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Full Length Research Paper

Functionalization of Biosynthesized Gold Nanoparticle from Aqueous Leaf Extract of *Catharanthus roseus* for Antibacterial Studies

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ABSTRACT

Synthesis of nanoparticles from various systems has been reported, but among all, biosynthesis of nanoparticles from plants is considered the most suitable method. The use of plant material not only makes the process eco-friendly or less toxic, but also makes it less expensive. This study investigated the ability of Catharanthus roseus as a reducing agent for gold nanoparticle biosynthesis and the effect of the functionalized gold nanoparticle on some human pathogenic bacteria. The biosynthesized nanoparticles and formulated nanodrug were characterized using UV-Vis spectrophotometry, Zetasizer, Scanning and transmission Electron Microscopy (SEM; TEM), Energy Dispersive spectrophotometry (EDAX) and Fourier Transform Infra-red Spectroscopy. Polyethylene glycol and Lincomycin were used to functionalize the surface of the Gold nanoparticle for antimicrobial properties. The absorption peak of the biosynthesized Gold nanoparticle was found to be 545.5 nm and Zetasizer analysis showed that the average particle size was 28.5 nm with morphological structure of spherical and triangular shapes using SEM and TEM. EDAX confirmed the presence of element gold, carbon, oxygen and copper. The Fourier transform infrared (FTIR) spectroscopy showed the strong band at 3417 cm-1 of hydroxyl (O-H) functional group in alcohols and phenolic compounds. The formulated nano-drug show antibacterial effects with maximum inhibition zones of 24 mm for Streptococcus pyogenes and 14.33 mm for Staphylococcus aureus. Therefore, this study demonstrated the bioreductive capability of aqueous leaf extract of *Catharanthus roseus* and the antibacterial activity of formulated gold nano-drug.

Keywords: Functionalization; polyethylene glycol; gold nanoparticles; Catharanthus roseus; lincomycin, antibacterial activity

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INTRODUCTION

Nanotechnology has provided research breakthrough in various industrial applications. Among those, it has attained remarkable levels in medicine such as in bio-diagnostic, drug delivery and cancer therapy. Nanomedicine is the interaction between the field of nanotechnology and medicine, which is concerned with the use of precisely engineered materials at the nanoscale to develop novel therapeutic and diagnostic modalities. Nanoparticles are particles with size range within the nanoscale, which exhibit new properties such as their large surface-to volume ratio, exceptional optical, physical and chemical properties. These particles have been found to be beneficial in the areas of drug delivery by overcoming the limitations of most conventional (Lucian, 2013). And of importance is the metallic nanoparticles, which being placed in the forefront as a novel platform for the target specific delivery of therapeutic agents (Joanne et al, 2011). Gold nanoparticles have advantages over other metal nanoparticles due to their biocompatibility and non-cytoxicity (Avnika and Garima, 2013). Gold nanoparticles have been synthesized by traditionally chemical and physical methods. However, these methods depend on severe reaction conditions which can be toxic and expensive (Umesh et al, 2011).

The use of plant extracts has been suggested as possible ecofriendly alternatives for the synthesis of nanoparticles for

medical and technological applications, due to their general abundance, non-toxic nature (Umesh et al, 2011). It has reported that nanoparticles can be synthesized using plant extracts at rates comparable to those of the chemical methods (Shankar et al, 2004).

Catharanthus roseus is a genus of flowering plants in the Apocynaceae family. They are known commonly as Periwinkles. The synonyms of the plant name include *Vinca rosea*, *Ammocallis rosea*, and *Lochnera rosea*. Other English names occasionally used include *Cape Periwinkle*, *Rose periwinkle Rosy periwinkle* (Monika and Vandana, 2013). There are eight known species, out of eight; seven are endemic to Madagascar (Brun et al, 2001). Though, one of the *Catharanthus roseus* is widely naturalized around the world (Shasi et al, 2006; Rahmatzadeh and Kazemitabar, 2013). The flowers are usually solitary in the leaf axils. Each has a calyx with five long, narrow lobes and a corolla with a tubular throat and five lobes. It grows up to about 20-80 cm high and blooms with pink, purple, or white flowers (Swanberg and Dai, 2008). There are over 100 cultivars of *Catharanthus roseus* known (Chuan et al, 2013).

The main active constituents of the plant have been reported to be phenolic acids, flavonoids, and alkaloids. These active substances perform a number of protective functions in the human organism and are involved in important anti-oxidative, anti-allergic, antibiotic, hypoglycemic and anti-cancer activities (Milan et al, 2010; Quideau et al, 2011; Stankovic et al, 2011; 2012). Alkaloids are the most potentially active chemical constituents of *Catharanthus roseus.* More than 400 alkaloids have been reported to be present in the plant, which is used as pharmaceuticals, agrochemicals, flavor and fragrance, ingredients, food additives, and pesticides. The alkaloids like actineo plastidemeric, Vinblastin, Vincrestine, Vindesine, Vindeline Tabersonine and Catharanthus are mainly present in aerial parts whereas, ajmalicine, vinceine, vineamine, raubasin, reserpine, catharanthine etc are present in roots and basal stem. Rosindin is an anthocyanin pigment found in the flower of *Catharanthus roseus* (Monika and Vandana, 2013). *Catharanthus roseus* have also shown the presence of these compounds along with other flavonoid (Povan and Fillipini, 2007).

Extracts from it have been used to treat numerous diseases, including Diabetes, Malaria, and Hodgkin's disease. The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia. Previous studies of Shankar and others have shown the rapid synthesis of gold nanoparticles with neem (*Azadirachta indica)* leaves and sun dried C. *campphora* leaves. They attributed the bioreduction capability to a water-soluble heterocyclic compound found in the plants (Rahmatzadeh and Kazemitabar, 2013; Swanberg and Dai, 2008). Chemical tannins have been used as a reducing agent for metallic gold to its nanoparticle size and the presence of phytochemical tannins in the aqueous extracts of this plant suggest their ability to reduce as suggested by Sivaraman *et al* (2010).

Based on previous researches that nanoparticles can be used to aid drug molecules to conjugate or exchange with a second organic molecule (the capping agent or the ligand of the AuNP), which could compliment the entire drug delivery to the targeted organ delivered directly (Kim et al, 2009; Gibson et al, 2007). Different types of antibiotic resistance are now evolving and spreading, thereby limiting the efficacy of current antibiotics with little or no effect against their target organisms.

Polyethylene glycol (PEG; HO-(CH_2-CH_2-O)$_n$-H) is a water soluble polymer compatible with the immune system of the human body with non-immunogenicity, non-antigenicity, and protein rejection properties (Alcanter et al, 2000; Ademovic et al, 2002). PEG is therefore a good candidate to serve as a soft spacer for modifying surfaces with different biomolecules.

PEG is widely used in providing nanoparticles with stealth properties, hence prolonging blood circulation times (Khlebtsov and Dykman 2011; Lipka et al, 2010). For example, Lipka et al showed that a longer PEG chain length of 10kDa improved nanoparticle blood circulation time as over 15% of applied volume was found after 24 hours in the bloodstream of mice subjects (Lipka et al, 2010.

Therefore, the use of *Catharanthus roseus* aqueous leaf extract in the biosynthesis of gold nanoparticles and its capability as drug carrier with covalent functionalization with polyethylene glycol was investigated

Plate 1: *Catharanthus roseus*

MATERIALS AND METHODS

Plants collection and identification: The leaves of *Catharanthus roseus* were obtained from Federal University of Technology (FUT), Bosso campus Minna, Niger State and Identified at Biological Sciences Department, Federal University of Technology, Minna, Niger State.

Chemicals: Tetra gold chloride ($HAuCl_4$**)** and polyethylene glycol was obtained from Sigma Aldrich and were freshly prepared with deionized water throughout the experiment.

Microorganisms: *Staphylococcus aureus* and *Streptoccocus pyogenes* were obtained from Center for Genetic Engineering and Biotechnology (CGEB), Federal University of Technology, Minna, Niger state. Two pathogenic bacterial strains were used in the current study.

Plant sample preparation: Fresh leaf of *Catharanthus roseus* was washed with clean water to ensure it is clean from dust and sand. The plant was air dried for three (3) weeks at room temperature to prevent the destruction of thermo labile constituent of the plant by direct Sun rays. The leaves were blended together into a powder and kept under safe condition.

Extract preparation and synthesis of gold nanoparticles: Five grams of the plant sample was weighed and added to 100 mL of sterile distilled water in Erlenmeyer flask and boiled for 5 minutes and filtered. About 0.5 mL of the filtrate was pipetted into a test tube and 9.5 mL of 1mM $HAuCl_4$ added for the reduction of Au^{3+} ions according to the method described by (Jae et al, 2009). Colour change was observed and UV Spectrophotometer was used to scan the wavelength.

Phytochemical analysis: The phytochemical analysis was carried out an accord to Trease and Evans (1989).

Characterization of gold nanoparticle synthesized: The reduced gold ions in aqueous solution were monitored and a UV–Vis Spectrophotometer (UV-1800 Shimadzu) was used to measure the wavelength between 200–800 nm using a quartz cuvette. The particle size and distribution of the Gold nanoparticles were measured using Dynamic Light Scattering (DLS) equipment (Malvern Zetasizer). The morphological visualization of the biosynthesized and functionalized nanoparticle was known through analysis with the Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) performed on a JEOL model 1200EX instrument operated at an accelerating voltage at 80 kV. The elemental components were analyzed on Energy Dispersive X-ray Spectroscopy (EDAX) S-3400N, Hitachi instrument according to Singh et al (2013). The Fourier Transforms Infrared (FTIR) Spectroscopy was used to identify the possible biomolecules responsible for the reduction of the Au ions and capping of the bioreduced gold nanoparticles synthesized by *Catharanthus roseus*. The synthesized Gold nanoparticle was centrifuged at 15, 000 rpm for 15 minutes and the pellet washed with deionized water to get rid of free proteins/enzymes that were not capped on the Gold nanoparticles. Thereafter, the purified suspension was freeze dried to a powder. The dried powder was analyzed using FTIR. The samples were dried and ground with KBr pellets and analyzed on Thermo Nicolet model 6700 spectrum instrument. A disk of 50 mg of KBr was prepared with a mixture of 2% finely dried sample and then examined under IR-spectrometer. Infrared spectra were recorded in the region of 500 - 4,500 cm^{-1}.

Functionalization of gold nanoparticle: The functionalization of the gold nanoparticle was carried out using a standard drug (Lincomycin) and polyethylene glycol (Molecular Weight = 3000). Three formulations of the three composites were made. First formulation: The standard drug coated nanoparticle and polyethylene glycol was prepared by the addition of 0.5 mL of biosynthesized *C. roseus* gold nanoparticle (AuNPs) with 5 g polyethylene glycol and 0.58 g of the standard drug with proper stirring of 1 hour.

- Second formulation: Similarly, 0.5 mL of biosynthesized *C. roseus* gold nanoparticle was added to 5 g polyethylene glycol and stirred for 1 hour.
- Third formulation: 1mL sterile deionized water was mixed with 0.58 g of standard drug and 5 g of polyethylene glycol with proper stirring of 1 hour.

All the formulations were made into tablet form and left for 24 hours in an incubator at 37^0c to enable digestion of the mixture, and then properly air dried (Joanne et al, 2011).

Antibacterial assay

Bacteria inoculation: 28g of the nutrient agar was dissolved in one-liter sterile distilled water and then autoclaved at 121^0C for 15 minutes. 4g of the nutrient broth was dissolved in 300 mL sterile distilled water and 10 mL of nutrient broth was dispensed in three test tubes for each organism. This was then autoclaved at 121^0C for 15 minutes. After sterilization, they were allowed to cool to about 45^0C -50^0C and the nutrient agar was then dispensed into petri dishes, which were left to solidify. Holes were then made on the agar containing petri dishes using a sterile cork borer with 10-millimeter diameter.

The organisms were then inoculated into the nutrient agar plate, and then 1 mL of each dissolved gold nano-formulation tube were dispensed into the holes made. The petri dishes were left to stand for 2 hours and then placed in the incubator for 24 hours. After incubation the diameter of inhibition zone (mm) was measured.

Drug release: Test tubes containing 3mL sterile deionized water were set up for the drug and also for each formulation. The height and diameter of each tablet were measured, and the tablets were placed in 3mL sterile deionized water for 3, 6, 9, 12, 15, 18 minutes. The standard drug concentration and the absorbance of various dissolved formulated tablets were measured spectrophotometrically and antibacterial activity was recorded.

Statistical analysis

Values are represented as mean ± Standard Error of Mean (SEM). The data were statistically analyzed using one-way analysis of variance (ANOVA) and Duncan multiple range test. Data from the antibacterial activities were compared with their respective controls and differences at $p<0.05$ were considered significant

RESULTS

Phytochemical constituents of the leaf extracts of plant

The qualitative phytochemical composition of the aqueous leaf extract of *Catharanthus roseus* indicates the presence of tannins, flavonoids, alkaloids, saponins, glycosides and phlobatanins (Table 1).

Biosynthesis of Gold nanoparticle using aqueous leaf extract of *Catharanthus roseus* : Plate 2A shows the tetra gold chloride was yellow colour before the reduction reaction. Plate 2B shows aqueous leaf extract of *Catharanthus roseus* with deep red colour while Plate 2C shows ruby red colouration formed after reaction of the aqueous leaf extract

of *Catharanthus roseus* with the tetra gold chloride solution resulting in the formation of gold nanoparticles

Table 1:
Phytochemical constituent of aqueous leaf extract of *Catharanthus roseus*

Phytochemicals	*Inference*
Anthraquinones	-
Alkaloids	+
Tannins	+
Flavanoids	+
Saponins	+
Steroids	-
Glycosides	+
Phlobatannins	+

Keys: (+) Present, (-) Absent.

UV-Visible spectrophotometry of gold nanoparticle synthesized using aqueous leaf extract of *Catharanthus roseus*: The absorption spectra of the biosynthesized gold nanoparticle are shown in Fig. 1 indicating a strong surface resonance which is visible at a peak of 545.5 nm.

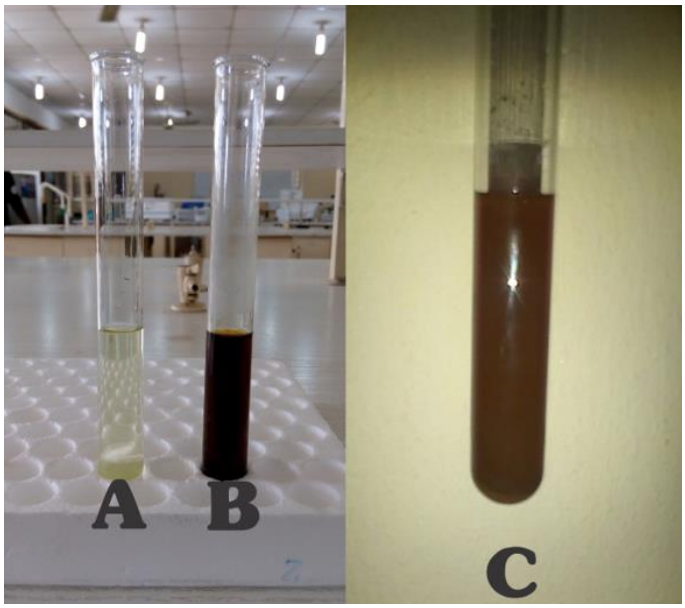

Plate 2:
(A) Tetra gold chloride solution; (B) Aqueous extract of *Catharanthus roseus*; (C) Gold nanoparticle

Particle size of Gold Nanoparticle synthesized using the aqueous leaf extract of Catharanthus roseus: Fig. 2 shows the average particle size of biosynthesized Gold nanoparticles of peak size 28.5 nm with the intensity of 17.5%.

Scanning and Transmission Electron Microscopy (SEM; TEM): The SEM and TEM monograph of biosynthesized and functionalized nanoparticle shown in Fig. 3 and 4. The image revealed the shape to be spherical and triangular.

Energy-Dispersive Spectroscopy Analysis of functionalized Polyethylene - AuNP with Lincomycin: The functionalized nanodrug was investigated using EDAX and confirmed the presence of gold and other elements (carbon, copper and oxygen) as shown in Figure 5. The vertical axis shows the number of X-ray counts and the horizontal axis shows energy in keV. The maximum optical adsorption peak was observed at approximately 2.30 keV and addition signals for carbon at 0.20, oxygen 0.50 and copper 1.0 (Fig.5)

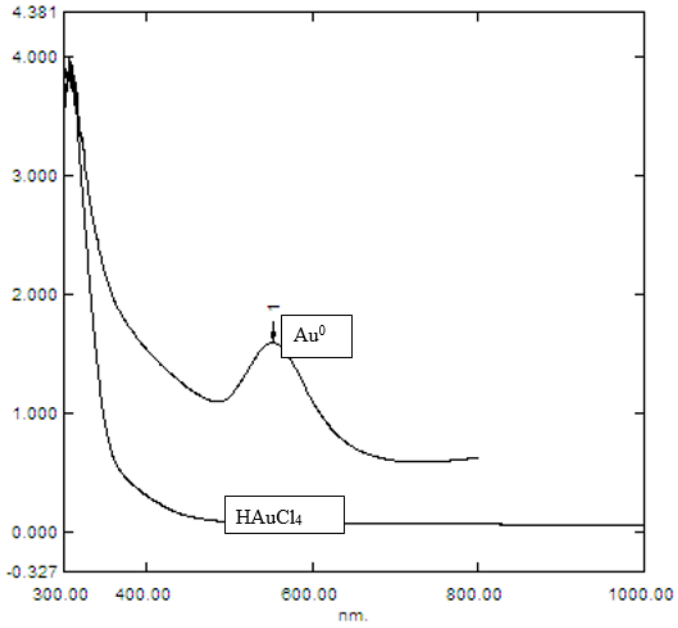

Figure 1:
Absorption spectra of tetra gold chloride and biosynthesized gold nanoparticle

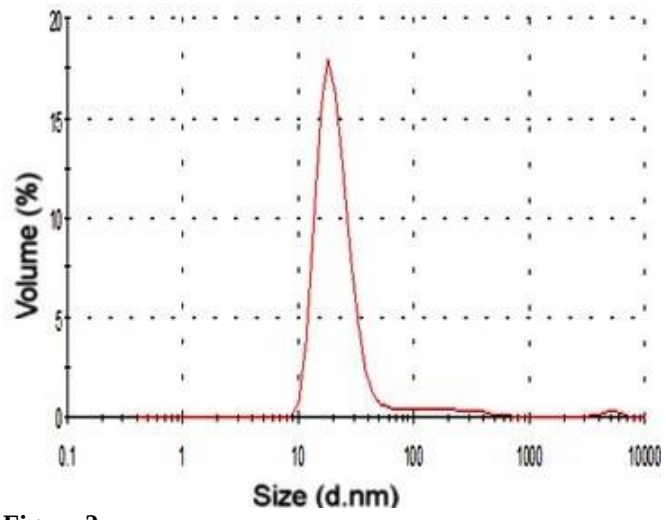

Figure 2:
Particle size of biosynthesized gold nanoparticle

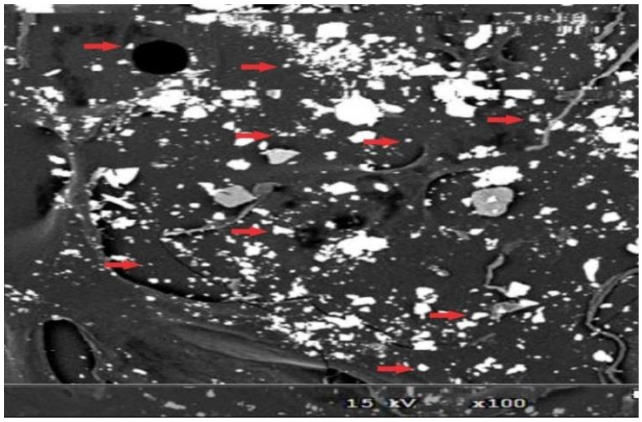

Figure 3:
Scanning Electron Morphology visualization of biosynthesized gold nanoparticle

Fourier Transform Infrared (FTIR) Spectroscopy: The FTIR spectrum in Fig. 6 reveals a strong band in functional group region at 3417 cm^{-1} and this strong band region corresponds to the hydroxyl group and the finger print region at 1624 cm^{-1} corresponds to the alkene (C=C) and carbonyl (C=O) group.

Drug release: The Fig. 7 shows the release capability of formulated nanodrug (mg/mL) versus time (minutes) for the three formulations (PD, PND, and PN). The formulated PND has the highest releasing efficiency with a peak at nine minutes (33.14 mg/mL) when compared to PD (control @ 16.73 mg/mL) and PN (0.78 mg/mL) formulated drugs.

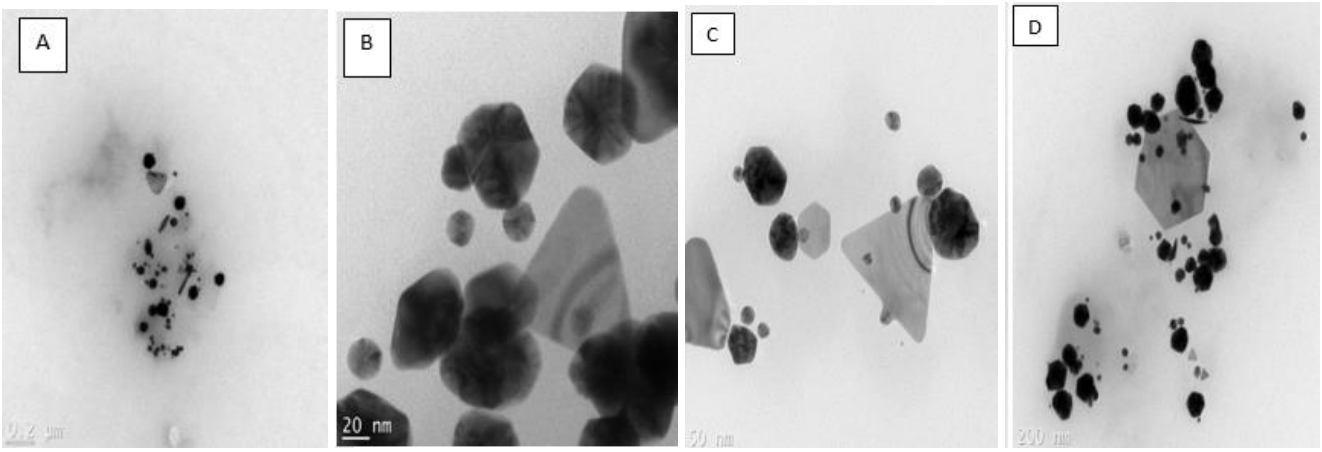

Figure 4:
Transmission Electron micrograph of formulated Polyethylene - AuNP with Lincomycin @ (A) 0.2 µm (B) 20 nm (C) 50 nm (D) 200 nm

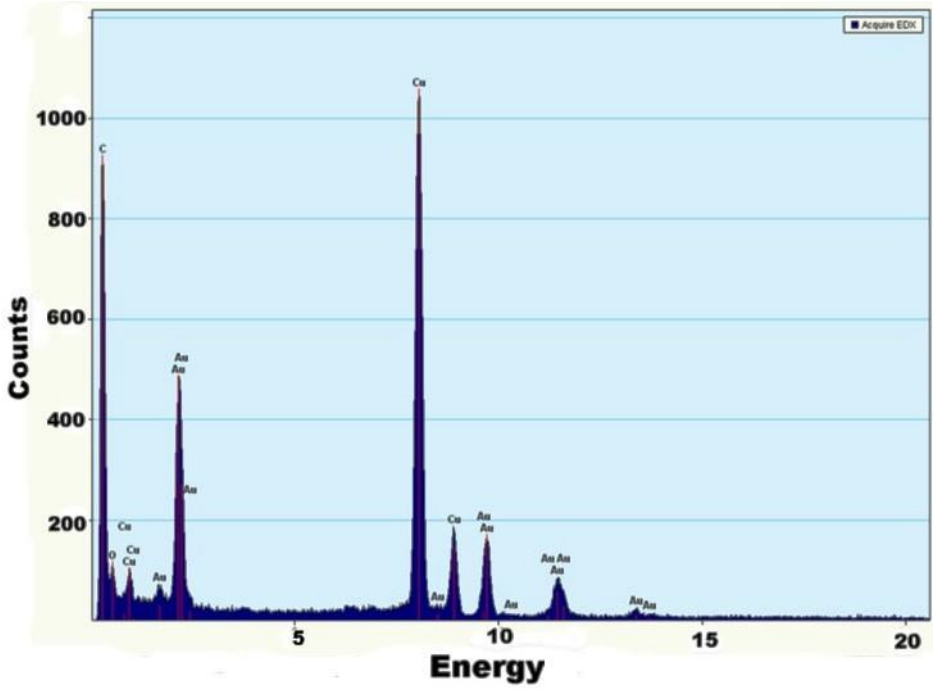

Figure 5:
EDAX spectra of functionalized Polyethylene - AuNP with Lincomycin

Antibacterial Activity

Antibacterial of formulations against Staphylococcus aureus

Table 2 shows the result of the antibacterial effect of the various formulations against *Staphylococcus aureus*. There was significant ($p<0.05$) increase in the zone of inhibition of *Staphylococcus aureus* growth by the formulated nanodrug PND compared to PD at various time intervals (3 to 9 mins). Whereas the Standard drug shows is maximum effect at zero minute while PND and PD at 18 and 12 minutes respectively.

Antibacterial effect of formulations against Streptococcus pyogenes: Table 3 shows the result of the antibacterial effect of the various formulations against *Streptococcus pyogenes.* There was significant ($p<0.05$) increase in the zone of inhibition of *Streptococcus pyogenes* growth by the formulated nanodrug PND compared to PD at various time intervals (3 to 9 mins). Whereas the Standard drug shows is maximum effect at zero minute while PND and PD at 18 and 12 minutes respectively.

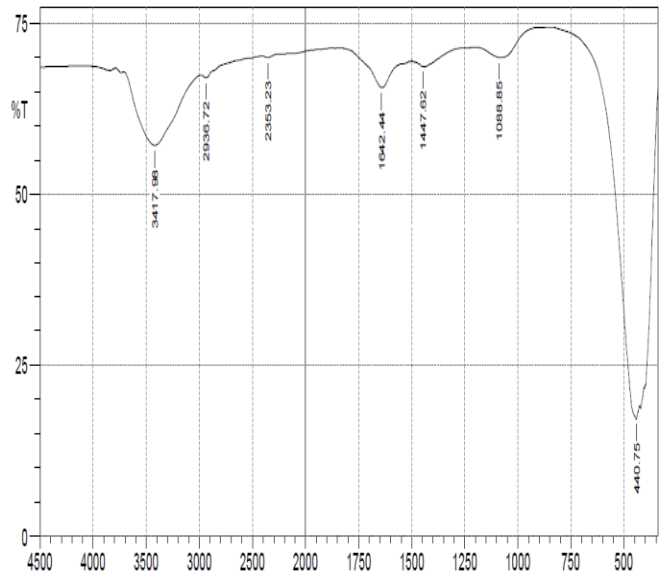

Figure 6:
FTIR spectra of *Catharanthus roseus* extract capped Gold nanoparticle

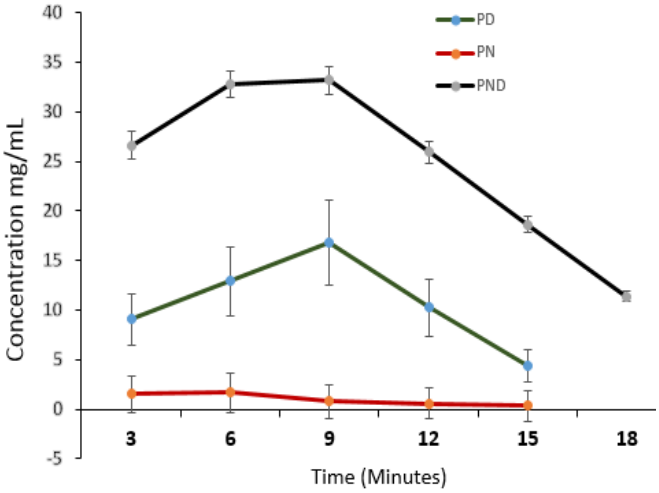

Figure 7:
Drug release with and without gold nanoparticle.
PD: Polyethylene glycol and Lincomycin
PN: Polyethylene glycol and nanoparticle
PND: Polyethylene glycol, Nanoparticle and Lincomycin

DISCUSSION

This study shows the phytochemical constituents of the aqueous leaf extract of *Catharanthus roseus* to include phenolic compounds (Flavonoid and Tannin) as shown in Table 1. The presence of these phytochemicals constituents in the aqueous extracts of the plant suggest its ability to reduce metallic gold to its nano size as in agreement with Huang *et al*. (2003) and eventually its therapeutic purposes reported by Edeoga *et al*. (2005). Therefore, it can be concluded that the phenolic compounds from the leaf extract of *Catharanthus roseus* have the ability to cap the gold nanoparticle by ionic interaction and thereby stabilizing them.

The observed colour change of light yellow (Plate 2A) to ruby red color (Plate 2C) after the addition of gold chloride to plant extract indicates the formation of gold nanoparticles. It has been reported that colour changes arise due to excitation of surface plasmon vibrations in the gold metal nanoparticles (Malvoney, 1996). This was also confirmed by a strong plasma resonance at 545.5nm for biosynthesized gold nanoparticle using UV–Visible spectrophotometer.

Table 2:
Antibacterial effect of the various formulations against *Staphylococcus aureus*

Formulation	Zone of Inhibition/ minute						
	0	**3**	**6**	**9**	**12**	**15**	**18**
PND	---	14.00 ± 2.51^{b}	14.00 ± 1.52^{b}	12.66 ± 1.66^{b}	13.00 ± 1.52^{b}	14.00 ± 2.51^{b}	24.33 ± 0.33^{c}
PD	---	11.00 ± 0.57^{ab}	7.66 ± 0.33^{a}	9.66 ± 0.33^{ab}	25.66 ± 0.88^{c}	---	---
SD	25.66 ± 0.88^{c}	---	---	---	---	---	---

Data were expressed as mean of 3 replicates ± SEM
PD: Polyethylene glycol and Lincomycin
PND: Polyethylene glycol, Nanoparticle and Lincomycin ; Standard drug: Lincomycin

Table 3:
Antibacterial effect of the various formulations against *Streptococcus pyogenes*

Formulation	Zone of Inhibition/ minute						
	0	**3**	**6**	**9**	**12**	**15**	**18**
PND	---	12.00±1.15^{ab}	16.66±0.66^{c}	15.00±0.57^{bc}	13.00±0.57^{ab}	11.33±1.45^{a}	4.00±1.15^{d}
PD	---	12.33±0.33 ^{abc}	10.33±0.33 ^{a}	13.66±0.33 ^{bc}	28.00±0.57 ^{d}	----	---
PD	25.66±0.88 ^{d}		---	---	---	---	--

Data were expressed as mean of 3 replicates± SEM; PD: Polyethylene glycol and Lincomycin
PND: Polyethylene glycol, Nanoparticle and Lincomycin ; Standard drug: Lincomycin

The size distribution curve of the biosynthesized gold nanoparticle obtained from light scattering experiments characterized with zeta sizer revealed the average particle size of 28.5 nm.

The morphology of the synthesized and functionalized gold nanoparticles were determined by SEM and TEM images. Typical, SEM images obtained for synthesized gold nanoparticles shows clearly spherical and triangular with aggregation of semispherical nanoparticles (Fig. 3). This confirms the inability of biomolecules to act as protecting agents for aggregation. This is in agreement with literature that citrate caped gold nanoparticles aggregated in physiological conditions (Nam et al, 2009).

The TEM images of functionalized gold nanoparticles shows a mixture of spherical, triangles, pentagons, and hexagons structures at various sizes. While the pentagons and hexagons structures are sizes more visible at 20 to 200 nm. This agrees with work of Jae *et al* (2009) that use Magnolia kobus and Diopyros kaki leaf extracts for gold nanoparticles synthesis. The EDAX profile of functionalized gold nanoparticles shows the strong signal of gold and other element (carbon and copper) and weak signal of oxygen which may be from the grid used as reported by Akinsiku *et al* (2015)

The FTIR spectra of gold nanoparticle show bands at 3417, 1624, 1447, and 1088 cm^{-1}. The band at 3417cm^{-1} corresponds to the O-H stretch H-bonded, strong broad of alcohols or phenols. And the 1624 cm^{-1} corresponds to carbonyl (C=O) strong stretching group while the 1447 and 1088 cm^{-1} bands correspond to the alkane (-C-H) bending variable group and alcohol (C-O) strong stretching group. The highest absorption peak 3417 cm^{-1} confirms that the hydroxyl group (O-H) is responsible for the reducing property of the *Catharanthus roseus* leaf extract and this functional group is a reflection of the presence of phenolic polymer known as Tannin. The Tannin has been reported to have antioxidant, antimutagenic and anticancer properties and it also reduces triglycerides. Besides its multipurpose applications, it has been reported to be used as a reducing agent and a protective colloid in noble nanoparticle synthesis (Ahmad, 2014; Ahmad and Khan, 2013; Yi et al, 2011). At its natural pH, tannin behaves as a weak reducing agent which can induce growth of nanoparticles at room temperature. Room temperature synthesis of silver or gold nanoparticles using tannin has also been demonstrated (Silvaraman et al, 2010).

The formulated nanodrug (PND) showed antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes* at different time interval with maximum activity at 18 minutes. Also, the PD had activity against bacterial species with maximum efficacy at 12minutes. On the other hand, the free standard drug (lincomycin) shows its maximum activity against bacterial species at zero minute. This implies that the PND still delivered activity at 18 minutes at target site compared to the free drug whose activity delivered at zero minutes. The activity of PND confirms the use of metal nanoparticles as a carrier and improves delivery of drug to target site. Also, gold nanoparticles have been reported to possess higher surface area and have affinity to adsorbed more drug forming a single group with a number of drug moieties acting against the microorganisms. This is in agreement with the work of Nirmala and Pandian (Nirmala and Pandian, 2007). Whereas, Polyethylene glycol and Lincomycin (PD) without gold nanoparticles has lower surface area efficacy at various time interval compared to PND except at 12 minutes.

In conclusion, this study has demonstrated the bioreductive capability of aqueous leaf extract of *Catharanthus roseus* on tetra gold chloride which may be attributed to the presence of a phenolic compound of the leaf extract. Various characterization methods confirmed the synthesis of gold nanoparticle and its conjugation with standard drug. Also, the functionalized that gold nanoparticles contribute to prolong release and efficacy of the PND drug against the microorganism.

REFERENCES

Ademovic, Z., Klee, D., Kingshott, P., Kaufmann, R. and Hocker, H. (2002). Minimization of protein adsorption on poly(vinylidene fluoride). *Journal of Biomolecular Engineering*, 19(2): 177-182.

Ahmad T. (2014). Reviewing the tannic acid mediated synthesis of metal nanoparticles. *J Nanotechnology* 54: 206.

Ahmad T. and Khan W. (2013). Size variation of gold nanoparticles synthesized using tannic acid in response to higher chloroauric acid concentrations. *World J Nano Science and Engineering* 03:62-68.

Akinsiku, A.A, Ajanaku, K.O., Adekoya, J.A and Dare, E.O (2015). Green Synthesis, Characterization of Silver Nanoparticles Using *Canna indica* and *Senna occidental is* Leaf Extracts. International Conference on African Development Issues (CU-ICADI) 2015: Materials Teclmology Track. Pp 154 - 157

Alcantar, N.A., Avdil, E.S. and Israelachvili, T. N. (2000). Polyethylene glycol-coated biocompatible surfaces. *Journal of biomedical materials research*, 51(3): 343-351

Avnika, T and Garima G (2013): Short review on Application of Gold nanoparticles. *Global J Pharmacology* 7(1):34-38

Brun, G; Bessiere, JM; Dijoux-Franca, MG; David, B; Mariotte, AM (2001). Volatile Components of *Catharanthus roseus* (L.) G. Don (Apocynaceae). *J Flavour Fragrance* 16(2):116-119.

Chuan Ku, Wan-Chia Chung, Ling-Ling Chen, and Chih-Horng Kuo (2013). The Complete Plastid Genome Sequence of Madagascar periwinkle *Catharanthus roseus* (L.) G. Don: Plastid Genome Evolution, Molecular Marker Identification and Phylogenetic Implications in Asterids. *Public Library of Science ONE* 8(6):1-11.

Daniel Huang,a Frank Liao,b Steven Molesa,b David Redinger,b and Vivek Subramanianb (2003). Plastic compatible low resistance printable Gold nanoparticle conductors for flexible electronics. *J Electrochemical Society* 150:412-417.

Edeoga HO, Okwu DE, Mbaebie BO.(2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 4(7):685-8.

Gibson, J.D., Khanal, B.P., Zubarev, E.R. (2007). Paclitaxel-functionalized gold nanoparticles. *J. Am. Chem. Soc.* 129: 11653–11661.

Jae Y, Hyeon-Kyeong, Beom S. (2009). Biological synthesis of gold nanoparticles using *Magnolia kobus* and *diopyros kaki* leaf extracts. *Process Biochemistry* 44:1133-1138.

Jae, Y. S., Hyeon-Kyeong., Beom, S. K, (2009). Biological synthesis of gold nanoparticles using *Magnolia kobus* and *diopyros kaki* leaf extracts. *Process Biochemistry* 44: 1133-1138

Joanne M, Kumar D, Meenan BJ, Dixon D (2011). Polyethylene glycol functionalized gold nanoparticle: the influence of capping density on stability in various media. *Gold bull* 44: 99-105.

Khlebtsov, N. and Dykman, L. (2011). Biodistribution and toxicity of engineered gold nanoparticles: A review of *in vitro* and *in vivo* studies. *Chemical Society Review*, 40(3): 1647-1671.

Kim, C.K., Ghosh, P. , Pagliuca, C. , Zhu, Z.J., Menichetti, S. ,Rotello, V.M. (2009). Entrapment of hydrophobic drugs in nanoparticle monolayers with efficient release into cancer cells. *J. Am. Chem. Soc*. 131: 1360–1361.

Lipka, J., Semmler-Behnke, M., Sperling, R.A., Wenk, A. and Takenaka, S. (2010). Biodistribution of PEG-modified gold nanoparticles following intratracheal instillation and intravenous injection. *Biomaterials*, 31(25): 6574-6581.

Lucian M. (2013). Drug delivery applications of Gold nanoparticle. *J Biotechnology, Molecular Biology and Nanomedicine* 1(1): 23-28.

Milan S. Stankovic,Marina Topuzovic, Slavica Solujic and Vladimir Mihailovic (2010). Antioxidant activity and concentration of phenols and flavonoids in the whole plant and plant parts of *Teucrium chamaedrys* L. var. *glanduliferum* Haussk. *J Medicinal Plant Research* 4 (20) :2092–2098.

Monika S, Vandana S.(2013). *Catharanthus roseus* (An anti-cancerous drug yielding plant) - A Review of Potential Therapeutic Properties. *Int J Pure and Applied Biosciences* 1(6):139-142.

Mulvaney P. (1996).Surface plasmon spectroscopy of nanosized metal particles. *Langmuir* 12(3):788-800.

Nam J, Won N, Jin H, Chung H, Kim S.(2009). pH-induced aggregation of gold nanoparticles for photothermal cancer therapy. *J Am Chem Soc* 131:13639–45.

Nirmala G. A. and Pandian K. (2007). Antibacterial efficacy of aminoglycosidic antibiotics protected gold nanoparticles—A brief study. Colloids and Surfaces A: Physicochem. Eng. Aspects 297: 63–70

Piovan A and Fillipini R. (2007). Anthocyanins in *Catharanthus roseus in vivo* and *in vitro*: A review. *Phytochemical Review* 6:235-242.

Quideau S, Deffieux D, Douat-Casassus C, Pouységu L. (2011). Plant polyphenols: chemical properties, biological activities, and synthesis. *Angew Chem Int Ed Engl* 50 (3):586–621.

Rahmatzadeh S, and Kazemitabar S. (2013). Biochemical and antioxidant changes in regenerated periwinkle plantlets due to mycorrhizal colonization during acclimatization. *International J American Chemical Society* 5(14):1535-1540.

Shankar S, Rai A, Ankamwar B, Singh A, Ahmad A, Sastry M (2004). Biological synthesis of triangular gold nanoprisms. *National Material* 3(7): 482 - 488

Shashi Pandey-Rai, Gopal Rao Mallavarapu, AA Naqvi, Anju Yadav, Sanjay Kumar Rai, Suchi Srivastava, Digvijay Singh, Raghvendra Mishra, Sushil Kumar (2006). Volatile components of leaves and flowers of periwinkle *Catharanthus roseus* (L.) G. Don from New Delhi. *J Flavour Fragrance* 21(3):427-430.

Singh, M., Kalaivani, R., &Manikandan, S. (2013). Facile green synthesis of the variable metallic gold nanoparticle using *Padinagymnospora*, a brown marine macroalga. *Applied Nanoscience*, 3, 145–151

Sivaraman S, Kumar S, and Santhanam V. (2010). Room temperature synthesis of gold nanoparticles – Size control by slow addition. *Gold Bulletin* 43: 275-286.

Stankovic, M.S., N. Niciforovic, V. Mihailovic, M. Topuzovic and S. Solujic, (2012). Antioxidant activity, total phenolic content and flavonoid concentrations of different plant parts of *Teucrium polium* L. subsp. *polium*. *Acta Societatis Botanicorum Polonaie* 81:117–122.

Stankovic, M.S.; Curcic, M.G.; Zizic, J.B.; Topuzovic, M.D.; Solujic, S.R.; Markovic, S.D. (2011). *Teucrium* plant species as natural sources of novel anticancer compounds: antiproliferative, proapoptotic and antioxidant properties. *Int J Molecular Sciences* 12:4190–4205.

Swanberg A, and Dai W.(2008). Plant Regeneration of Periwinkle (*Catharanthus roseus*) via Organogenesis. *J HortScience* 43(3):832-836.

Trease G, and Evans W. (1989). Pharmacology. 11th Ed Bailliere Tindall Ltd London 60- 75.

Umesh K, Birendra K, Padmalochan N.(2011). Green synthesis and characterization of Gold Nanoparticles using onion (*Allumcepa*) extract. *World J Nano Science and Engineering*1:93-98.

Yi Z., Li X., Xu X., Luo B., Luo J., Wu W., Yi Y., Tang Y.(2011). Green, effective chemical route for the synthesis of silver nanoplates in tannic acid aqueous solution. Colloids and Surfaces: *Physicochemical and Engineering Aspects* 392:131-136