



# Evaluation of Antioxidant Activities from Biosynthesized Copper Nanoparticles (AO-Cunps) Using *A. Officinalis* Leaf Extracts

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## Abstract

Nanotechnology is an expanding area of research where the materials are dealt with in nano- dimensions. The conventional procedures for synthesizing metal nanoparticles (NPs) need sophisticated and costly instruments or high-priced chemicals which is a major disadvantage of this research. Going forward with the chemical techniques may not be environmentally safe as the generated chemical waste could be a demerit to develop nanoparticles by any chemical means. Therefore green technologies for the synthesis of nanoparticles are always preferred. The biological method of synthesizing nanoparticles from inactivated plant tissue extracts is gaining more interest nowadays in comparison to synthesis by physical or chemical means. Synthesis of metal nanoparticles by biological means is predominantly cost-effective, which in turn can be a viable method for the large-scale development and production of metal nanoparticles. Plant extracts are mostly useful in nanoparticle synthesis as both reducing and capping agents, because of their bioactivity and lack of toxicity. As a result, many researchers started using phytochemicals as reducing agents and stabilizers in nanoparticle synthesis, resulting in the development of phytochemical-based nanoparticles. The mangrove plants which are well known for their rich content of chemically diversified and unique phytochemicals have been least exploited for their potency of nanoparticle synthesis. In the present study, the aqueous leaf extracts of a medicinally important mangrove plant *Avicennia officinalis* are being utilized to synthesize copper nanoparticles. Further, the antioxidant potential of this synthesized nanoparticle is evaluated employing DPPH scavenging assays. The copper nanoparticles (AO-CuNPs) were prepared from an aqueous solution of  $\text{CuSO}_4$ . The absorption peak at 300–400 nm confirmed the synthesis of AO-CuNPs. The DPPH free radical scavenging antioxidant assay showed all the three NPs could scavenge the DPPH radical in a dose-dependently. The present study showed that the synthesized nanoparticle AO-CuNPs showed antioxidant potentials which could be utilized in various biological applications by the pharmaceutical and food industries.

**Keywords:** Nanotechnology; Nano- dimensions; Nanoparticles

## Introduction

“Nanotechnology has wide applications in almost every field with the most exploitable properties being chemical, optical, mechanical, thermal, specific surface area, electrical, magnetic, diagnostics and medical etc [1]”. It has a huge potential to play an important role in global manufacturing and commercialization. Nanotechnology has brought scientific revolution and remarkable enhancement in the twenty first century due to elaboration and details exploration in all over the world. Particles having size less than 100 nm at most belongs to the range of nanoparticles.

“Colloids, precipitates, spherical nanoparticles, fullerenes, and dendrimers are examples of nanostructures available in three dimensions, which show all dimensions at the nanoscale” [2]. These plant-derived nanoparticles have novel catalytic, magnetic mechanical, absorption, sensitivity, and bio imaging properties, as well as industrial, agricultural, and medical applications [3]. Nanomaterials have the potential to be used as lubricants. They're used in a variety of products, including paints and coatings, ceramics, batteries, clays, and fuel cells. Due to their numerous applications in a variety of fields, metal nanoparticles are extremely important. Copper nanoparticles are gaining popularity

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among nanoparticles because of their optical and electrical conducting properties as well as their lower cost than gold and silver [4,5]. Copper nanoparticles are useful in a variety of applications, including catalysts, printed circuit boards, flexible electronics, light emitting diodes, and biocompatibility [6]. They're mixed into lubricant oil to help repair worn surfaces by lowering friction [7]. Microorganisms such as *S. aureus*, *S. cerevisiae*, *E. coli*, and *Listeria* can be inhibited by bioactive coatings made of copper Fluoropolymer nanocomposites [8]. Copper oxide is used in textiles as an antimicrobial [9]. Hospital wastewater is disinfected with copper and silver ions [10]. "Copper nanoparticles were found to be more active than silver nanoparticles against single *B. subtilis* and *E. coli* strains [11]. "Copper nanoparticles have been reported to be bactericidal when supported on polyurethane foam, carbon, sepiotile, and polymers" [12]. "Another study found that copper nanoparticles has superior activity against *B. subtilis*, *S. aureus*, and *E. coli* strains" [13]. Copper ions may interact with DNA molecules and nucleic acid strands. However, the high oxidizing property of copper nanoparticles when exposed to air and water makes their synthesis difficult. Nanoparticles' biological effectiveness is proportional to their surface area. Because of their size and shape, nanoparticles have new and improved properties. For the synthesis of copper nanoparticles, various methods such as direct electrochemical reduction, thermal reduction and decomposition, electro exploding wire (EEW), in situ synthesis in polymers, polyol process, mechanic-chemical process, ion beam radiation, and chemical reduction have been used; however, most of the reduction methods require a large amount of energy and chemical input. The Mumbai coast is home to 21 species, the most prominent of which are *Avicennia Sonneratia*, *Rhizophora*, and *Acanthus* [14]. Mangrove plants have unique characteristics such as the ability to survive in high salinity, extreme temperatures, anaerobic & unstable substrates, resulting in unique environments and floral faunal assemblages, and thus may produce unique types of bioactive compounds not found in terrestrial plants. According to recent research, Indian mangroves have antiviral, antibacterial, antifungal, and mosquito larvicidal, and antioxidant properties [15]. Mangroves are a salt-tolerant intertidal group of plants that have long been used in folk medicine for their anthelmintic, anti-inflammatory, and other medicinal properties. To adapt to their constantly changing environment and, indeed, to survive in such conditions, the mangrove plant community synthesizes a variety of secondary metabolites. The mangrove system is a vast ecosystem that contains an enormous amount of valuable products with green applications in medical biology, pharmacology, and other fields. They are mostly found in the world's tropical and subtropical intertidal zones, primarily between 30° north and 30° south of the equator. Their ability to survive in high-stress environments has resulted in unusual morphology and physiological adaptations.

Many researchers have shown interest in investigating various bio prospects of mangroves, as these plants are used for green synthesis of CuNPs, due to their unique characteristics [16]. Sundarban, the world's largest estuarine mangrove forest (covering 350 km in width), is shared by India and Bangladesh. Bio prospecting of mangroves from this unique habitat is a relatively new field of study. The goal of this study was to use and establish a new green synthesis procedure for CuNPs induced by mangrove leaves while evaluating their bio reductant potential. The medicinal and antimicrobial properties of the mangrove plants used in this study have previously been reported. The novel green synthesis of metal nanoparticles using this unique vegetation will add a new dimension to their numerous applications and bio prospects. Several studies have found that specially formulated CuNPs have potent antibacterial properties [17]. CuNPs are effective antimicrobials due to their unique ability to target microorganism cell walls, cytoplasmic membranes, and proteins. Several previous studies have looked into the antimicrobial and antiviral properties of CuNPs, as well as copper ions and compounds.

## Review of Literatures

Nanotechnology is transforming many fields of science, technology, and industry. The power to convert phytochemicals into nanoparticles via green chemistry has been harnessed in the field of nanotechnology as a way to mitigate nanotechnology's impact on environmental sustainability. Nanotechnology is causing an increase in environmental concerns, such as saving fuels, reducing production materials, toxic effects in medical care, monitoring pollutants in the environment, and green manufacturing. Due to quantum effects and a large surface-to-volume ratio, metal nanoparticles have fascinating ultraviolet-visible sensitivity, electrical, catalytic, thermal, and antibacterial properties. Due to the smaller particle size, a large number of atoms are present on the surface. The shape and size of nanoparticles, as well as their ultraviolet-visible sensitivity and conductivity, affect the surface area-to-volume ratio. The change in surface area modulates the electronic energy levels, electron affinity, electronic transitions, magnetic properties, phase transition temperature, melting point, and affinity to polymers, biological, and organic molecules, among other properties. The charge on nanoparticles is imparted by quantum effects, which are caused by a combination of quantum size and Coulomb charging effects. When the Coulomb charge effect is combined with quantum size, a variety of fascinating properties emerge that are not seen in bulk materials. In spherical particles and particles with sharp edges, quantum effects are prominent. Nanoparticles are used in catalysis, sensing, and imaging because of these effects and their size-dependent nature.

## Green Synthesis of Nanoparticles

Nanotechnology uses key methods to generate new products and to enhance the properties of broad range of market products of electronics, packaging, healthcare and coatings [18]. This use of nanotechnology is enhanced by Green nanotechnology. There are two methods for the synthesis of nanoparticles, one includes Chemical synthesis and other focused on Green synthesis [19,20]. The nanoparticle synthesized through the chemical method involves chemical reduction using different metal ions and chemicals such as sodium citrate, ascorbate, sodium borohydride, etc., whereas in green way for the synthesis of nanoparticle, green reducing agents are employed using phytochemical extracts of different natural products such as leaf extract, juice extract, extract from medicinal plants etc [21,22]. To provide unlimited opportunities for new discoveries. Green synthesis involves the synthesis of nanoparticles through aqueous extract of green product (such as plant extract of *Musa balbisiana* (banana), *Azadirachta indica* (neem) and *Ocimum tenuiflorum* (black tulsi), etc.) and metal ions (such as silver ions) [23]. The fixed ratio of plant extract and silver ions were mixed and kept at room temperature for reduction the change in color was noticed at regular intervals of time from yellow to reddish brown or dark brown that confirmed the formation of nanoparticles. Further, the synthesized nanoparticles were characterized by using UV, XRD and FTIR data [24]. Nowadays, the more focus is to develop an eco-friendly processes, to reduce the toxic chemicals in the process of nanoparticles synthesis. This marks the development in field of green chemistry to eco-friendly procedures for the synthesis and congregation of metal NPs. Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, Tollens because plant extract termed as phytochemical are rich in phenolic compounds, alkaloids, diterpenoid, steroid and other compounds which inhibit the development of various microorganisms as phytochemicals act as reducing and capping agent in the reduction of metal ions to metal nanoparticles. The Green nanoparticles synthesized via green principle provides important applications to prevent waste, less hazardous chemical, renewable feedstock, reduce derivatives. Green nanotechnology not completely fits into the picture of sustainability whereas there is a need to go beyond environmental protection for sustainability. Green synthesized nanomaterial could help to alleviate major sustainability issues of climate change, renewable energy, natural resources and toxic products.

### **Factors Influencing Nanoparticles Formation by Green Technology pH**

In both extracts and living plants, the pH of the medium has an impact on the size of nanoparticles forming [25]. PH is an important factor in the bio formation of colloidal gold, according to alfalfa biomass which has been proved earlier. investigated the formation of AuNPs using *Avena sativa* biomass and discovered

that the size of AuNPs can be controlled by adjusting the pH of the medium. The initial pH of the solution had no effect on the adsorption of Au ions to alfalfa biomass. However, the size of the resulting nanoparticles varied greatly depending on pH.

### **Temperature**

The fact that NPs biosynthesis takes place at room temperature is one of the most intriguing aspects of the process. The temperature of the reaction medium, on the other hand, is a critical factor that determines the nature of nanoparticles formed; a study using the leaf extract of *Cymbopogon flexuosus* revealed that at higher temperatures, the percentage of gold nanotriangles relative to spherical particles is significantly reduced, whereas lower reaction temperatures primarily promote nanotriangle formation [26].

### **Reducing agents**

Phytochemicals (such as; primary and secondary metabolism products like antioxidants, flavonoids, flavones, isoflavones, catechins, anthocyanidins, isothiocyanates, carotenoids, polyphenols) are known as an important natural resource for the synthesis of metallic nanoparticles among the various categories of compounds synthesized in plants that have potent biological activities [27]. A large number of papers have been published on the biosynthesis of nanoparticles using phytochemicals found in a variety of plant extracts [28].

### **Materials and Methods**

#### **Collection of mangrove plant**

The leaves of *Avicenna officinalis*, a mangrove plant, were gently collected from the Mahanadi Delta mangrove forest in Odisha and identified by the Department of Biotechnology, CET, Bhubaneswar, and Odisha.

#### **Synthesis of nanoparticles**

3 ml of leaf extract was finely added to an aqueous solution of copper sulphate,  $\text{CuSO}_4$  (20 mM, 7 ml) and heated at 80°C for 10 minutes in a water bath, then stirred for 4 hours in a magnetic stirrer. After 4 hours of continuous stirring, the reduction of  $\text{CuSO}_4$  to AO-CuNPs was confirmed by a change in the colour of the solution, followed by UV-Vis spectrophotometric determination [29-38].

#### **Characterization of nanoparticles**

The *A. officinalis* mediated synthesized nanoparticles are characterized by different analytical techniques like UV-Visible spectroscopy, dynamic light scattering (DLS) technique etc [39-45].

#### **UV-Visible spectral analysis**

CuNPs synthesised by *A. officinalis* leaf extracts had their UV–Vis absorbance spectra measured using the UV–Vis spectrophotometer UV–117 (SystronicsTM). The characteristic peaks were detected by measuring the absorbance in the 200–700 nm range with a 1 nm step. The spectra were taken at 1 to 10 minute intervals, with distilled water serving as a baseline [46-50].

### Dynamic Light Scattering (DLS) analysis

The size of AO-CuNPs was measured by using a particle size analyser. All measurements were performed at 25°C. Each measurement was obtained as an average of 20 runs.

### Bioactivity studies

The synthesized nanoparticles were evaluated for their antioxidant activity by DPPH free radical scavenging assay.

### DPPH scavenging activity

The method developed by was used to calculate the free radical scavenging activity of the synthesised nanoparticles on the stable radical 1, 1- diphenyl-2-picrylhydrazyl. Synthesized nanoparticles were mixed with 3.0 ml of a DPPH methanol solution at various concentrations (0.1 mg/ml to 0.5 mg/ml) (0.1 mM). A UV-Visible spectrophotometer was used to measure the absorbance at 517 nm against methanol as a blank after 30 minutes of reaction at room temperature in the dark. The percentage (percent) of inhibition of the free radical DPPH was calculated as follows:

$$\% \text{ of inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Abs control is the absorbance of the control reaction and Abs sample is the absorbance of the sample.

## Results

### Preparation of plant extract

The leaves of the *Avicennia officinalis* mangrove plant were collected in Odisha's Mahanadi Delta mangrove forest. The leaves of *A. officinalis* were cut and washed in distilled water. The leaves of *A. officinalis* were then pulverized after being shade dried for 15 days. 10g of leaves were boiled for 20 minutes in 100 ml of distilled water. Whatmann filter paper was used to filter the plant extract. A total of 40 mL of aqueous extract was collected and stored at 4 degrees Celsius for later use (Figure 1).

### Synthesis of Copper nanoparticle (AO-CuNP)

Copper nanoparticles were synthesized by adding *A. officinalis* leaf extract to a copper sulphate solution and stirring rapidly for 4 hours at 37 oC. The colour change of the solution and UV-spectral analysis confirmed the reduction of copper sulphate to CuNPs.

### Characterization of Nanoparticles

### Spectral analysis

Nanoparticles were first characterized by using a UV-Visible absorption spectrometer with a wavelength range of 400 to 800 nm. The absorbance spectra of nanoparticles formed in reaction media show that different synthesized nanoparticles have different absorbance peaks.

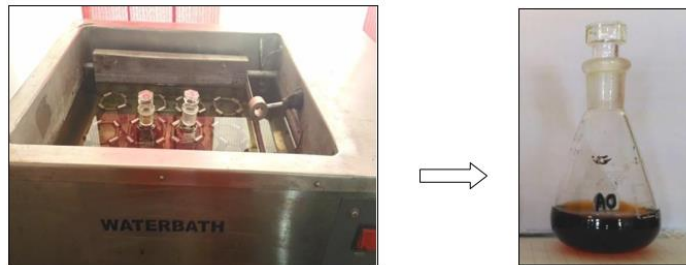


Figure 1: Preparation of leaf extract of *A. officinalis*.

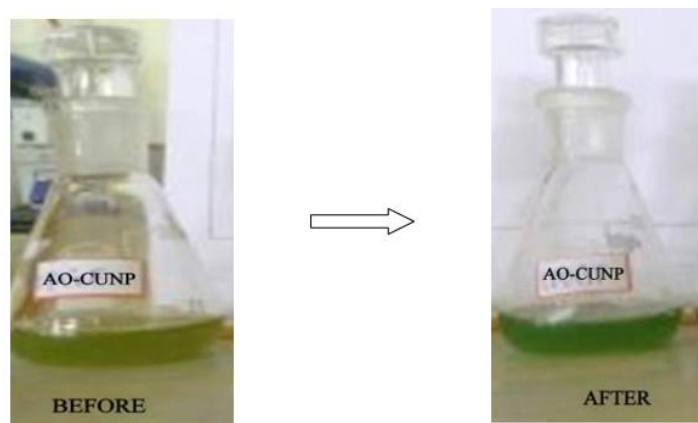


Figure 2: Copper nanoparticles (CuNP) synthesized using leaf extract of *A. officinalis* exhibiting change in colour.

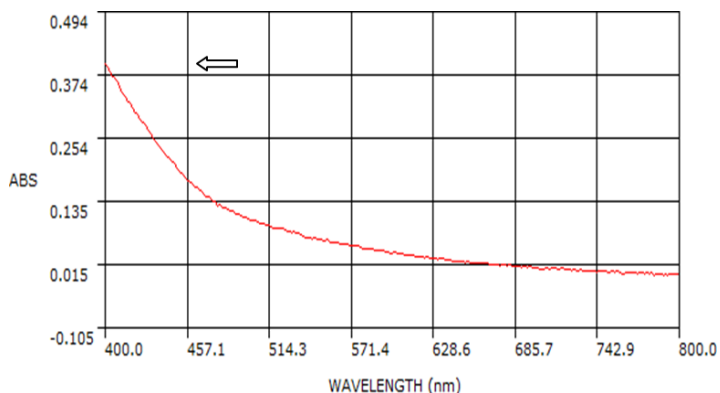


Figure 3: UV–Vis absorption spectra of copper nanoparticles (AO-CuNPs).

### Spectral analysis of Copper nanoparticles

UV-Visible Absorption spectra of copper nanoparticles formed in the reaction media have showed an absorbance peak which ranges

between 400 nm to 500 nm indicating the formation of copper nanoparticles (AO-CuNPs) (Figure 2,3).

### DLS analysis

DLS is a relatively new and widely used method for calculating the hydrodynamic diameter and differential potential of nanoparticle suspensions based on Brownian motions. DLS was used to examine the random changes in the intensity of light scattered from our solution in order to confirm the presence of a specific compound at a given wavelength.

### DLS analysis of copper nanoparticles

The size distribution of the nanoparticles given by zeta sizer for CuNPs. The average hydrodynamic diameter as calculated by DLS of copper nanoparticle is 772.1 nm with an intensity of 100 % (Figure 4).

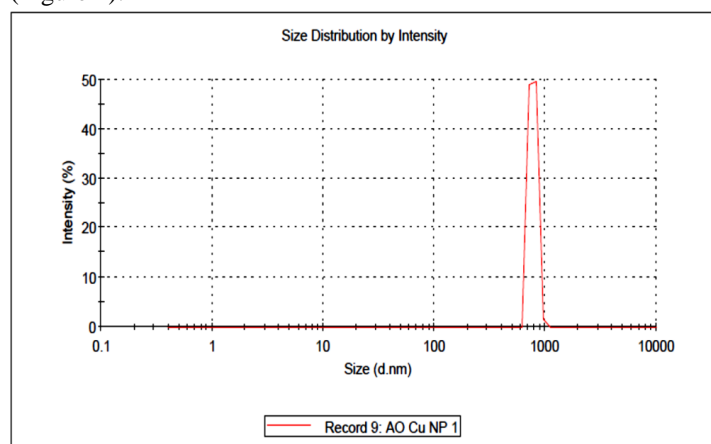


Figure 4: DLS results of copper nanoparticles (AO-CuNPs).

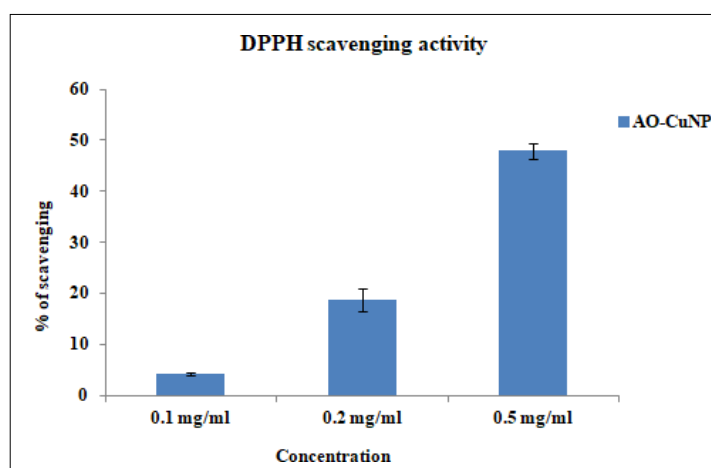


Figure 5: DPPH scavenging activity of copper nanoparticles (AO-CuNPs).

### Bioactivity Studies

In the present study, AO-CuNPs were evaluated for their in vitro antioxidant potential by studying their DPPH scavenging potential

### DPPH Scavenging Assay

The stable free radical DPPH was used to test the ability of samples to donate hydrogen. The nanoparticles' DPPH radical scavenging activity is shown below (Table 1).

Table 1: DPPH scavenging assay copper nanoparticles (AO-CuNPs).

Sample	Concentration	Percentage of Inhibition
AO-CuNP	0.1 mg/ml	4.19 ± 1.32
	0.2 mg/ml	17.90 ± 1.26
	0.5 mg/ml	49.83 ± 1.58

The radical scavenging activity of AO-CuNPs was found to increase as the concentration of AO-CuNPs synthesised by *A. officinalis* increased (Figure 5).

### Conclusion

Copper nanoparticles were synthesized in this study using *Avicennia officinalis* leaf aqueous extract, a medicinally important mangrove plant having a potential history of traditional remedies. Nanoparticle synthesis by biological means is a simple, quick, and environmentally friendly method that does not use any toxic chemicals. Different analytical techniques, such as UV-VIS spectroscopy and DLS analysis, were used to characterize AO-CuNPs. The antioxidant properties of the green synthesized NPs were tested by DPPH scavenging assay. In an in vitro antioxidant study, all three NPs were found to be able to scavenge DPPH free radicals in a dose-dependent manner. The mangrove plant's synthesized NPs could have applications in the biomedical and pharmaceutical industries, indicating the importance of further research. Thus, combining medicinal phytochemicals derived from *A. officinalis* leaves with NPs could lead to unprecedented opportunities for the discovery of a less expensive and more effective treatment for hyperglycemia, oxidative stress-induced diabetic complications, and inflammatory and bacterial infections.

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