed with plant and bark extraction solution and $AgNO_3$. The tube was covered with aluminum foil and was homogenized gently. After 10 min, the $AgNO_3$ changed its color from light yellow to brown color. The reaction mixture was then centrifuged at 10,000× *g* for 10 min using a microcentrifuge. After the centrifuge process, the supernatant was discarded, and the pellet of silver nanoparticle remained in the tubes. 500 µl of the supernatant was added to the Eppendorf tubes and the pellet was washed thoroughly. After that, distilled water was added and centrifuged again at 10,000× *g* for 10 min. After the final washing, the pellet was dried and stored at 4°C for further experiment.

### 2.4. Conjugation of silver nanoparticles and gold nanoparticles

Gold nanoparticles were bought from the commercial source. Gold nanoparticles were functionalized with 5 mM of 16-Mercaptohexodecanoic acid (16-MHA). When gold nanoparticle was mixed with 16-MHA, the surface of the gold nanoparticles was produced with tethered with carboxylic groups on functionalized. After the mixture of 16-MHA and gold nanoparticles, 5 ml of silver nanoparticles that capped with plant extract conjugate was added. During conjugation of silver and gold nanoparticles, both particles are linked by 16-MHA. These conjugated particles were evaluated by analytical characterization and the antimicrobial activity of these conjugated particles was assessed.

### 2.5. Characterization of silver nanoparticles, gold nanoparticle and conjugation of silver and gold nanoparticles by FTIR, SEM, TEM, and UV-Vis spectrophotometry analysis

A sample of plant extract capped silver nanoparticles, gold nanoparticle, and conjugation of gold and nanoparticles were evaluated using FTIR, TEM, SEM, and UV-Vis spectrophotometry. After the process of the conjugation of silver and gold nanoparticles, three different samples first evaluated by SEM. All the samples were observed under SEM to characterize the size and the structure. The magnification of image was seen with 20× and 15 kV power. After SEM observation, these samples were analyzed using FTIR. The purpose of FTIR

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analysis is to determine the functional groups of the samples. The range of the infrared region used is between 1600 and 400 $cm^{-1}$. The sample was then scanned, and the data was collected at each of the scanning. UV-Vis spectrophotometry analysis was used to determine the absorbance level of three different samples. These three different samples were placed in the cuvette that contained 5 ml of samples each and placed them into the UV-Vis spectrophotometry for result observation.

### 2.6. Standardization of microbial suspensions

A standard microbial suspension was prepared at a concentration of $2 \times 10^7$ CFU/ml. First, microbial suspension which had been incubated overnight was calculated for the total number of the colonies per ml (CFU/ml) using hemocytometer. The suspension was then adjusted to the desired concentration by diluting with nutrients broth or inoculates with bacteria. CFU/ml of microbial suspension = (average of colonies/$2.5 \times 10^{-7}$/ml) × (1/dilution factor).

### 2.7. Disk diffusion method

Agar well diffusion method was used to determine the antimicrobial effect of green synthesized silver nanoparticles and conjugated silver and gold nanoparticles against the strains of *E. coli* and *A. niger.* Approximately 2 ml from the bacteria and fungi stock suspension ($2 \times 10^7$ CFU/ml) were taken and thoroughly mixed with sterilized 25 ml of NA and PDA. This solution was poured into the sterilized Petri dishes and allows to solidify. After that, 4-disc papers (sterile) were used by placing on the PDA and NA plates using the forceps. Then, 20 µL of samples were transferred to each of the discs that placed in the petri dish. The plate was incubated in an upright position at 30°C for 24 h for bacteria and 3 – 7 days for fungi. The plant extract, silver nanoparticles that capped with plant extraction, conjugated gold nanoparticles, and control (antibiotic) were transferred onto the surface agar. Three replicates were used against each tested organism. The primary mechanism involved in this experiment is by diffusion. On adding equal volumes on the discs there will be a uniform diffusion and bacterium cannot survive in the diffused areas and the clear zone varies with the inhibition ability of samples.

### 2.8. Determination of the minimum inhibitory concentration (MIC)

The MIC values of conjugation silver and gold nanoparticle were examined using standard broth dilution method. The MIC was determined by using serial dilutions of silver nanoparticles and gold nanoparticles with adjusted bacteria concentration to $2 \times 10^7$ CFU/ml. Seven dilution factors of synthesized silver nanoparticle were prepared and tested against *E. coli* and *A. niger*. Approximate 3 mL volumes for each of the dilution factor were transferred to the tubes, accordingly, containing 12 mL. The positive control used contained nutrient broth medium with tested bacterial concentration and negative control contained only inoculated broth and the time and temperature of incubation being 24 h at 33°C. The MIC was noted by visual turbidity of the test tube before and after incubation. All values were calculated from the replicates and averaged by the standard deviation.

### 2.9. Statistical analysis

The antimicrobial potential with the conjugation of different samples ($n$ = 5) was calculated for the probability. The analysis done was against the bacterial growth. For this calculation, values from different experiments were considered and omni online software was used.

## 3. RESULTS

### 3.1. Synthesis of silver nanoparticle

In this study, *G. sylvestre* was used to produce silver nanoparticle by capping with silver nitrate solution. The $Ag^+$ ions are reduced to Ag nanoparticles as the process occurred. **Figure 1A** shows that the color changes with 1 mM silver nitrate and plant extract *G. sylvestre.* The prepared silver nanoparticle was tested for antimicrobial activity alone and by conjugating with gold nanoparticle.

### 3.2. Antimicrobial activity

#### *3.2.1. Disc diffusion assay*

In this study, disk diffusion assay was carried out to examine the antimicrobial activity of the plant extract, silver nitrate, silver nanoparticle, and conjugation silver and gold nanoparticles against *E. coli* and *A. niger*. The conjugation of silver and gold

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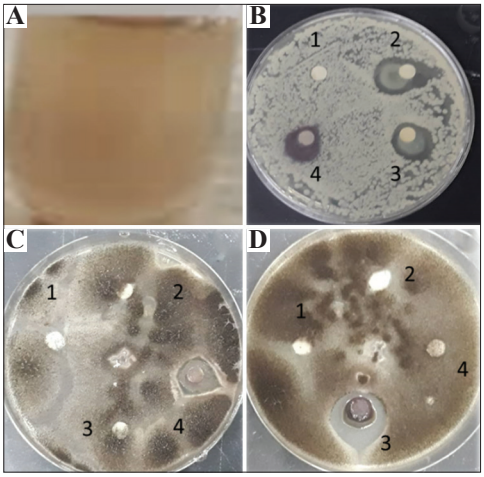



**Figure 1.** (A) Color changes with silver nitrate solution on addition of plant extract. (B) Inhibition of (1) blank, (2) conjugation silver nanoparticle and gold nanoparticle, (3) silver nanoparticle, (4) plant extract of *Gymnema syvelstre* on *Escherichia coli.* (C) Inhibition: (1) Blank; (2) plant extract; (3) silver nitrate; (4) silver nanoparticle. (D) inhibition: (1) blank; (2) plant extract; (3) conjugated silver and gold nanoparticle, and (4) gold nanoparticle on *Aspergillus niger* inoculated agar plate.

**Table 1.** Evaluation of antibacterial activity of different samples used to determine the clear zone

| **Concentration of *Gymnema syvelstra* (1 mM)** | **Diameter of inhibition zone (mm)** | |
|---|---|---|
| | ***Escherichia coli*** | ***Aspergillus niger*** |
| Plant extract | 1.3±0.01 | 1.12±0.01 |
| Silver nitrate | 1.6±0.01 | 1.25±0.01 |
| Silver nanoparticle | 1.8±0.02 | 1.45±0.01 |
| Gold nanoparticle | 0 | 0 |
| Conjugated silver & gold nanoparticle | 2.0 ± 0.01 | 2.32 ± 0.04 |

*Data represent means±standard error at 95% (*n*=3–6). Mean that to do not share letter are significantly different using Turkey's Honest significant different test. *P*=0.85

nanoparticles exhibited the excellent antimicrobial activity against *E. coli* and *A. niger* (**Figure 1B-D**). **Table 1** shows that the zone of inhibition of plant extract of *G. syvelstre,* synthesized silver nanoparticle, gold nanoparticle, and conjugation of silver and gold nanoparticle against *E. coli* and *A. niger*. The results show that different types of samples exhibit the admirable zone of inhibition.

Synthesized silver nanoparticle from 1 mM is significant compared with 2 mM. Both concentrations resulted in different inhibitions with the inhibition decreases as the concentration increases. Furthermore, at higher concentration, the synthesized silver nanoparticle was able to inhibit the bacterial growth, but a lower concentration it induces them to synthesize silver nanoparticle. The optimum concentration for biosynthesis silver nanoparticle occurred at a concentration of 1 mM silver nitrate.

Antimicrobial activity of plant extract of *G. sylvestre*, silver nanoparticle, gold nanoparticle and conjugated silver and gold nanoparticles against pathogens, *E. coli* and *A. niger* was evaluated. The gold nanoparticle was availed from the commercial source, while silver nanoparticle was from silver nitrate capped plant extract of *G. sylvestre.* From **Table 1**, the conjugation of silver and gold nanoparticles showed the largest clear zone compared to other three samples and exhibited the best antimicrobial activity, followed by synthesized nanoparticle and silver nanoparticle alone.

The synthesized silver nanoparticles showed the larger zone of inhibition compared to silver nitrate solution and plant extract. In this study, the silver nanoparticle concentration used was low, that is 1 mM, the result showed that it slightly increased the zone of inhibition when reduced the concentration of silver nitrate. The evaluation of antimicrobial activity of synthesized silver nanoparticles against *E. coli* showed that this pathogen can grow at lower concentration because exposure of metal ions can induce toxicity. Further, it was demonstrated that the AgNP at 20 µg/disc showed the moderate antibacterial activity against *E. coli*, indicated by a diameter of inhibition zones of 1.8–2.8 mm. Among the pathogenic bacteria, AgNP were more active against *E. coli* compared to other bacteria that always been used, for example; *Bacillus subtilis*.

The clear zone appears to be larger if silver nanoparticle conjugated with gold nanoparticle was used. Gold nanoparticle reacts with the capped silver nanoparticle by the linker 16-mercaptodecanoic acid (16-MHA). 16-MHA helps to conjugate these two nanoparticles and makes the clear zone more obvious against *E. coli* and *A. niger*. The clearance zone size is larger than silver nanoparticle with a difference more than 2.8 mm. This shows that the conjugation of silver and gold nanoparticle slightly increased the zone of inhibition compared to the silver nanoparticle only, which shows a better inhibitory process.

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**Table 2.** Determination of minimum inhibition concentration

| Dilution factor | *Escherichia coli* | *Aspergillus niger* |
|---|---|---|
| Initial sample | – | – |
| $10^{-1}$ | + | – |
| $10^{-2}$ | + | – |
| $10^{-3}$ | – | – |
| $10^{-4}$ | – | – |

Positive (+): With turbidity shows the growth; Negative (–): No turbidity in the absence of growth

### *3.2.2. Minimum inhibition concentration (MIC)*

MIC of the synthesized silver nanoparticle (1 mM) was determined in this study. **Table 2** shows that the bacterial growth of 1 mM synthesized silver nanoparticles after incubation at 37°C for 24 h. The turbidity of the sample was observed, and this indicated the growth of microbes. For *E. coli*, the turbidity was observed with three-fold dilution (dilution factor $10^{-2}$). For the turbidity of *A. niger*, it can be observed that only one-fold of dilution is required. The presence or absence of the turbidity was denoted as positive (+) and negative (–), respectively. The biological effect of silver nanoparticle is believed to be closely related to the silver ions. In addition, silver nanoparticle shows a stronger bactericidal effect than larger particles as they have a larger surface of interaction.

## 3.3. Characterization of conjugated silver and gold nanoparticles

### *3.3.1 FTIR analysis*

FTIR spectroscopy analysis was carried out in this experiment. The purpose of FTIR is to reveal the functional groups of three different samples, which are plant extract (*G. sylvestre*) capped with silver nitrate (silver nanoparticle), gold nanoparticle and conjugation of silver and gold nanoparticles. **Figure 2** shows that the FTIR spectrum of three different samples as stated above. The spectrum of the silver nitrate capped with plant extract, *G. sylvestre* showed three different peaks, which were at wave number 3259.1, 2156.33, and 1634.09 $cm^{-1}$. The highest peak with this sample was 3259.1 $cm^{-1}$, which is characterized as O-H group of phenolic and alcoholic molecules. The silver nanoparticle in this sample was stabilized by hydrophilic flavonoids, which came from the natural components of the plant extract. Further research found the hydrophilic flavonoids at range 3307 $cm^{-1}$. The sample of silver nitrate capped with *G. syvelstre* plant extract is near to previous research. The absorption band of this sample is at 2156.33 $cm^{-1}$, corresponding to the O-H stretching with a carboxylic acid. The weakest peak of the sample was 1634 $cm^{-1}$ located as a functional group of alkynyl C=C stretch. This peak was the weakest because of amide-I bond protein was combined. Besides that, this weak peak was caused by secondary stretching vibrated in the secondary structure of the proteins. This variation of the absorption bands can result in a bioreduction process that was catalyzed by the phenolic compounds [18]. The plant extract of *G. sylvestre* showed that there is a protein present in the plant extract. Silver nitrate capped with plant extract acts as reducing agents and stabilizer for the silver nanoparticle to prevent agglomeration.

The spectrum of gold nanoparticles also showed three different peaks and at similar wavelength with the silver nanoparticle. Three peaks of the gold nanoparticle are 3254, 2140, and 1634.32 $cm^{-1}$, respectively. The highest peak of the gold nanoparticles is at 3254 $cm^{-1}$, which is located at O-H group of the phenolic and alcoholic molecules. This peak indicative of bonded –OH hydroxyl group. The middle peak was at 2140 $cm^{-1}$ and the lowest peak of the gold nanoparticles was at 1634.32 $cm^{-1}$, which means that it corresponds to the asymmetric stretching of C=O group stretching. The functional groups identified were alcohol/phenol O-H stretch, amide C=O stretch, and alkynyl C=C stretch (**Figure 2A-C**).

### *3.3.2. SEM analysis*

The morphology of three samples have been observed by SEM. **Figure 3A** shows the SEM images of the silver nanoparticle that already capped with *G. sylvestre* plant extract. Uniform shapes can be seen with the silver nanoparticle while for the gold nanoparticle, the shape was spherical. In this experiment, only one type of shape was found in gold nanoparticle because the gold nanoparticle was produced by chemical synthesis (**Figure 3B**). It is just solely a gold nanoparticle and does not react with another material such as plant extract. Furthermore, the bactericidal activity of gold nanoparticle relies much on the size of the particle. SEM analysis confirmed the conjugation of silver

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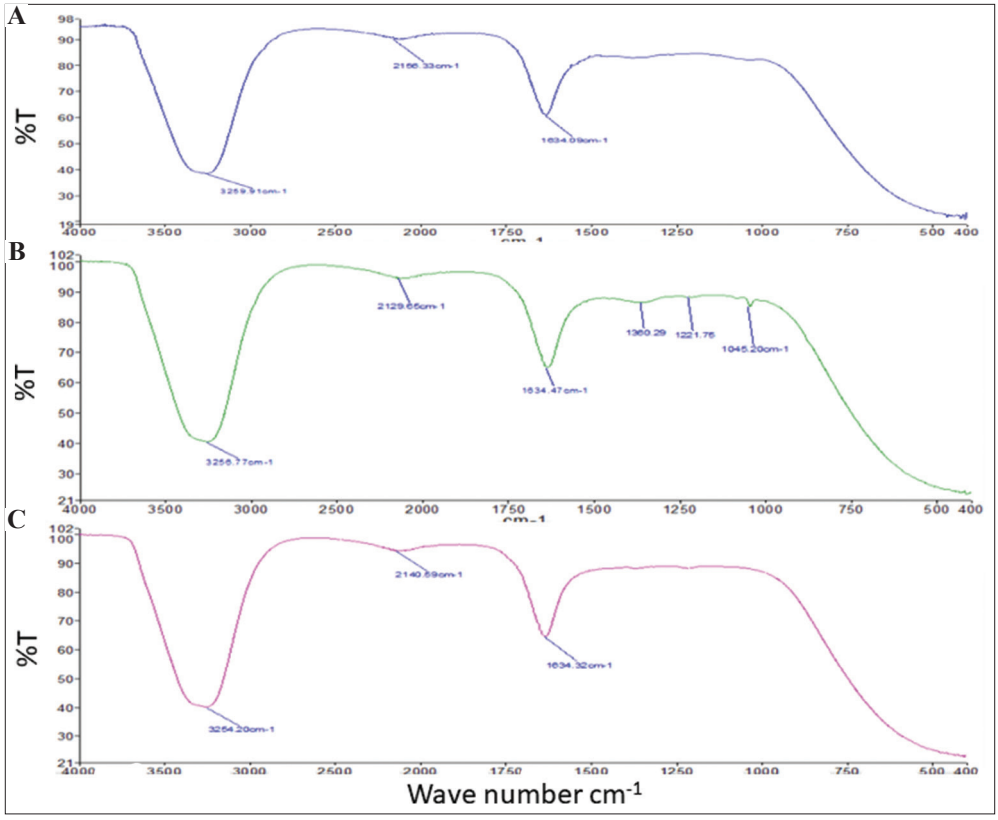



**Figure 2.** FT-IR spectrum of (A) plant extract *Gymnema sylvestre* capped with silver nitrate (silver nanoparticle), (B) gold nanoparticle, (C) conjugation silver and gold nanoparticle.

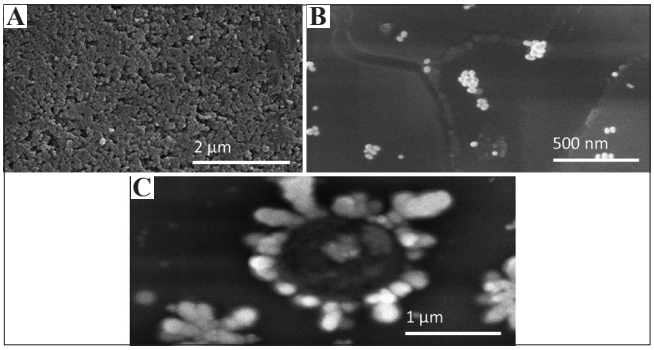



**Figure 3.** Scanning electron microscopy analysis of (A) silver nanoparticle, (B) gold nanoparticle, (C) conjugated silver and nanoparticle.

and gold nanoparticles. Types and shapes found on the conjugated silver and gold nanoparticles were spherical. This implies that the conjugation of silver and gold nanoparticle can be achieved with the help of 16-MHA. 16-MHA plays the main role to help silver and gold to conjugate within a good condition. When different samples are conjugated such as metal with metal, the size shape can be varied (**Figure 3C**). The metal has different types of shapes that can be seen under SEM analysis especially when the metal reacts with natural sources such as plant extract.

### *3.3.3. TEM*

TEM utilizes the energetic electron that can provide morphological, compositional, and crystallographic information on the sample. TEM also provide a higher resolution, black, and white images from the interaction that occurred in the sample. In this study, two samples that include silver nanoparticle and gold nanoparticle. Were investigated using TEM. The size of the silver nanoparticle was 20 and 100 nm under TEM analysis. From that range of size, it was shown that, silver nanoparticle contained uniform silver in the sample. Based on the scale given, the size of the silver nanoparticle is 25 nm which indicates the presence of silver nanoparticle in that particular size (**Figure 4A**). One of the characteristics of silver nanoparticle that can be observed under TEM was polycrystalline. This characteristic can be seen under selected area of electron diffraction. For the gold nanoparticle, the size that had been investigated through TEM analysis was 30 nm with scale 20 nm and 1 µm (**Figure 4B**). Through TEM analysis, the size and shape of the gold nanoparticle were poly-dispersed and spherical in shape. Overall, both the samples, silver nanoparticle and gold nanoparticle

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have different sizes which can be seen under TEM analysis.

### *3.3.4. UV-visible spectroscopy*

UV-visible is used to investigate three different samples, including silver nanoparticle, gold nanoparticle, and conjugated silver and gold nanoparticle. The function of UV-Vis is to analyze the chemical structure of the substance and measure the intensity of the light, which is proportional to the wavelength. Silver nanoparticle formation has been observed under UV-vis spectroscopy analysis. The absorption of silver nanoparticle is at 430 nm (**Figure 5A**). For the gold nanoparticle, the highest spectrum was observed at 520 nm (**Figure 5B**). For the conjugation silver and gold nanoparticle, the absorbance of the sample shows the higher absorbance compared to gold nanoparticle (**Figure 5C**).

## 4. Discussion

The synthesis of nanoparticles using plant extract, particularly in herbal plants is gaining attention due to the presence of active phytochemicals in the plants [6,19,20]. In this study, the extracted compounds from *G. sylvestre* were used to synthesis the silver nanoparticles. One of the approaches that can be used to detect the presence of silver nanoparticle is by color identification. Color identification is one of the visual observations to confirm the formation of silver nanoparticles. The color of the solution will change from faint light to yellowish brown indicates the formation of silver nanoparticles and the color will intensify with increasing amount of the extracts. The color change from yellowish to brown color in the aqueous solution is due to the excitation of the surface plasmon vibration in silver nanoparticles [21]. This is essential and will course the vibration of the group conduction of the electron. The surface plasmon membrane vibration was found when a metal particle is exposed to the light, induce a collective coherent oscillation of the free electrons (conduction band electron) of the metal. The electron oscillation is around the particle surface causes a charge separation with respect to the ionic lattice, forming a dipole oscillation along the direction of the electric field of light. The surface plasmon vibration induces a strong absorption of the incident light and thus can be measured using a UV-Vis absorption spectrometer. The surface plasmon vibration band is much stronger for plasmon nanoparticle (noble metal, especially gold and silver) than other metals.

The functionalized silver and gold nanoparticles are considered as the universal antimicrobial substances that can produce a good clearance zone for antimicrobial activity. However, the use of silver nanoparticles as antimicrobial agents is limited due to interfering effects of silver nanoparticles and antimicrobial mechanism of silver ions from the metal. The limitation with silver nanoparticles as antimicrobial agents can be overcome using

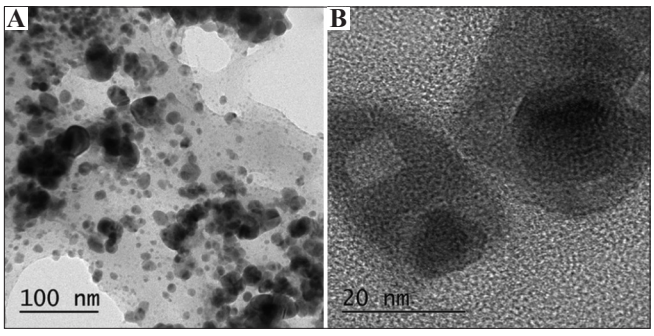



**Figure 4.** Transmission electron microscopy analysis on (A) silver nanoparticle (B) gold nanoparticle.

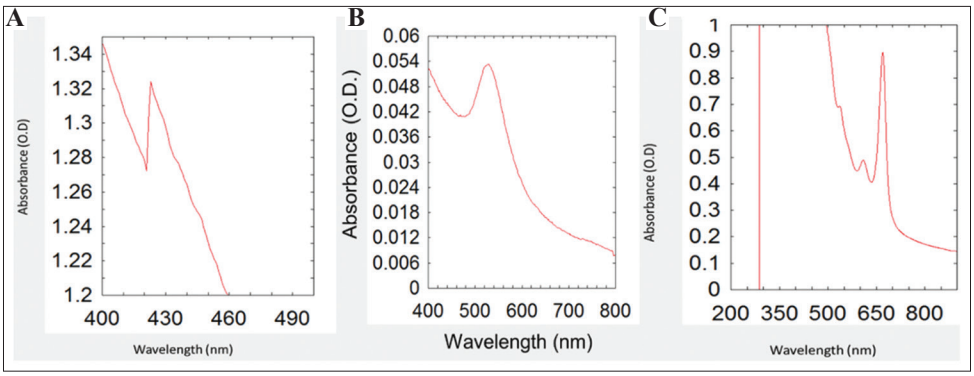



**Figure 5.** Ultraviolet-visible analysis of (A) silver nanoparticle, (B) gold nanoparticle, (C) conjugation silver and gold nanoparticle.

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synthesized silver nanoparticle. This can be applied using the biological method from the natural sources such as the compounds from plant extract which is considered to be environmentally friendly technique. Silver nitrate may also induce apoptosis (death of cell) that may occur at the same time with the normal and control treatments on the bacterial growth. Ahmad *et al*. [22] reported the antibacterial activity of silver nanoparticle against *E. coli* and antifungal activity against *A. niger*. In their study, the synthesized nanoparticle had the greatest antimicrobial activity against *E. coli*. They also claimed that, the higher the concentration of silver nanoparticle, the lesser its antibacterial and antifungal activity.

**Table 1** revealed that clearance zone of the antibacterial *E. coli* is larger than the antifungal zone of inhibition of *A. niger*. This was supported by Ahmad *et al*. (2016) who claimed that the clearance zone against *E. coli* usually resulted in the diameter range between 1.1 and 1.8 mm compared to *A. niger* where the clear zone is lesser than 1 mm with the same amount of sample that was dropped on the disc (20 μg/disc). The growth curves of bacterial cell treated with different concentrations of silver nanoparticle also differ. The growth curves of bacterial cells treated with AgNP indicate the growth of bacterial cells. The growth curve of *E. coli* treated with 100 and 150 μg/ml silver nanoparticle was shown to inhibit the growth of *E. coli*. Interestingly, on comparison of the bacterial curves, the growth curves of silver nanoparticle-treated bacteria indicated a faster growth inhibition compared with other antibacterial agents. Overall, the present study highlights the antibacterial activity of conjugated silver and gold nanoparticle, and synthesized silver nanoparticle and gold nanoparticle are promising agents against plant pathogen. The green-synthesized silver nanoparticle is a good source, which is easier to produce and extensively useful. Furthermore, the gold nanoparticle can also be easily purchased. So that, these two nanoparticles can be conjugated as a perfect combination for preventing the microbial activity of pathogenic microbes with the help of lower concentration of the silver nitrate capped with *G. sylvestre* plant extract.

With the characterization, the size of nanoparticles used in this study was in the range of 20 – 100 nm. This was supported by Jyoti and Singh (2016) [23], they claimed that the pure silver nanoparticle contained in the sample ranges from 20 to 100 nm. FTIR was also performed to characterize the surface chemical groups on the prepared nanoparticles. In the previous study, it was confirmed that presence of hydroxyl and carboxyl groups in the gold nanoparticle. However, with the third sample of the conjugated silver nanoparticle with the gold nanoparticle showed six different peaks with FTIR spectrum at the same wavelength as in other samples. The peaks that were collected from the experiment are 3256.77, 2129.65, 1634.42, 1360.29, 1221.75, and 1045.20 $cm^{-1}$. The highest peak that presents in this sample was displayed at 3256.77 $cm^{-1}$, while the weakest peak is at 1045.20 $cm^{-1}$. From this variation of the peak, it can be concluded different types of functional group were present, indicating different types and characteristics. According to Jyoti and Singh (2016), the authors reported that silver nanoparticle had a single orientation which they can form a cluster of the silver particle. Apart from above morphological and surface functional characteristics, optical study was done with UV-Vis spectrophotometer analysis. The peak showed a good surface Plasmon's band center. This statement was supported by Jyoti and Singh (2016) and claimed that the best surface Plasmon band center is at 450–700 nm. The brownish color is formed when it is capped with plant extract *G. sylvestre* with silver nitrate. This means that the surface plasmon vibration is present in the process and clearly showed that the capping agents are involved in this process. The absorbance wavelength of gold nanoparticle is longer than a silver nanoparticle. This is because the difference in the absorbance that causes the wavelength of silver nanoparticle is different compared to the gold nanoparticle. The surface plasmon band was showed at the absorption of conjugation silver and gold nanoparticle. This statement was supported by Jyoti and Singh [21], where they stated that whenever the conjugation process happened, the excitation of the surface of plasmon membrane will occur. The entire nanoparticle can stay in a good condition for 4 – 6 months and this was confirmed by the UV-Vis analysis. The biogenic synthesis of silver nanoparticle usually needs more than 12 h for the process to complete.

## 5. Conclusion

The aim of this project is to evaluate the conjugation of silver and gold nanoparticles by using *G. sylvestre* plant

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extract. The silver nanoparticle had changed its color when reacting with plant extract of *G. sylvestre*. The antibacterial properties of silver and gold nanoparticle were performed by disc diffusion method on *E. coli* and *A. niger*. The samples used include plant extract, silver nanoparticle, gold nanoparticle, and conjugated silver and gold nanoparticles. This result proved that conjugation of silver and gold nanoparticle showed the biggest clear zone of inhibition compared to other samples. MIC has been performed to determine the lowest concentration of sample that can inhibit the growth of the microorganism. *G. sylvestre* plant used in this study pose huge beneficial effects including antimicrobial, antioxidant, anti-diabetic, wound healing, and gastro-protective properties. Synthesized silver nanoparticles are better than the extract of *G. sylvestre* in terms of inhibition. These beneficial properties indicate that plant extract of *G. sylvestre* has a great potential for development in a wide range, for example, in health and food supplements. Besides, it can participate in several down-stream applications and inter-disciplinary sciences in nanotechnology.

## Conflict of interest

There is no conflict of interest to declare.

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