RESEARCH ARTICLE

Production of silver nanoparticles synthesis of *Couroupita guianensis* plant extract against human pathogen and evaluations of antioxidant properties

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ABSTRACT

Couroupita guianensis, Silver nanoparticles synthesis of Plant extract were confirmed by UV-vis and FTIR. Further it was tested against various pathogenic microorganism and followed by antioxidant properties. The *Couropita guinanensis* flower extract mediated nanoparticles showed absorbance peaks at 318--323nm region in the spectral analysis.Fourier transform infrared spectroscopy analysis of the silver nanoparticles showed absorption peaks of reduced silver at 1631.95 cm−1. At concentration of plant extract with silver nanoparticles. The (12 mm) clear inhibitory zone appeared around 100µl against *Pseudomonas aeroginosa* MTCC 2453after incubation for 24h followed by *Bacillus subtilis* MTCC121 (23 mm), *Staphylococcus aureus*MTCC96 (13 mm), *Klebsiella pneumonia* MTCC109 (15 mm) and *E.coli* MTCC 912 (20 mm).In *Couroupita guianensis* total antioxidant was found to increase with increase in concentration in standard, plant extract and $AgNO_3$.

Keywords: *Couroupita guianensis,* FTIR, UV and Antioxidant.

INTRODUCTION

The new field of nanotechnology has become a major thrust in scientific research. It has adapted itself to various field of science and technology including physics, chemistry, etc. It is expanding and continues to change the way we perceive and execute things and has a pronounced effect on therapeutics and shaping the ever evolving society and influencing our daily lives (Chakraborty *et al*., 2011). These nanoparticles due to their targeted action increase the efficacy of the drug. Their small size gives them an edge while evading the immune responses and also gives them the ability to cross relatively impermeable membranes (Uchegbu and Schatzlein, 2012).

Nanoparticles result in significantly low toxicity on adoption of this technique, it can be used for encapsulation of drug molecules. Further research in the field of nanomedicine with respect to AgNPs is going on worldwide. Bacterial strains, both gram positive and gram negative, have been employed in the non-enzymatic production of AgNPs through the interaction of silver ions with the organic compounds present on the bacterial cells. For example, *Lactobacillus*, *Enterococcus*, *Pediococcus pentosaceus* and *Enterococcus faecium* reduce silver ions in alkaline conditions (Ahmad *et al*. 2013). AgNPs synthesized by *Plectonema boryanum* precipitates spherical AgNPs of size 200 nm. *Bacillus subtilis* yields AgNPs of 5–60nm on microwave irradiation. Bio-reduced diamine silver complexes of *Corynebacterium* strain, SH09, results in silver nanoparticles of size ranging between 10 and 15 nm (Bhattacharjee *et al*. 2005). *Spirulina platensis* is also used for the extra cellular synthesis of nanoparticles. AgNPs of size7–16nm and gold nanoparticles of size 6–10nm are obtained at optimum conditions, i.e.37 ◦C, and 120h and pH5.6 (Sintubin *et al*. 2009).

Fungi have been immensely used for the green synthesis of nanoparticles. AgNPs are known to be excellent antimicrobial and anti-inflammatory agents and are thus used to enhance wound healing. Compared to bacteria, fungi have been known to secrete much higher amounts of bioactive substances and so fungi are considered more suitable for large-scale production. *Fusarium oxysporum* synthesizes bioactive substance extracellularly by reducing silver nitrate. The process includes stabilization of AgNPs in a solution with the help of protein secreted by the fungal strain and the metal ions produced are reduced by nitrate-depend reductase and quinines huttle.

The AgNPs thus produced are tested for their bactericidal effect against *S.aureus* on cotton and silk cloth (Fu *et al*. 2006). Algae are employed for the synthesis of nanoparticles which reduces the Ag+ ions by means of proteins released by them and these proteins reduce the nanoparticles and help in maintaining AgNP's stability. In *Chorella vulgaris*, the proteins in the extract have dual function of Ag+ ion reduction, and shape controlled synthesis of NPs. The Ag nano plates are obtained at room temperature. Reduction of Ag+ ions is done by the hydroxyl groups in Tyr residues and carboxyl groups in Asp/Glu residues. It is responsible for the isotropic growth of Ag nano plates which yields rod-like particles with a mean length of 44nm and width of 16–24nm. By way of this background we are reported that the edifice of silver nanoparticles synthesis of *Couroupita guianensis* plant extract effective against human pathogen and its antioxidant properties.

MATERIALS AND METHODS

Collection of Plant Materials

Couroupita guianensis are evergreen trees, native to tropical northern South America, southern Caribbean and also India. Its flowers are orange, scarlet and pink in colour, and form large bunches. Floral parts of *C. guianensis* was collected from Sorimuthu Ayyanar Koil , Pabanasam, Thirunelveli District, Tamil Nadu, India (8º 39' N and 77 º 20' E) with elevation of 1500 m above sea level.

Plant Extract Preparation

The fruit pulp (white in color which converts into blue to brown within minutes) was collected for the synthesis of nanoparticles (Torresdey, 2003).

Preparation of 1mM Aqueous Solution of Silver Nitrate

0.017gm of Silver Nitrate ($AgNO_3$) was added to the 100 ml of distilled water and the solution was stirred well continuously until the silver nitrate is dissolved. This 1mM Silver Nitrate solution stored in brown bottle at 4° C for further use for the synthesis of Silver Nanoparticles from *Couroupita guianensis.*

Synthesis of AgNPs

1mM aqueous solution of silver nitrate (Himedia, Mumbai) was prepared for synthesis of silver nanoparticles. For the synthesis of AgNPs, two boiling tubes were taken, one containing 10ml of 1Mm AgNO3 solution as control and the second containing 9ml of 1mM silver nitrate solution and 1ml of plant leaf extract as test solution. These were incubated at room temperature for 1-2 hours. The color change of the leaf extracts from pale yellow to dark brown was checked periodically. The silver nanoparticles were confirmed by color changes and qualitatively characterized by UV-Visible spectrophotometer.

Characterization of silver nanoparticles

a) UV-visible spectroscopy:

Synthesis of silver nanoparticles by reducing, the respective metal ion solution with leaves extract may be easily observed by UV- Vis spectroscopy.

b) FT-IR chemical analysis:

The interaction of Ag-NPs obtained with PEG and gluconic acid products by reduction of sugar compound were confirmed by FT-IR spectra.

c) Antibacterial Activity:

The synthesized AgNPs was evaluated against human pathogenic bacteria (*Pseudomonas aeroginosa* MTCC 2453, *Bacillus subtilis*MTCC121, *Staphylococcus aureus*MTCC96, *Klebsiella pneumonia*MTCC109 and *E.coli* MTCC 912) using the agar well diffusion method (Perez *et al*. 1990).

d) Determination of total antioxidant capacity (TAC):

Total antioxidant activity of sulfated polysaccharides from seaweeds will be determined according to the method (Prieto *et al*. 1999).

e) DPPH radical scavenging assay:

The free radical scavenging activity of sulfated polysaccharides from seaweeds were measured by the 1-1-Diphenyl-2-picryl-hydrazyl (DPPH) following method (Blois, 1958).

f) Hydroxyl radical scavenging assay:

Hydroxyl radical scavenging activity will be measured by studying the competition between deoxyribose and test compounds for hydroxyl radical generated by Fe^{3+}- Ascorbate EDTA H_2O_2 system. The free radical damage imposed on the substrate, deoxyribose (TBARS) will be measured by the method (Yamaguchi *et al*. 2006).

RESULTS

The *Couropita guinanensis* flower extract mediated nanoparticles showed absorbance peaks at 318--323nm region in the spectral analysis shown in Fig.1.The peaks were stable with time duration also.It indicates that the synthesis of silver nano particles reqires the reduction of α-NADPH to α- $NADP^+$ and the hydroxy quinoline probably acts as electron shuttle tranforming the electron generated during the reduction of nitrate to Ag^+ ions translating them to Ag^0

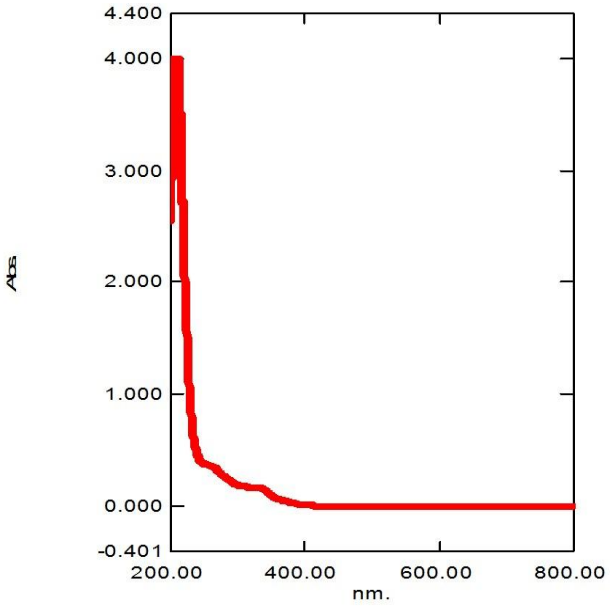

Fig.1: UV-spectrophotometer for $AgNo_3$ Synthesis of *Couroupita guianensis*

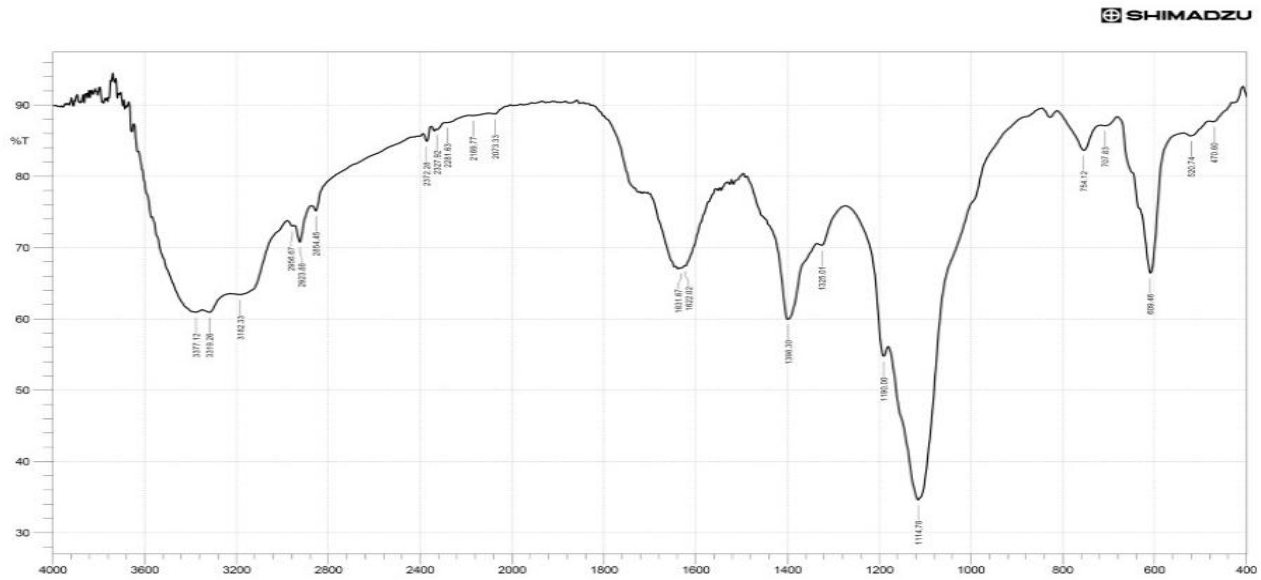

Fig.2: FTIR analysis for $AgNo_3$ synthesis of *Couroupita guianensis*

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy analysis of the silver nanoparticles showed absorption peaks of reduced silver at 1631.95 cm−1 (Fig.2). The stretching vibration of C=C obtained at 1622 and the single absorbance peak located at 1114 cm−1 is assigned to C-O Polyols Further distinct peaks in the region of 2372.28 cm−1 correspond to C≡N stretch for nitrile groups, while 3377.12 and 3319.26 cm−1 corresponds to O-H and N-H stretching vibration. The aromatic C-H stretching vibrations obtained at 2956.67 and 2923.88 cm−1 respectively.

ANTIBACTERIAL STUDIES

The antimicrobial activity of silver nanoparticles *Couroupita guianensis* against various pathogenic organisms including bacteria and yeast was investigated. Compared with the control, the diameters of inhibition zones increased for all the test pathogens. At concentration of plant extract with silver nanoparticles. The (12 mm) clear inhibitory zone appeared around 100µl against *Pseudomonas aeroginosa* MTCC 2453after incubation for 24h followed by *Bacillus subtilis* MTCC121(23mm), *Staphylococcus aureus*MTCC96 (13 mm), *Klebsiella pneumonia* MTCC109 (15 mm) and *E.coli* MTCC 912 (20 mm).(Fig.3. and Table.1)

Total antioxidant properties

Free radical scavenging activity of the silver nanoparticles was assessed by DPPH solution exhibited a deep purple colour with a maximum absorbance at 517nm. The disappearance of purple colour on adding synthesized silver nanoparticles might due to presence of antioxidant in the medium.

In *Couroupita guianensis* total antioxidant was found to increase with increase in concentration in standard, plant extract and $AgNO_3$. $AgNO_3$ showing a minimum activity at 19% at 100µg/ml and maximum activity observed 40% at 500µg/ml followed by the plant extract showing a minimum activity at 10% at 100µg/ml and maximum activity observed 60% at 500µg/ml(Fig.4).

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of *Couroupita guianensis flower extract of* silver nanoparticles exhibit higher activity at 15% in600µg/ml, likewise lower activity observed at 200 µg/ml with 3%followed by flower extract showing minimum activity at 200 µg/ml with 10% and also maximum activity observed at 600 µg/ml with 35%(Fig.5).

Table.1: Mean zone of inhibition of synthesized silver nanoparticles from *Couroupita guianensis*

Pathogens	Zone of inhibition (mm)			
	Water	Plant extract	$AgNO_3$	Plant extract with $AgNO_3$
Pseudomonas aeruginosa MTCC 2453	-	-	9	12
Bacillus subtilis MTCC121	-	-	11	23
*Staphylococcus aureus*MTCC96	-	-	7	13
Klebisella pneumoniae MTCC109	-	-	5	15
*E.coli*MTCC912	-	12	-	20

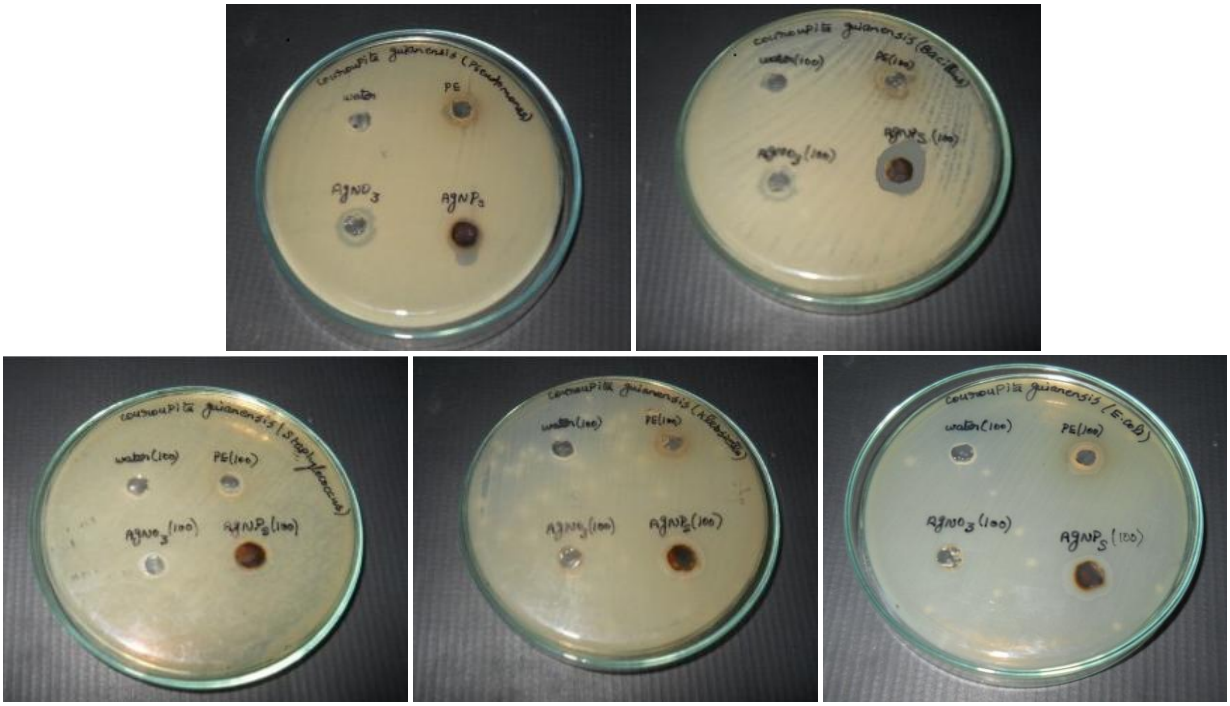

Fig.3: Activity of silver nanoparticles against different microorganisms depicting zones of inhibition of (**A**) Water control. (**B**) Plant extract-positive control (**C**) Silver nanoparticle positive control **(D)**Silver nano particle mediated plant extract synthesis at 100µg.

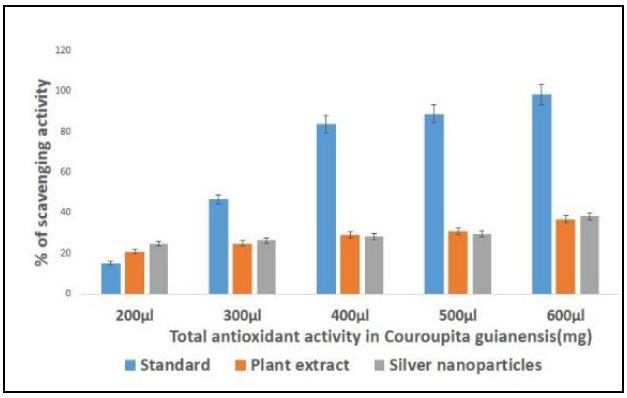

Fig. 4: Total antioxidant activity of *Couroupita guianensis.*

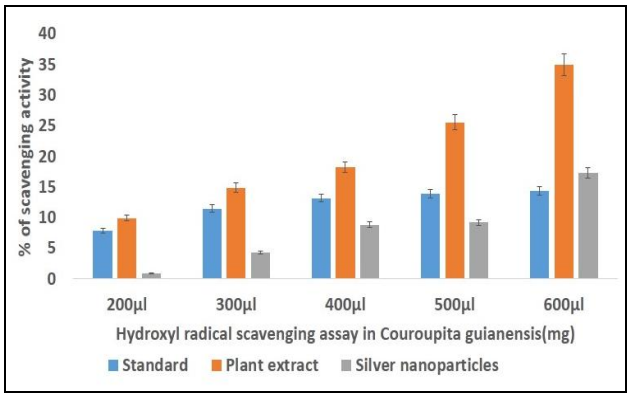

Fig.5: Hydroxyl radical scavenging assay in *Couroupita guianensis*

Hydrogen peroxide scavenging activity

In *Couroupita guianensis* $AgNO_3$ showing a minimum activity at 4% at 200µg/ml and maximum activity observed 9% at 500µg/ml followed by the plant extract showing a minimum activity at 37% at 200µg/ml and maximum activity observed 60% at 600µg/ml(Fig.6).

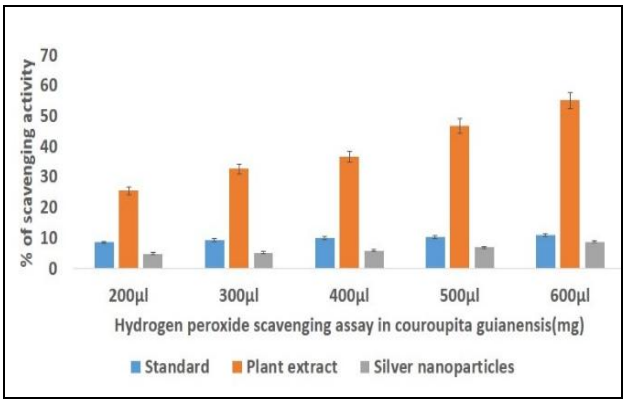

Fig.6. Hydrogen peroxide scavenging assay in *Couroupita guianensis*

DISCUSSION

The present exploration stataed that the *Couropita guinanensis* flower extract, mediated nanoparticles showed the peaks were stable with time duration also.It indicates that the synthesis of silver nano particles reqires the reduction of α-NADPH to α- $NADP^+$ and the hydroxy quinoline probably acts as electron shuttle tranforming the electron generated during the reduction of nitrate to Ag^+ ions convert into Ag^0. This is identical to the characteristics UV-visible spectrum of metallic silver.

Likewise, Bhat *et al*. (2011) stated that the bio reduction was achieved and attributed to the metabolites present in the plant. Synthesis of gold nanoparticles was pronounced when 15 mL of root extract was used for bioreduction, while 10 mL of the same root extract was sufficient for production of silver nanoparticles. Nanoparticles may grow in a process involving rapid bio reduction and strongly influence surface plasmon resonance in the water extract. Similarly it was correlated by *Artemisia nilagirica*, indicating occurrence of a silver band at 340–400 nm followed by Vijayakumar *et al*. (2012).

Couropita guinanensis flower extract mediated nanoparticles determined by the Fourier transform infrared spectroscopy analysis of the silver nanoparticles showed absorption peaks of reduced silver at 1631.95 cm^{-1}. The stretching vibration of C=C obtained at 1622 and the single absorbance peak located at 1114 cm^{-1} is assigned to C-O Polyols and further distinct peaks in the region of 2372.28 cm^{-1} correspond to C≡N stretch for nitrile groups, while 3377.12 and 3319.26 cm^{-1} corresponds to O-H and N-H stretching vibration. The aromatic C-H stretching vibrations obtained at 2956.67 and 2923.88 cm^{-1} respectively. The FTIR spectra of the silver nanoparticles depicted presence of functional groups like C-N, C-O-C, amide linkages and –COO-. These functional were found to play an important role in the capping of nanoparticles and their further stability in aqueous solution.

Fourier transform infrared spectroscopy analysis of the silver nanoparticles showed absorption peaks of reduced silver at 1653.96 cm^{-1} and 1027.44 cm^{-1} in the region of 1000–1800 cm^{-1}. Two absorption peaks located at 1653 cm^{-1} are associated with the stretch vibration of C=C and the single absorbance peak located at 1027 cm^{-1} is assigned to C-N stretching vibrations of amine. Further distinct peaks in the region of 2343.97–2362.27 cm^{-1} correspond to C≡N stretch for nitrile groups, while 3447.86 cm^{-1} corresponds to O-H stretching vibration by Geethalakshmi *et al*. (2012) Therefore, the present study showed same functional groups of silver nanoparticles

In the contemporary study reported that the *Couropita guinanensis*, plant extracts are also effective against selected human pathogenic organisms. In this junction, the antimicrobial activity effect of silver was identified. Hence there are a number of studies in the field of silver nanoparticles by using different type of procedure. *Couropita guinanensis*, silver ion and

silver based compounds are highly toxic to microorganisms showing a strong biocidal effect against microbial species.

These clearly indicate that the enhancement of efficacy was due to the synergistic antibacterial action between antibiotics and silver nanoparticles. Silver nanoparticles facilitate the transport of antibiotics to the cell surface acting as a drug carrier. More recently it is shown that silver chelation prevents unwinding of DNA. Silver nanoparticles are composed of silver atoms. Silver nanoparticles are larger in size than silver ions, which makes them react with more molecules, leading to more antimicrobial activity. In the identical way, the antibacterial effect was more pronounced in Gram-negative bacteria than Gram-positive ones. The antimicrobial activity of colloidal silver particles is influenced by the particle dimensions Silver has long been recognized as having an inhibitory effect on microbes present in medical and industrial processes. The most important application of silver and silver nanoparticles is in medical industry, such as in topical ointments to prevent infection against burns and open wounds by Kaviya *et al*. (2011).

In the present examination, *Couropita guinanensis* was studied for antioxidant properties. DPPH is a stable and well characterized synthetic solid radical for evaluation of antioxidant potential of compounds. The DPPH will be reduced by accepting the hydrogen or electron, the DPPH reducing ability of silver nanoparticles was quantified spectrophotometrically by changing the DPPH color from purple to yellow. Inhibition was found to be high in silver nanoparticles, when compared with gold nanoparticles, which may be due to the facts that silver act as a good oxidant can easily lose electrons. Similar observations with enhanced DPPH scavenging activity by selenium, platinum, silver nanoparticles have been reported by Saikia *et al*. (2010).

In the present analysis *Couropita guinanensis*, shows the superoxide scavenging activity of both the plant extract and AgNPs as determined by the PMS-NBT reduction system. Superoxide (O_2 −) radicals easily react with DNA and protein which necessitate their immediate clearance in living systems. The superoxide radical quenching activity of plant extract and AgNPs was found to be increased with increasing concentrations and the average inhibition was about 40%. Similarly, the superoxides radical inhibition has been reported for platinum and selenium nanoparticles by Ramamurthy *et al*. (2013).The potential superoxide scavenging activity of gold and silver nanoparticles report supported our findings by Pacher *et al*. (2007).

Couropita guinanensis, of H_2O_2 scavenging activity of Plant extract and AgNPs are active in quenching H_2O_2 radicals and the average inhibition was found to be 80 % By the same token, PLAgNPs were as effective as PLFE in quenching H_2O_2 radicals and the average inhibition was found to be 96% as compared to PLFE. In this study, it could be noted that the superoxide radical quenching activity and NO quenching activity of PLAgNPs was 60% and 70% respectively as compared to PLFE which can be explained on the fact that the concentration of phytocompounds responsible for the scavenging activities was higher in the extract than adhered to the nanoparticles. On the other hand, the observed increase in H_2O_2 scavenging activity of PLAgNPs (96%) may be because of the plant condensed tannins present in the extract that are involved in the formation of nanoparticles. Similar observations were made with silver nanoparticles prepared with stem bark of *Shorearox burghii* previously observed by Subramanian *et al* (2013).

CONCLUSION

Silver nanoparticles synthesis of *Couropita guinanensis* exhibited high antioxidant properties.

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