Antimicrobial and antioxidant activities of algal-mediated silver and gold nanoparticles

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Abstract

This study focused on the synthesis and application of nanoparticles using Neodesmus pupukensis (MG257914). Cell free extracts of the microalga was employed to synthesize both silver (AgNPs) and gold nanoparticles (AuNPs). The nanoparticles were characterized using UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), and Scanning electron microscopy (SEM). The nanoparticles were screened for their antimicrobial potential and free radical scavenging activity against stable free radical (2,2-diphenyl-1-picrylhydrazyl). The UV absorption spectra showed absorbance peaks of 430 nm and 530 nm for AgNPs and AuNPs respectively. The FTIR spectra at 3283, 2102.87, 1636.02 for AgNPs and 3264.86, 2104.49, 1636.62, 1232.39, 1028.97 cm⁻¹ for AuNPs confirms the participation of proteins in the capping and stabilization of the nanoparticles. The zone of inhibition indicating the activity of the NP-AgNPs were: Pseudomonas sp (43 mm); Escherichia coli (24.5 mm); Klebsiella pneumoniae (27 mm), Serratia marcescens (39 mm) while AuNPs showed activity to only Pseudomonas sp (27.5 mm) and Serratia marcescens (28.5 mm). The antifungal potency of NP-AgNPs was confirmed with mycelial inhibition of 80.6, 57.1, 79.4, 65.4 and 69.8% against Aspergillus niger, A. fumigatus, A. flavus, Fusarium solani and Candida albicans respectively, while NP-AuNPs had 79.4, 44.3, 75.4, 54.9 and 66.4% against A. niger, A. fumigatus, A. flavus, F. solani and C. albicans respectively. Appreciable free radical scavenging properties was obtained with NP-AuNPs (68.9%) and NP-AgNPs (41.21%). The nanoparticles of Neodesmus pupukensis showed appreciable potential as...
antimicrobial and antioxidant agents and could be explored for various applications in biotechnology.

**Keywords**: *Neodesmus pupukensis*, Nanoparticles, Antimicrobial activity, Antioxidant activity

1. **Introduction**

Multidrug resistance of pathogenic organisms and the occurrence of different human diseases emerging with time have called for researches in search of compounds from inorganic sources which can serve as potential bioactive agents [1]. Various pathogenic organisms have developed resistance to some of the available drugs due to drug abuse. The alarming occurrence of diseases caused by these multidrug resistance microorganisms has called for the need to obtain biologically active compounds from biological materials. These compounds must possess the potential that inhibit the growth of pathogenic organisms and not harmful to human. Similarly, relative oxygen species (superoxide anion, hydroxyl radicals and hydrogen peroxide) which are produced when metabolism takes place starts up a process where membrane lipids and other biomolecules becomes peroxidized; this results in several diseased conditions in the body [2]. There is a need to introduce antioxidants that have the ability to stop and scavenge free radicals in the body thereby resulting in the protection of the body against diseases. The body is being protected against diseases by antioxidants; they are able to achieve this by scavenging ROS thereby preventing oxidant-induced damaging of cell structures and molecules [3]. Antioxidants are important in building the body against diseases in that they enhance the physiology of the human body [4]. Although the human system contains some effective antioxidants which are able to handle oxidative stress against the body, there is need for additional antioxidants that are non-indigenous [4].

Microalgae are organisms that contain rich metabolites reservoir of various chemical components and biologically active substances [5]. They contain materials which are biochemically active such as polyunsaturated fatty acids, polyphenols, carotenoids, vitamins, and phycobiliproteins which have been identified as antioxidants responsible for getting rid of the action of ROS that are produced when photosynthesis takes place [6-9]. Microalgae possess mechanisms with which they defend themselves against the resulting photo-induced oxidative damage which is caused by the antioxidative mechanics to detoxify and remove highly reactive oxygen species [10].

Exogenous antioxidants are present in photosynthetic microalgae and these include: polyphenols, carotenoids, sterols or vitamins [11]. Microalgae and macroalgae contain
carotenoids such as: fucoxanthin, diatoxanthin, siphonaxanthin, and diadinoxanthin [8,12]. Apart from carotenoids with high bioactivity, phycobiliprotein which are also present in microalgae possess both antioxidant and antimicrobial activity [13]. Aside from the known applications of microalgae biomass as a potent antimicrobial and antioxidant agent, green synthesis of nanoparticles from microalgae is also a promising research area which has been underexplored [14-15]. Microalgae are able to produce nanoparticles using various metal ions as precursors; these metals include silver, gold, platinum, and cadmium [16-19]. Phyco-nanotechnology which involves nanoparticles synthesis from algae as bioagent is gaining attention because of the potential applications of these algal mediated nanoparticles [20-24]. Synthesis from algae has shown some advantages which are reduction in duration of synthesis [25-26], its ease of application in optimization of silver nanoparticles and absence of culture and maintaining process [27]. Algae are abundantly rich in proteins, lipids, carbohydrates; these molecules play vital roles in the reduction of metals [28]. Stability and capping of these metallic nanoparticles in solution is the major responsibility of proteins [29]. In the current study, an attempt was made to synthesize both silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) from the cell-free extracts of *Neodesmus pupukensis* (MG257914). The synthesized AuNPs and AgNPs of each microalga were screened for both antimicrobial and antioxidant potentials.

### 2 Materials and Methods

#### 2.1 Maintenance and Harvesting of Microalgal cultures

*Neodesmus pupukensis* (MG257914) which was previously isolated from fish pond water in our laboratory was cultured in BG 11 medium. The algal biomass was harvested on day 21 using a centrifuge at 5000 rpm for 20 min. Sterile distilled water was used five times to achieve the removal of BG II medium from the microalgal biomass [22]. Five pathogenic bacteria and five fungi were collected from the Department of Science Laboratory Technology (SLT), LAUTECH, Ogbomoso, Oyo State, Nigeria. These test organisms include: bacteria (*E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *S. aureus* and *S. marcescens*) and fungi (*A. niger*, *A. flavus*, *A. fumigatus*, *F. solani* and *C. albicans*).

#### 2.2 Synthesis of Silver and Gold Nanoparticles from Microalgae

Algae powder was obtained from the microalga by drying the filtrate obtained above in a glass Petri dish at 80 °C. One gram of algal powder was dissolved in 100 ml of distilled water, boiled at 100 °C for 20 min [22]. The cell-free extract of the microalga was separated from the mixture in a centrifuge at 5000 rpm for 10 min. Synthesis of silver nanoparticles
from the extract was done by adding 90 ml of 1 mM AgNO$_3$ to 10 ml of the extract while the same quantity of 1 mM gold chloride was used to synthesize gold nanoparticles. The mixtures were incubated and the colour change was monitored until it stabilized [30]. The nanoparticles were designated with identification codes according to the precursor used for synthesis as follow: *Neodesmus pupukensis*-based silver nanoparticles (NP-AgNPs) and *Neodesmus pupukensis*-based gold nanoparticles (NP-AuNPs)

2.3 Characterization of nanoparticles

Bioreduction of silver nanoparticles was first observed with the change in colour and the spectra of the reaction mixtures were measured using Double beam spectrophotometer with a quartz cuvette of 1 cm optical path length with spectra range from 300 and 1000 nm. The functional groups present in each nanoparticle were evaluated with the use of FT-IR spectrophotometer. The sample solution was centrifuged at 7000 rpm for 10 min. The resulting pellets were washed in double distilled water twice. The algal pellets were freeze dried and ground to powder with KBr. The FT -IR analysis was done on avatar 330 FT-IR instrument in the region of 4000 to500 cm$^{-1}$ at a resolution of 4 cm$^{-1}$.

The silver nanoparticles of the algal were prepared for SEM analysis in order to determine the size and morphology of the nanoparticles. The microphotograph was recorded using Concise FEGSEM 6100 Zeiss Ultra Plus (Germany) at an accelerated voltage of 20.0 kV with secondary electrons in low vacuum mode. The gold nanoparticles were also prepared and a drop of each sample was deposited on copper grids already coated in carbon, the solution was dried and the micrograph of each sample was taken with JEOL JEM 2100 HRTEM at accelerating voltage of 200 kV. The elemental compositions of the silver and gold nanoparticles were measured with the same equipment.

2.4 Antimicrobial activity

The Antimicrobial activity of gold and silver nanoparticles of microalgae was determined by agar well diffusion method. One hundred microliter of each bacterium was spread uniformly on Mueller Hinton Agar (MHA) plate, and five different wells were made on the plate using a cork borer. Four different volumes (25, 50, 75, and 100 µl) of the nanoparticles were prepared. Each well represented each concentration while the fifth hole served as control where no nanoparticles were added. Standard antibiotic disc were used as control.
2.5 Antifungal assay

The antifungal potency of AgNPs and AuNPs were evaluated by adding graded concentration of each AgNPs and AuNPs into potato dextrose agar plates which were subsequently inoculated with 6 mm agar plug of 48-h old cultures of each fungus [29]. In the control experiments, fungal plugs were inoculated on PDA plates without the incorporation of nanoparticles. All the plates were incubated at 28 °C for 72 h. The diameter of fungal growth in all the plates were measured and used to determine the percentage growth inhibitions as follows:

\[
\text{Mycelia inhibition (\%)} = \frac{D_{\text{control}} - D_{\text{test}}}{D_{\text{control}}} \times 100
\]

D is the diameter of fungal growths on the potato dextrose agar plates.

2.6 Antioxidant assay

The antioxidant property of the NPs was done using DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) assay. DPPH (1.97 g) was measured and dissolved in 5 ml of methanol. One millilitre of this solution was added to 3 ml of hydrogel solution (1 g of prepared hydrogel in 10 ml of distilled water). The mixture was shaken and left to stand for 30 min at room temperature. The absorbance at 517 nm was measured by using a UV-visible spectrophotometer. Antioxidant activity was estimated by calculating the % inhibition by the following formula:

\[
\text{DPPH Scavenging effect (\%)} = \frac{\text{Control}_{\text{absorbance}} - \text{Sample}_{\text{absorbance}}}{\text{Control}_{\text{absorbance}}} \times 100
\]

3 Results and Discussion

3.1 Synthesis of nanoparticles and UV-visible spectroscopy

The colour change indicates the synthesis of nanoparticles. It was monitored immediately after the addition of silver nitrate and gold chloride to the cell free extract of *N. pupukensis*. There was colour change from pale green to brown after 25 min of incubation for NP-AgNPs with the UV-visible absorption spectrum at 436 nm (Figure 1a). The colour change in NP-AgNPs is in agreement with previous reports on the synthesis of nanoparticles from algae and also the surface plasmon resonance is in agreement with earlier reports (22, 31). Similarly, for gold nanoparticles, the colour change from pale green to purple was observed within 2 h of incubation and the surface plasmon resonance peak was obtained at 554 nm (Figure 1b). The absorbance peak obtained for NP-AuNPs is similar to previous
reports [18-19, 23]. The absorption spectrum of UV-visible is a strong confirmation that metallic nanoparticles have been formed in the mixture.

**Figure 1.** UV-visible absorption spectra of NP-AgNPs (A) and NP-AuNPs (B)

### 3.2 Electron microscopy and elemental composition of nanoparticles

The scanning electron microscopic image of NP-AgNPs showed spherical shape with particle size ranging from 52-179 nm (Figure 2a), while the transmission electron microscopy of NP-AuNPs showed circular shaped particles with size ranging from 5-34 nm (Figure 2b). The micrographs obtained confirm the bioreduction of silver ions. The nanoparticles of both silver and gold have broad absorption peaks and this accounted for the polydispersed nature of the nanoparticles.

**Figure 2a.** SEM image of NP-AgNPs

**Figure 2b.** TEM image of NP-AuNPs
The EDX spectra of NP-AgNPs revealed that silver was the most abundant with weight composition of 33.33% with a strong energy signal strength between 3-3.2keV (Figure 3a) while a strong signal strength was obtained for gold in NP-AuNPs with a weight composition of 96.62% (Figure 3b). The absorption peak displayed at energy signal strength between 3 and 4 keV is similar to previous report for metallic silver nanoparticles [14].

### 3.3 Fourier Transform Infrared Spectroscopy of Algal Nanoparticles

The FTIR spectroscopy revealed prominent peaks at 3283.0, 2102.87, and 1636.02 cm\(^{-1}\) for NP-AuNPs (Figure 4a), while NP-AuNPs had prominent peaks at 3264.86, 2104.49, 1636.62, 1232.39 and 1028.97 cm\(^{-1}\) (Figure 4b). The absorbance bands present in the nanoparticles at 3283, 2102, 1636, 3264.86, 2104.49 and 1636.62 cm\(^{-1}\) indicated that alcohol and unsaturated hydrocarbon (O-H stretching vibrations in polyphenols and stretching vibrations of the (NH)==O group) were present, while absorbance band at 1232 cm\(^{-1}\) showed Aryl and Vinyl ethers (C-O stretching vibrations), and 1028 cm\(^{-1}\) shows the presence of Diakyl ether (C-O stretching vibrations). The different peaks which represent various functional groups were responsible for the reduction and capping of resulting nanoparticles.
3.4 Antimicrobial activities of nanoparticles

The antimicrobial potential of both NP-AgNPs and NP-AuNPs was investigated against five pathogenic bacteria and toxigenic fungi. NP-AgNPs showed a high antibacterial potency of 43 mm, 24.5 mm, 27 mm and 39 mm against *Pseudomonas aeruginosa*, *E. coli*, *K. pneumoniae* and *S. marcescens* respectively, while NP-AuNPs was mildly active against both *Pseudomonas* sp and *S. marcescens* with zone of inhibition of 27.5 mm and 28.5 mm respectively (Figure 5a). The activity of NP-AgNPs and NP-AuNPs against *Staphylococcus*
The comparison of standard antibiotic disc with nanoparticles of *N. pupukensis* shows high activity of silver nanoparticles against the five test pathogens. The highest zone of inhibition was obtained against *Pseudomonas aeruginosa* (43 mm), while the lowest was obtained with *E. coli* (24.5 mm). The silver nanoparticles had higher antibacterial activity compared to their gold counterparts which was fairly active against *E. coli* and *S. marcescens*. Gold nanoparticles of *Glacilaria corticata* were reported to be inactive against some bacteria but a high activity was reported when combined with antibiotics [19]. Although the AgNPs have been reported to be dose dependent in their antibacterial activity [22], the report in this work shows that silver nanoparticles of *N. pupukensis* were active at all doses compared against the test pathogens (Figure 6). However, the cell-free extract *N. pupukensis* did not inhibit any of the test isolates.

**Figure 5a.** Antibacterial activity of some antibiotics and nanoparticles against Gram negative pathogenic bacteria {OFX (10 µg Tarivid), S (Streptomycin 30 µg), SXT (Septrin 30 µg), CH (Chloramphenicol 30 µg), SP (Sparfloxacin 10 µg), CPX (Ciprofloxacin 10 µg), AM (Amoxacillin 30 µg), AU (Augmentin 30 µg), CM (Gentamycin 10 µg) PEF, (Pefloxacin 10 µg), AgNPs (Silver nanoparticles) and AuNPs (Gold nanoparticles)}. 
Figure 5b. Antibacterial activity of some antibiotics and nanoparticles against *Staphylococcus aureus* {E (10 μg Erythromycin), PEF, (Pefloxacin 10 μg), CN (Gentamycin 10 μg), APX (Ampiclox 30 μg), Z (Zinnacef 20 μg), AM (Amoxicillin 30 μg), R (Rocephin 25 μg), S (Streptomycin), SXT (Septrin 30 μg), CPX (Ciprofloxacin 10 μg), AgNPs (Silver nanoparticles) and AuNPs (Gold nanoparticles)}.  

Figure 6. Antibacterial activity of silver nanoparticles synthesized from *N. pupukensis* against (F) *P. aeruginosa*; (G) *E. coli*; (H) *K. pneumoniae*; (I) *S. aureus*, and (J) *S. marcescens* at different concentrations of A (25 μl), B (50 μl), C (75 μl), D (100 μl) and E (cell free extract of *N. pupukensis*).  

The antifungal potency of NP-AgNPs was confirmed with mycelial inhibition of 80.6, 57.1, 79.4, 65.4 and 69.8% against *A. niger*, *A. fumigatus*, *A. flavus*, *F. solani* and *C. albicans* respectively, while NP-AuNPs had 79.4, 44.3, 75.4, 54.9 and 66.4% against *A. niger*, *A. fumigatus*, *A. flavus*, *F. solani* and *C. albicans* respectively (Figure 7). The highest mycelial inhibition was obtained against *A. niger* by NP-AgNPs (80.6%) and the least was against *F.*
solani by NP-AuNPs (44.3 %). Both silver and gold nanoparticles of N. pupukensis were highly potent against the toxigenic fungi investigated and some of the plates showing mycelial inhibition are shown in Figure 8.

![Graph showing mycelial inhibition](image)

**Figure 7.** Antifungal activities of silver and gold nanoparticles of *Neodesmus pupukensis*

![Figure 8: Potato dextrose agar plates showing mycelial inhibition](image)

**Figure 8.** Potato dextrose agar plates showing mycelial inhibition of silver and gold nanoparticles of *Neodesmus pupukensis.*
3.5 Antioxidant activities of gold and silver nanoparticles

The ability of NP-AgNPs and NP-AuNPs to scavenge free radical against 2,2-diphenyl-1-picrylhydrazyl was high for NP-AuNPs at 68.96%, followed by NP-AgNPs (41.21%), while the least of 37.56% was obtained from the cell free extract of *N. pupukensis* (Figure 9). The ease and rapidity that comes with the use of DPPH has presented it as a tool in the determination of antioxidant potential of a compound. An increased antioxidant activity was obtained with the nanoparticles than the cell free extract of *N. pupukensis*; this agrees with a report on the antioxidant activity of silver nanoparticles of * Isochrysis* sp and the cell extract [31].

![Figure 9](image_url)

**Figure 9.** Free radical scavenging activity of silver nanoparticles, gold nanoparticles and cell-free extract of *N. pupukensis*

4.0 Conclusion

The green synthesis of both silver and gold nanoparticles from *N. pupukensis* was carried out. It was demonstrated that the silver nanoparticles were able to actively inhibit the growth of both Gram positive and negative bacteria, while the gold nanoparticle was fairly active against two of the five pathogens. The antifungal and antioxidant activities of the nanoparticles were also outstanding and this presents nanoparticles of *N. pupukensis* as a prospective candidate for several biomedical and biotechnological applications.
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