

Effect of Volume of Gold Chloroauric Acid on Size, Shape and Stability of Biosynthesized AuNPs using Aqueous *Elaeis guineensis* (Oil Palm) Leaves Extract

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ABSTRACT

Biosynthesis of gold nanoparticles (AuNPs) using plant extracts has been receiving considerable attention of researchers due to their environmental friendly, low cost and renewability features. AuNPs were successfully synthesized at ambient conditions using aqueous *Elaeis guineensis* (oil palm) leaves extract without addition of any chemical agent. UV-Vis spectrophotometer, DLS and TEM were used to characterize the phytosynthesized AuNPs. FTIR was carried out to identify the functional groups involved in reduction of gold ions and stabilization of AuNPs. Hydrodynamic diameter was found to be reduced from 69 ± 39.80 nm to 55.22 ± 42.86 nm with an increase in the volume of gold chloroauric acid (1.53 mM) from 1.0 mL to 2.0 mL with fixed volume of oil palm leaves extract respectively. Size enlargement was observed from 60 ± 43.76 nm to 123.5 ± 110 nm with further increase in the volume of gold chloroauric acid from 3.0 mL to 5.0 mL respectively. Zeta potential (surface charge) of AuNPs was found to be reduced from -20 ± 6.80 mV to -15 ± 6.15 mV with an increase in the volume of gold chloroauric acid from 1.0 mL to 5.0 mL respectively. TEM results showed the formation of well scattered spherical, triangular and nonspherical shaped AuNPs by using 3.0 mL of gold chloroauric acid with an average particle diameter of 36.84 ± 9.0 nm. FTIR results revealed the role of phenolic, carboxylic and amides functional groups in reduction of gold ions to form AuNPs and their stabilization. Hence, polydisperse AuNPs synthesized using *Elaeis guineensis* (oil palm) leaves with alterable morphology and good stability can be efficiently employed in medical and industrial applications.

Keywords: Gold nanoparticles; *elaeis guineensis* (oil palm); hydrodynamic diameter.

INTRODUCTION

Nanomaterials have injected a revolutionary inspiration in almost every field of life due to their outstanding physiochemical characteristics than bulk materials. Metallic nanoparticles are playing a vital role in electrical equipment [1], heat transfer applications [2, 3] and grinding machines productivity [4]. Synthesis of various metallic nanoparticles including gold, silver platinum titanium dioxide, palladium iron and copper have been widely dealt by researchers because of their indispensable role in industrial applications [5-11]. However, AuNPs have gained special position among all other metallic

nanoparticles due to their historic medicinal applications like cancer and arthritis treatment [12, 13].

Conventionally, AuNPs are being synthesized through physical, chemical methods with well-defined characteristics. AuNPs synthesized through aforementioned protocols become unfit for medical applications and economically unfeasible due to involvement of hazardous reagents and expensive equipment in their synthesis procedures [14, 15]. AuNPs are also being synthesized through biological resources including plants extracts and microbes [16, 17]. However, biological synthesis of AuNPs through microorganisms is reported to contain various operation impediments, such as long time required to synthesize nanoparticles, critical conditions for growth of microbes and inflexible process conditions [18]. Hence, there is an urgent need to develop ecofriendly and cost-effective mode of synthesis to form AuNPs in order to meet their exponentially increasing industrial applications. Keeping in view, plants and their extracts have shown a great potential to synthesize AuNPs due to their low cost, renewable, biocompatible and environmental friendly features. Recently various plant extracts have been successfully used to synthesize AuNPs employing green chemistry-based principles [19-22]. Plants are reported to contain various phytochemicals such as phenolic compounds, flavonoids, carbohydrates and terpenoids with active hydroxyl, aldehyde and carboxyl functional groups, which hold the capability to bioreduce the gold ions stabilize them after their formation [23].

In Malaysia, *Elaeis guineensis* (oil palm) is an important industrial crop. In the last 20 years oil palm industry has been emerged as an important part of Malaysian economy. Oil palm industry is generating a huge amount biowaste in form of oil palm trunks, oil palm fronds, empty fruit bunches and oil palm leaves, which are creating the severe disposal problems to environment [24].

Elaeis guineensis (oil palm) leaves, a major portion of oil palm bio-waste are trimmed down from plants in harvesting season and left on ground to decompose for nutrient enhancement of soil. Oil palm leaves are reported to contain various important phytochemicals such as phenolic acids, glycosylated flavonoids and catechins, which have shown their potential for medicinal applications [25, 26]. The presence of abovementioned bio-compounds in oil palm leaves has explored its potential to be employed as a strong candidate for green synthesis of metallic nanoparticles [27]. Hence, no longer decaying of oil palm leaves to increase nutrition value of soil can be afforded in presence of more profitable alternative utilizations.

In our previous study, successful biosynthesis of AuNPs using aqueous *Elaeis guineensis* (oil palm) leaves extract with spherical shaped morphology and excellent stability has been reported [28]. Hence, current research work was planned for the first time, to study the influence of volume of gold precursor with constant volume of oil palm leaves extract on size, morphology and stability of AuNPs using aqueous *Elaeis guineensis* (oil palm) leaves extract, which can be useful to obtain nanoparticles with controllable morphology for potential applications.

METHODS AND MATERIALS

Materials

Gold chloroauric acid with 99.99 % purity was procured from Sigma-Aldrich Malaysia. Stock solution (12.69 mM) was made by dissolving 500 mg of gold chloroauric acid in

100 mL of deionized water. Gold chloroauric acid of required concentration was obtained by doing suitable dilution of stock solution. *Elaeis guineensis* (oil palm) leaves free from fungal infections were brought from a local oil palm mill Felcra Berhad Nasaruddin Oil Palm Mill located in Perak Malaysia.

Preparation of Aqueous Oil Palm Leaves Extract

Elaeis guineensis (oil palm) leaves were washed with distilled water and dried under sun light for one week. Dried oil palm leaves were washed again with distilled water and oven dried at 70 °C for 8 h. Oven dried oil palm leaves were grinded using IKA@ grinder to obtain fine powder. Aqueous oil palm leaves extract was made by mixing 1.0 g of oil palm leaves powder in 20 mL of distilled water at 70 °C for 10 min and performing gravity filtration using whatman no 40 filter paper. Filtrate was kept as oil palm leaves extract at 4 °C for additional investigations.

Characterization

The Absorption spectra of biosynthesized AuNPs was obtained by using Perkin Elmer *UV-Vis* spectrophotometer in range of 400 to 700 nm. Particle size distribution and morphological behavior was studied using Transmission Electron Microscopy (TEM) LIBRA 200FE. Hydrodynamic diameter and stability study was measured by using Dynamic Light Scattering (DLS) Malvern Zetasizer Nano ZSP. The Identification of functional groups involved in reduction reaction and stabilization of AuNPs was done through Perkin Elma spectrum in 450 to 4500 cm^{-1} .

Synthesis of AuNPs

AuNPs were successfully synthesized by stirring the reaction mixture containing 2.0 mL of aqueous oil palm leaves extract, gold chloroauric acid (1.53 mM) with an addition 5 mL of distilled water at room temperature (30 °C), 500 RPM for 60 min. The volume of gold chloroauric acid was varied from 1 mL to 5 mL keeping amount of oil palm leaves (2 mL) constant

RESULTS AND DISCUSSION

Role of volume of gold precursor on size, shape and stability of AuNPs was investigated by changing volume of gold chloroauric acid (1.53 mM) from 1 mL to 5 mL keeping volume of aqueous oil palm leaves extract constant. The progress in formation of AuNPs was monitored by scanning the reaction mixture in range of 400 nm to 700 nm. Reaction solution containing 2 mL of oil palm leaves extract, 5 mL distilled water and 2 mL of gold chloroauric acid (1.53 mM) changed its color from light yellow to light pink at room temperature (30 °C) after 8 min of stirring due to surface plasmon resonance (SPR) [29]. The color change confirmed the formation of AuNPs due to reduction of gold ions through phytochemicals present in oil palm leaves extracts [30]. The *UV-vis* spectra also showed the SPR peak between 500 nm to 550 nm, which further confirmed the occurrence of reduction reaction to synthesize AuNPs.

Our previous study has confirmed the completion of reduction reaction after 60 min of reaction time to synthesize AuNPs using aqueous oil palm leaves extract [31].

Hence, reaction solution was stirred at room temperature for 60 min at 500 RPM to get complete conversion of gold ions into gold atoms. The *UV-vis* spectra showed a broader SPR peak using 1.0 mL of gold chloroauric acid (1.53 mM), which revealed the formation of larger size AuNPs. The SPR peaks were found to be increased with further increase in volume of gold chloroauric acid from 1 mL to 3.0 mL as shown in Figure-1. The higher SPR peaks observed for larger volume of gold chloroauric acid (1.0 mL – 3.0 mL) could be due to an increase in formation of AuNPs due to higher amount of gold ion present in reaction mixture [32]. The *UV-vis* spectra peaks started to decrease with further increase in volume of gold chloroauric acid from 4.0 ml to 5.0 mL most likely due to unavailability of sufficient amount of bio-compounds to convert all gold ions into gold atoms as shown in Figure 1 [33]. Similar results were also reported by Nazar Ul Islam et al. [34], where reduction in SPR peaks were observed for using more than 15 mL of gold chloroauric acid with constant volume of *Salix alba* leaves extract (1.0 mL).

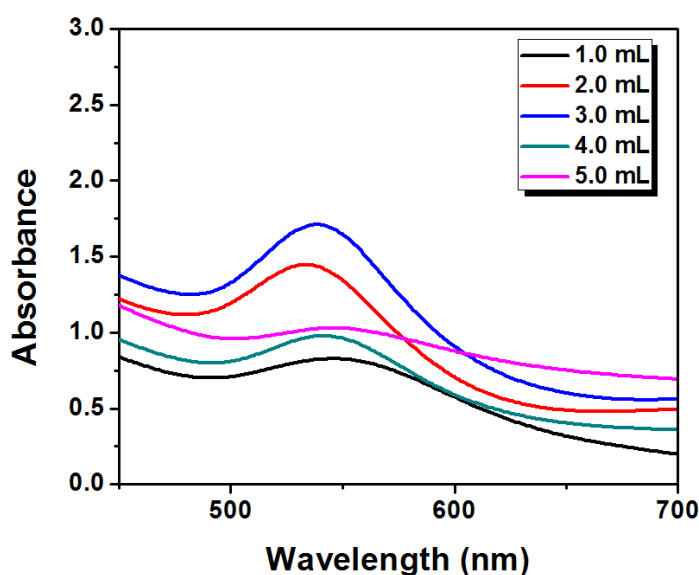


Figure 1: *UV-Vis* spectra against different volume of gold chloroauric acid keeping oil palm leaves extract [30].

The *UV-vis* spectra results showed a shift in maximum wavelength towards lower wavelength region with an increase in volume of gold chloroauric acid from 1 to 2 mL, which revealed the formation of smaller size AuNPs as shown in Figure 2 [35]. It might be due to presence of sufficient amount of reducing and stabilizing agents in reaction solution to convert all gold ions into AuNPs and stabilize them. Further increase in volume of gold chloroauric acid from 3.0 to 5.0 mL exhibited the shifting of maximum wavelength towards higher wavelength region due to formation of larger size AuNPs as shown in Figure 2 [36]. It could be due to unavailability of enough amount of reducing and stabilizing agents left in reaction mixture to form and stabilize AuNPs.

Dynamic Light Scattering (DLS) is used to measure size of AuNPs along with thickness of bio-compounds capped on surface of nanoparticles. DLS results showed reduction in hydrodynamic diameter from 69 ± 39.80 nm to 55.22 ± 42.86 nm with an increase in volume of gold chloroauric acid from 1.0 mL to 2.0 mL as shown in Figure 2 respectively. Further increase in volume of gold chloroauric acid from 3.0 to 5.0 mL

caused size enlargement of AuNPs from 60 ± 43.76 nm to 123.5 ± 110 nm respectively. Hence, DLS measurements showed the complete agreement with *UV-Vis* spectra trend regarding size variations of AuNPs.

Zeta potential is considered to play an important role in determining the stability of AuNPs. Hence, the stability of AuNPs was determined through zeta potential (surface charge) analysis. It was found that zeta potential value reduced from -20 ± 6.80 mV to -15 ± 6.15 mV with an increase in volume of gold chloroauric acid from 1.0 mL to 5.0 mL respectively as shown in Figure 3.

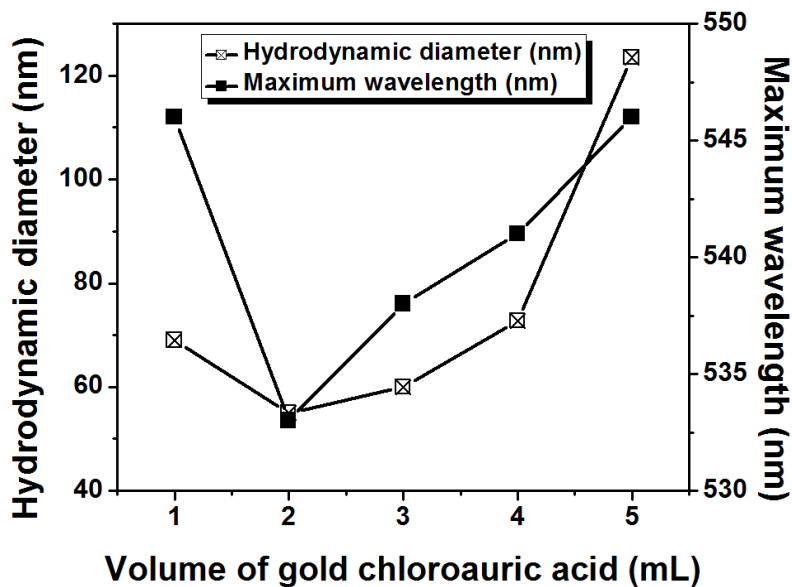


Figure 2: Change in hydrodynamic diameter and maximum wavelength with increase in volume of gold (1.53 mM) solution

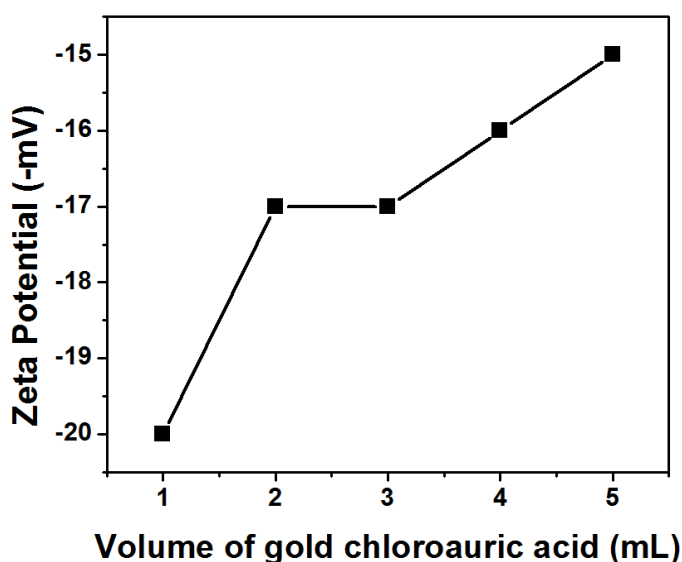


Figure 3: Change in zeta potential with function of volume of gold chloroauric acid (1.0 - 5.0 mL)

It could be due to reduction in amount of available reducing and stabilizing agents in reaction mixture with continuous increase in volume of gold chloroauric acid [37], as the SPR peaks were increasing with an increase in volume of gold chloroauric acid from 1 to 3 mL as shown in Figure 1. Hence, it is quite evident that amount of capping agents present in reaction mixture were continuously consuming in order to avoid aggregation of AuNPs. Negative values of zeta potential revealed the formation of negatively charged AuNPs. Electrostatic repulsive forces among negatively charged AuNPs may stop the aggregation of nanoparticles resulting enhancement of stability [38].

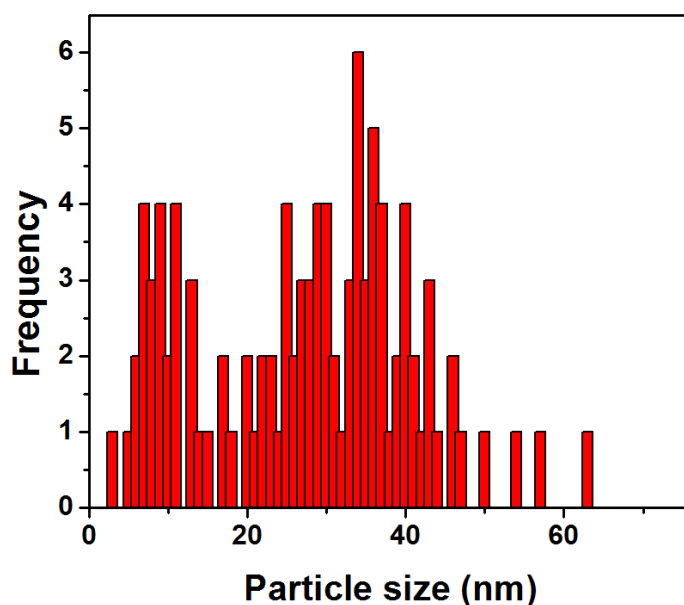


Figure 4: Size distribution histograms of AuNPs synthesized using 3.0 mL of gold chloroauric acid with 36.84 ± 9.0 nm of average diameter calculated from HRTEM images

Morphological behavior and particle size distribution of AuNPs was done through Transmission Electron Microscopy (TEM). TEM size measurements showed formation of polydisperse AuNPs with an average particle diameter of 36.84 ± 9.0 nm using 3.0 mL volume of gold chloroauric acid as shown in Figure 4. The difference in particle size measurements done through TEM and DLS might be due to dissimilarity in their working principles. TEM measures the exact size of AuNPs excluding thickness of bio-compounds present on surface of AuNPs. TEM images revealed formation of spherical, nonspherical and triangular shaped AuNPs were formed by using 3.0 mL of gold chloroauric acid as shown in Figure 5. Formation of nonspherical AuNPs might be due to presence of less amount of stabilizing agents left in reaction mixture. Few spherical shaped AuNPs were also formed by using 3.0 mL of gold chloroauric acid.

The shape of AuNPs is of great importance due to their shape dependent characteristics and applications [39]. Spherical shaped AuNPs can play an important role in drug delivery and antibacterial activity applications due to their high surface area to volume ratio [40]. Nonspherical shaped AuNPs can also be utilized in catalytic applications [41]. Hence, it is evident from the TEM images that the biosynthesized AuNPs using oil palm leaves (oil palm biowaste) with different morphology can be efficiently utilized in various suitable medical applications. The presence of bio-

compounds capped on surface of AuNPs preventing them from aggregation can also be seen in TEM images as shown in Figure 5 (a) and (b).

FTIR results showed the possible involvement of functional groups taking part in reduction of gold ions as well as stabilization of AuNPs. A broader and intense peak observed at 3390 cm^{-1} as shown in Figure 6, (C) is due to O-H stretching vibrations indicating presence of phenolic, and alcoholic functional groups based compounds in oil palm leaves extract respectively [42]. FTIR spectra revealed a sharp peak at 1639 cm^{-1} exhibited stretching vibration of C=O, N-H indicating existence of carboxylic group and amides [43, 44].

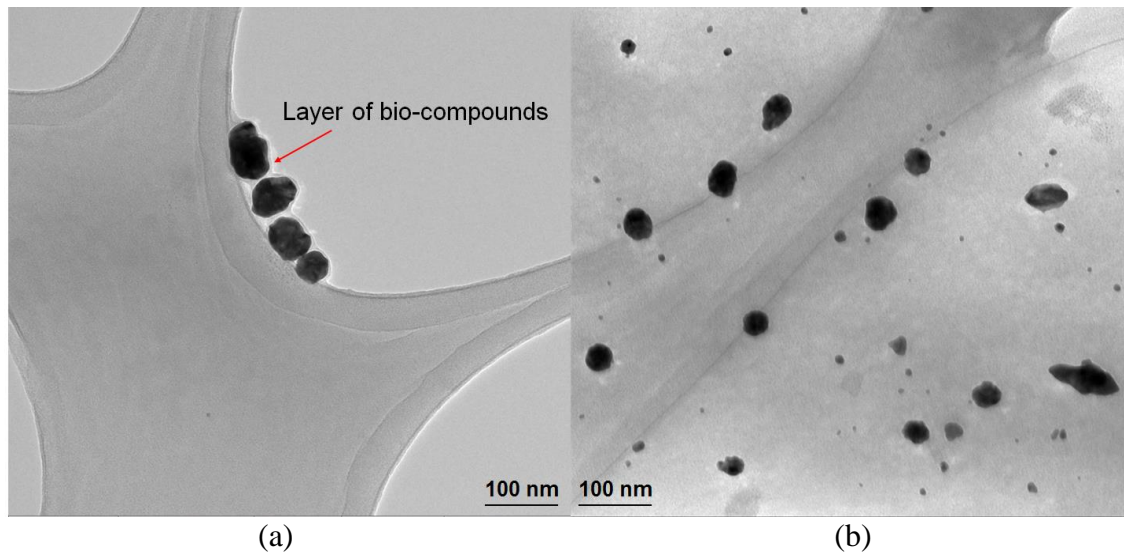


Figure 5: TEM images (a) and (b) of AuNPs obtained using 3.0 mL of gold chloroauric acid.

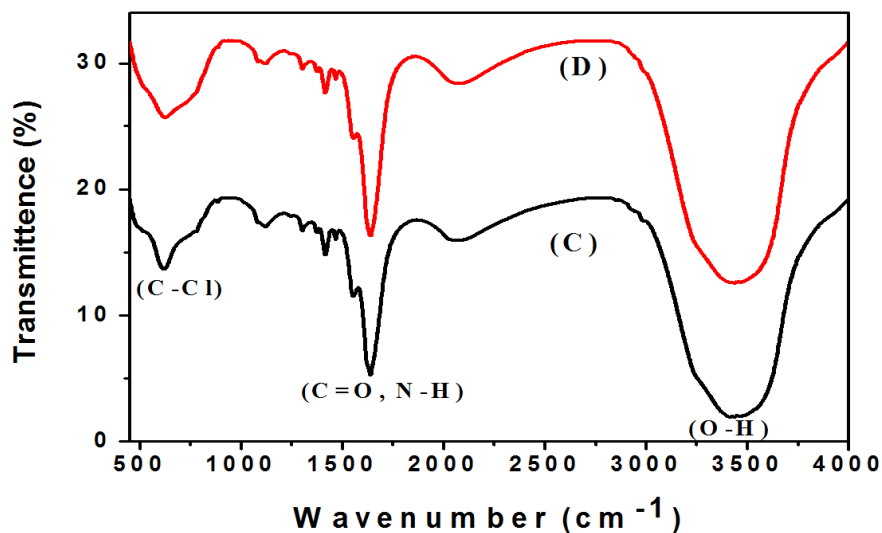


Figure 6: FTIR spectra of (C) oil palm leaves extract and; (D) reaction solution

A smaller peak was also observed at 1466.62 cm^{-1} indicates presence of -NH amines stretch vibrations in protein amides [45]. Peak observed at 1372.38 cm^{-1} is

produced due aromatic amines present in aqueous oil palm leaves extract [46]. FTIR spectra results of aqueous *Elaeis guineensis* (oil palm) leaves extract revealed the existence of phenolic, alcoholic, carboxylic, amides-1 of proteins, alkynes and aromatic functional group based compounds as reported in previous studies [25, 36, 47, 48]. After synthesis of AuNPs, reduction in intensities of peaks showed possible involvement of above mentioned functional groups in reaction synthesis and stabilization of AuNPs. Maximum reduction in intensities of FTIR peaks was detected at 3390 to 3412.21 cm^{-1} and 1639 to 1636 cm^{-1} after reaction respectively, which could be due possible involvement of phenolic, alcoholic, carboxylic, amides and proteins in synthesis as well as stabilization of AuNPs as shown in Figure 6, (D).

CONCLUSION

AuNPs were successfully synthesized by using aqueous *Elaeis guineensis* (oil palm) leaves extract. Volume of gold chloroauric acid has a significant impact on shape, size and stability of AuNPs keeping volume of oil palm leaves extract constant. Hydrodynamic diameter was decreased from 69 ± 39.80 nm to 55.22 ± 42.86 nm with an increase in volume of gold chloroauric acid (1.53 mM) from 1.0 mL to 2.0 mL with constant volume of oil palm leaves extract. Addition of higher volume of gold chloroauric acid from 3.0 mL to 5.0 mL showed size enlargement from 60 ± 43.76 nm to 123.5 ± 110 nm respectively. Zeta potential (surface charge) of AuNPs was decreased from -20 ± 6.80 mV to -15 ± 6.15 mV with an increase in volume of gold chloroauric acid from 1.0 mL to 5.0 mL respectively. Spherical, triangular and nonspherical shaped AuNPs were formed using 3.0 mL of gold chloroauric acid (1.53 mM) with average particle diameter of 36.84 ± 9.0 nm. FTIR results showed possible involvement of phenolic, alcoholic, carboxylic, proteins and amide functional groups in reduction of gold ions and stabilization of AuNPs. Biosynthesized AuNPs using one form of oil palm biowaste at room temperature with well-defined morphology, shape and stability can be utilized as strong candidate in various medical applications.

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