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BIOLOGICAL SYNTHESIS OF Ag-Au ALLOY NANOPARTICLES USING *Cola nitida* EXTRACT AND ITS APPLICATIONS AS ANTIOXIDANT, LARVICIDAL, AND DYE-DEGRADING AGENT

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Abstract

Seed extract of *Cola nitida* were used to synthesize bimetallic nanoparticles (Ag-Au alloy). Stable nanoparticles of the alloy were formed in a ratio of 20:80 respectively, by adding 2 ml of HAuCl_4 to 8 ml of AgNO_3 . Seed extract was used as the reducing agent of Au^{3+} to Au^0 and Ag^+ to Ag^0 . Ultraviolet-visible spectroscopy was used to monitor the formation of alloy particles. Synthesized alloy nanoparticles were characterized with Scanning electron microscope (SEM), energy dispersive x-ray spectroscopy (EDX) and Fourier transform infrared spectroscopy (FTIR). In the larvicidal assay, varying concentrations of Ag-Au alloy nanoparticles was prepared (60, 80, and 100 $\mu\text{g/ml}$) by dispersing the nanoparticle in sterile distilled water to which mosquitoes at larvae stage were exposed. Methylene blue, malachite green and congo red dyes were used in the dye degradation assay. In the antioxidant assay, different gradient concentrations of DPPH (20, 40, 60, 80 and 100 $\mu\text{g/ml}$) was prepared and mixed with 1 ml of synthesized alloy nanoparticles. This study presents an environmentally friendly method of biological alloy nanoparticles production. It also gives a much faster rate of synthesis in comparison to the chemical methods and potentially can be used in various human contacting areas such as food and medical applications.

Keywords: Biosynthesis, Plant extract, *Cola nitida*, Green synthesis, Ag-Au nanoparticles

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Introduction

Several methods including chemical, physical and biological methods have been employed in the synthesis of nanoparticles (Iravani *et al.*, 2014; Gudikandula and Maringanti, 2016; Elias and Saravanakumar, 2017). Chemical approach, such as chemical reduction,

electrochemical techniques, and photochemical reduction is most widely used, but these methods seem to be hazardous, toxic and costly (Song and Kim, 2009; Patra and Baek, 2014; Kuppusamy *et al.*, 2016; Khan *et al.*, 2017). Metals have also been used extensively

in the synthesis of nanoparticles (Song and Kim, 2009; Mody *et al.*, 2010), but composite materials (Tanahashi, 2010; Khan *et al.*, 2017) have also in recent times been the more focused which has proven to have better results than individual metals. However, there have recently been renewed interests in the synthesis of nanoparticles using biological methods (Raveendran *et al.*, 2006) that involve using plant or plant extracts (Shankar *et al.*, 2004; Lateef *et al.*, 2015a, 2016a), microorganisms (Konishi *et al.*, 2011; Li *et al.*, 2011; Iravani, 2014; Lateef *et al.*, 2015b; Singh *et al.*, 2016) and enzymes (Willner *et al.*, 2006; Lateef and Adeeyo, 2015; Lateef *et al.*, 2015c; Adelere and Lateef, 2016; Elegbede *et al.*, 2018a, b; Elegbede *et al.*, 2019).

Biological means of nanoparticles production is the eco-friendliest means of synthesis as compared to chemical and physical methods (Sastry *et al.*, 2004; Sharma *et al.*, 2009). Ag-AuNPs have been synthesized using biological extracts (Lateef *et al.*, 2016b; Ojo *et al.*, 2016; Elegbede *et al.*, 2019). Among other biological processes, it can be more advantageous when plant extracts are used in nanoparticles synthesis, by getting rid of the ambiguous cell culture maintenance processes. This is not only environmentally friendly, safe for use, but also cheap.

Cola nitida is from the family Malvaceae, mostly found in the rainforests of tropical West Africa. Caffeine is a major constituent in the seeds and is chewed as a stimulant and also employed in the production of soft drinks. *Cola nitida* seeds, shells, as well as pods have also found application in biosynthesis of Ag nanoparticles and reported to yield encouraging outcomes with outstanding antibacterial properties yielding good results with remarkable antibacterial properties (Lateef *et al.* 2015a; Lateef *et al.*, 2016b, c).

Furthermore, bimetallic alloys are of state-of-the-art due to the possibility to manipulate the optical and electronic characteristics as a subject of the alloy composition (Mallin and Murphy, 2002). Metallic nanoparticles are important in modern materials science research owing to their chemical, physical and biological properties (Jain *et al.*, 2008). Bimetallic alloy nanoparticles of silver and gold have drawn so much attention of researchers as quite a handful of synthesis have yielded Ag-Au composite nanoparticles (Chen *et al.*, 2006; Shah *et al.*, 2009). The preparation of bimetallic silver-gold nanoparticles typically involves a mixture of AgNO₃ and HAuCl₄ which are reduced either simultaneously or subsequently.

However, only distinct techniques will synthesize alloy nanoparticles and not core-shell particles, as a result of phase separation. One of the methods employed in the production of Ag-Au bimetallic alloy nanoparticles include varying the ratio of the component metals (Ag-Au) from 25:75, 50:50, 75:25, to 80:20 (Daima *et al.*, 2012).

Metallic nanoparticles have vast potential applications in biomedical, optical and electronics fields. Nanobiotechnology also serves as a means of developing efficient procedures to produce different types of nanoparticles. It is highly recommended that this field of study be explored more, for several prospective applications in health care, cosmetics, food and feed and biomedical sciences. In this study, seed extract of *C. nitida* was used to produce Ag-AuNPs.

Materials and Methods

Collection of Materials

Cola nitida seeds (Vent.) Schott & Endl. were collected from Ogbomoso, Nigeria and air-dried (30 ± 2 °C) for two weeks. The seeds

were milled to powdered form and about 0.1g of the powdered seeds was dissolved in 10 ml of distilled water and the solution was heated to 60 °C for 1 h. The mixture was then centrifuged at 4000 rpm for half hour. The supernatant was decanted and ready for use while the residue was discarded. Chloroauric acid (HAuCl₄) (Kem Light, Mumbai, India) of 1 mM concentration was made by dissolving 0.34 g of the salt in 1000 ml of distilled water. Silver nitrate (AgNO₃) (BDH Chemicals, Poole England) of 1 mM concentration was derived by suspending 0.17 g of the salt in 1000 ml of distilled water, the salt solution was mixed. Silver-gold mixture was prepared in a ratio of 20:80 respectively, by adding 2 ml of HAuCl₄ to 8 ml of AgNO₃. A milliliter of the seed extract was introduced into the solution and exposed to sunlight for photoactivation for 1 h and was visually observed for colour change which indicates bio-reduction of Au³⁺ to Au⁰ and Ag⁺ to Ag⁰.

Characterization of Ag-Au Nanoparticles

After photoactivation, formation of nanoparticles is confirmed by the color change observed. UV-visible spectrophotometer (Cecil, USA) was used within 190–1000 nm range to determine the wavelength at which there is maximum absorbance. Fourier Transform Infrared spectroscopy analysis was conducted on the sample using IR Affinity-1S Spectrophotometer (Shimadzu, UK) adjusted to 4 cm⁻¹ resolution to determine the biomolecules accountable for the production and stabilization of the nanoparticles.

Scanning electron microscopy (SEM) was used to detect the shape and size of the component metal nanoparticles. Energy dispersive spectroscopy (EDS) spectra showed the signals indicative of the metal nanoparticles due to surface Plasmon resonance, while selected area electron diffraction (SAED) pattern reveal lattice

planes that are peculiar to the nanoparticle synthesized. SEM and EDS analyses were carried out to view the nanoparticles and ascertain the component metals respectively.

Applications of Synthesized Ag-Au Alloy Nanoparticles

Larvicidal Activity

In this assay, varying concentrations of Ag-Au alloy nanoparticles was prepared (60, 80, and 100 µg/ml) by dispersing the nanoparticles in sterile distilled water. Ten anopheles mosquitoes were picked into Petri plate and were subjected to 10 ml of each graded concentration of the nanoparticles at room temperature (30 ± 2 °C). Mortality rate trailed a dose response pattern and percentage mortality was plotted against time in hours. Mosquito larvae were introduced into distilled water without addition of nanoparticles to serve as the control experiment.

Dye Degradation

Synthesized nanoparticles (5 µg/ml) were mixed with each of the dye in the ratio of 1:9, put on shaker at 100 rpm for effective mixing. Absorbance rate were noted at 660, 619 and 498 nm for methylene blue, malachite green and congo red respectively at intervals to determine the level of degradations. Control experiment was prepared, and the absorbance of each dye was taken. The percentage degradation was computed with equation:

$$\% \text{ Degradation} = \frac{AbsC - AbsT}{AbsC} \times 100\%$$

Where; *AbsC* is the absorbance of control; and *AbsT* is the absorbance of the test material.

Antioxidant Assay

Different gradient concentrations of synthesized Ag-Au alloy nanoparticles were prepared (20, 40, 60, 80 and 100 µg/ml). A millimeter synthesized Ag-AuNPs was withdrawn from each of the concentration and mixed with 4 ml of methanolic DPPH, mixed and incubated in the absence of light for half hour after which optical density was checked using spectrophotometer at wavelength of 517 nm. The control was prepared as 4 ml of methanolic DPPH and 1 ml of methanol and the optical density taken using the spectrophotometer.

A standard antioxidant, ascorbic acid (LOBA Chemie, Mumbai, India), prepared using 0.01 g dissolved in 10 ml of distilled water. The gradient concentrations (µg/ml) of 2, 4, 6, 8 and 10 of the standards were prepared. A milliliter of each concentration was mixed with 4 ml of the methanolic DPPH and incubated in the absence of light at room temperature for 30 minutes. Spetrumlab 752S UV-vis spectrophotometer was then used to measure the optical densities.

Results and Discussion

Biosynthesis of Ag-Au Alloy Nanoparticles

There was reduction from Au^{3+} to Au^0 and Ag^+ to Ag^0 to signal the biosynthesis of Ag-Au alloy nanoparticle with kola seed extract as the bioreductant. A visible colour change was observed from light orange to intense dark brown. The colour change in the reaction mixture was observed after 1 min, intensified after 5 min and eventually stabilized after 1 h of sunlight exposure. Colour changes in Figure 1 implied the formation of alloy nanoparticles. Singh *et al.* (2005) reported a violet color for Ag-Au alloy nanoparticles synthesized using bovine serum, while Daima *et al.* (2012) also documented a brown colour for Ag-Au alloy nanoparticles synthesized using tyrosine.

UV-vis Absorption Spectrum of Ag-Au Alloy Nanoparticles

The UV-visible spectroscopic results revealed that the reaction solution has maximum absorption attributable to the surface plasmon resonance (SPR) band of the alloy nanoparticles. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave.

Figure 1: Biosynthesis of Ag-Au nanoparticles (A, Control; B, immediately after adding seed extract of *Cola nitida*; and C, 1 h after adding seed extract of *Cola nitida*)

The sharp bands of alloy nanoparticles, an indication that new nanoparticles have been

formed (neither gold nor silver) were observed around 523.5 nm as shown in the Figure 2

which is at par with the results reported by Raveendran *et al.* (2006). Daima *et al.* (2012) experimented on Ag-Au alloy nanoparticles with maximum absorbance wavelength of 425 nm. However, the bulk silver nitrate-gold

chloride solution did not show any absorbance at this range as shown in Figure 3. This also confirms the synthesis of Ag-Au alloy nanoparticles.

Figure 2: A plot of UV-vis absorption spectrum of the synthesized silver-gold (Ag-Au) nanoparticles

Figure 3: Plot showing UV-vis absorption spectrum of bulk AgNO₃- HAuCl₃ mixture (control)

Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier transform infrared spectroscopy was employed to identify the biomolecules accountable for the synthesis of the Ag-Au nanoparticles. Figure 4 shows the spectra values observed during the analysis, which were 3396.64, 2088.91, 1826.59, 1647.21, 1508.33, 1396.46, 1228.66, 677.01, and

435.91 cm^{-1} . The peak values 1647.21, 1508.33, and 1228.66 cm^{-1} indicated an alkene, nitro compound and amine, respectively while 677.01, 3396.64 cm^{-1} indicated the presence an alkyne and 1°, 2° amines, amides (N-H) stretch (Singh *et al.*, 2005). This result confirms that the seed extract of *Cola nitida* is capable of synthesizing Ag-Au alloy nanoparticles.

Figure 4: FTIR spectrum of the synthesized Ag-Au bimetallic nanoparticles

Scanning electron micrograph of Ag-Au alloy Nanoparticles

Scanning electron micrograph indicated the morphology of Ag-Au alloy nanoparticles synthesized. The nanoparticles formed showed aggregate of particles as displayed in Figure 5. Song and Kim (2009) reported a scanning electron micrograph showing the synthesized nanoparticles with an aggregate of nanoparticles thereby confirming their presence.

Energy Dispersive X-ray Spectroscopy (EDS)

The EDX profile indicated strong signals for Ag-Au which confirms the presence of the alloy. The silver displayed an optical absorption band peak at 3 KeV, as reported by Shahverdi *et al.* (2007) and the gold peaks at around 1.5 KeV. This result also confirms the reduction of the metals from their ionic state to their elemental state. The spectral result is shown in Figure 6. This is another indication of the synthesis of Ag-Au alloy nanoparticles by seed extract of *Cola nitida*.

Figure 5: SEM snapshot of synthesized Ag-Au alloy nanoparticles

Figure 6: Energy dispersive X-ray signal of the synthesized Ag-Au nanoparticles

Larvicidal Activity

A different degree of effectiveness of Ag-Au alloy nanoparticles was observed. 100% mortality was documented for larvae exposed to concentration at 60 $\mu\text{g}/\text{ml}$ and 80 $\mu\text{g}/\text{ml}$ within the period of 24 h. At the end of 72 h, all the concentrations Ag-Au nanoparticles prepared revealed 100% mortality rate as shown in Figure 7, which could be due to the

vitality of the larvae, as they were randomly selected from the environment to mimic the natural condition of their existence. Larvicidal activity of silver nanoparticles has been established by Rajakumar and Abdul Rahman (2011), but this study has shown the larvicidal activity of Ag-Au alloy nanoparticles which implies that the alloy nanoparticles can find application in the control of *Anopheles* through the killing of its larvae.

Figure 7: Larvicidal effect of Ag-Au nanoparticles on mosquito larvae

Degradation of Dyes

The synthesized nanoparticles were shown to have high degrading capability based on the resulting optical densities obtained. Congo red has 22% degradation after 24 h, although this dye is recalcitrant in nature to degradation. Malachite green and methylene blue also had 97% and 61.8% degradation, respectively after 24 h when treated with the synthesized Ag-Au nanoparticles. Roy and Das (2015) reported a

remarkable degradation of methylene blue by silver nanoparticles. Percentage degradations of these dyes indicate that Ag-Au bimetallic alloy nanoparticles are applicable in dye degradation, especially in industrial application. The degradation patterns for congo red, methylene blue and malachite green are shown in Figure 8. These results show that the biosynthesized Ag-Au alloy nanoparticles are applicable in textile industry.

Figure 8: Degradation pattern of congo red, malachite green, and methylene blue by Ag-Au nanoparticles

Antioxidant Activity

The biosynthesized Ag-Au alloy nanoparticles displayed DPPH radical scavenging activities of 72.2, 76.3, 79.5, 72.2, and 75.1 % with concentration range from 20-100 µg/ml as depicted in Table 1. However, the standard antioxidant; ascorbic acid displayed DPPH

radical scavenging activities of 39.0-67.3 %, but at concentration of 2-10 µg/ml. Lateef *et al.* (2016c) confirmed these effects to be exhibited by Ag nanoparticles synthesized using *Cola* pod extract. These results show that the biosynthesized Ag-Au alloy nanoparticle can be used as antioxidant.

Table 1: The DPPH-radical scavenging activity of Ag-Au alloy nanoparticles

	% DPPH scavenging activity				
Ag-AuNPs (µg/ml)	20	40	60	80	100
% Scavenging	72.2	76.3	79.5	72.2	75.1
Standard (µg/ml)	2	4	6	8	10
Ascorbic acid	39	58.0	64.0	66.1	67.4

Conclusion

It can be concluded that the seed extract of *Cola nitida* can synthesize Ag-Au bimetallic alloy nanoparticles. Biosynthesized alloy nanoparticles absorbed maximally at 523.5 nm. The synthesis was also monitored by SEM, FTIR and EDX, which affirmed the synthesis Ag-Au alloy nanoparticles. The synthesized nanoparticles were aggregate and has found its application in larvicidal activity with a 100% mortality rate recorded for larvae exposed to 60 and 80 µg/ ml concentration of the synthesized nanoparticles within 24 h, and after 72 h, all concentrations showed 100% mortality rate. In dye degradation, the synthesized nanoparticles showed high degrading capability with congo red, malachite green and methylene blue having 22, 97 and 61.8% respectively after 24 h. Furthermore, in antioxidant assay, the biosynthesized alloy nanoparticles displayed DPPH radical scavenging activities of 72, 76.3, 79.5, 72.2 and 75.1% at concentration of 20-100 µg/ ml.

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