# Biosynthesis and Antioxidant of Gold Nanoparticles by Endophyte Fungus Aspergillus Fumigatus Isolated from Sanna Surattensis Leaves

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## Abstract

In the modern era, the fabrication of nanoparticles is anew and advanced approach in the field of nanotechnology. Studies that include the description of gold nanoparticles produced by endophyte microorganism are important and novel in the field of technology, especially. In this study Aspergillus fumigatus was isolated from the healthy leaves of Sanna surattensis plant as endophyte fungus. The fungal isolate was identified by using advance molecular approach.GDNA was isolated, the target fragments of 18S rRNA amplified and send for sequencing following .A.fumigatus cell-free filtrate was used in the biosynthesis of gold nanoparticles (AuNPs) via reduction of gold salt (HAuCl4)solution to nanocrystal. The formation of AuNPs was monitored by observing the color change. The UV-VIS spectrum confirmed the reduction of gold ions. The resulting nanoparticles were characterized by Transmission electron microscopy(TEM) and X-ray diffraction (XRD).AuNPs were synthesis using A.fumigatus filtrate and tested for free radical scavenging activity by 1,1- diphenyl 2-picrylhydrazyl (DPPH) method .The size and shape of AuNPs determined by TEM were mostly spherical, and the average size 23 nm. The result emphasized of (XRD) indicates that the resulting material is pure gold nanoparticles. AuNPs stated significant antioxidant activity was comparable to the standard ascorbic acid.

Keywords: Biosynthesis, Gold nanoparticles, endophyte fungi, nanoparticles characterization, antioxidant.

## 1. Introduction

Nanotechnology is multidisciplinary science that can be defined as the manufacture and application of materials at the Nano level in various fields including physics ,chemistry ,engineering and medicine [1].The synthesis of nanoparticles is one the main aspects of nanotechnology due to their unique chemical ,physical ,electrical and optical properties compared to those of the large scale .The process of synthesis of nanoparticles has evolved from physical and chemical processes to biological processes because of the unsatisfactory and costly conditions of chemical and physical methods(e.g. Hazardous waste generation ,toxic chemical ,temperature requirements and high energy ).Biological sources have been exploited in the synthesis of nanoparticles as alga ,fungi , bacteria,actinomycetes and plant [2, 3].Fungi are excellent choice among microorganism for nanoparticles biosynthesis due to it their ability to secrete a large amount of enzymes outside their cells [4]. Alternaria alternate, Penicillium rugulosum, Aspergillus spp and other fungi have been tested in the biosynthesis of AuNPs [5-7]. Gold is a metal that belong to precious metals and has been used not only in the jewelry industry but also for industrial uses and application biology and medicine [8-10].The use of gold at the nanoscale shows interesting physical and chemical properties in contrast to the bulk metal, which is chemically inert and little use in scientific research [11].AuNPs properties that depend on their size (diameter), surface , structure , agglomeration state and shape, the most common method to describing AuNPs are UV-Visible spectroscopy and transmission electron microscopy [12, 13].Because of their oxidation resistance ,stability and biocompatibility, they are widespread research tool [14].Endophyte fungi are microorganisms that lives inside the tissues of healthy plant organs without causing any disease symptoms [15, 16].It has been isolated from stems, root and leaves of many plant such as cotton, canola ,ginseng and others [17].It is capable of producing a wide range of biologically active compound that can be used to treat many of illnesses and as reduction agent in the synthesis AuNPs [15, 18].Free radicals cause degenerative reaction that damage cell and biological molecules ,antioxidants attack free radicals, thus protecting cells and biological molecules from damage [19].Inorganic nanoparticles have been shown in recent research to effectively scavenge free radicals, especially highly effective oxygen [20]..This study was performed to synthesis the gold nanoparticles from endophyte fungus A.fumigatus isolated from Sanna surattensis.The potential antioxidant activity of thes myconanoparticles was also studied. . The sequences of nitrogenous bases of the multiplexed DNA (PCR amplified products) were determined to be compared and to know the similarities and genetic differences with isolates registered in the National Center for Biotechnology Information (NCBI).

## 2. Material and Method

### Collection of plant sample

The healthy mature leaves were collected from the garden of the Pharmacy collage at the University of

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Missan.It was placed in and transported to the laboratory. The host plant was identified by Sadiqi Sabeeh Kareem Assistant Professor at science collage Missan University.

### Isolation of endophyte fungi

Endophyte fungi were isolated from healthy leaves of Sanna surattensis plant according to the protocol for surface sterilization outlined in [21]. Each sample was washed with running water to remove residues, then the leaves were cut into 6mm- diameter pieces. These pieces were sterilized by ethyl alcohol 75% for 3minte,5% NaOCl for 2minte and ethyl alcohol 75% for 30 sec,respectively then washed with sterile distilled water(D.W), and dried on sterilized blotting paper .The prepared pieces of S.fumigatus were placed on potato dextrose agar (PDA) supplemented with chloramphenicol and incubated at 27°c for 7-10 days. Part of developing fungus was transferred to PDA medium pre-prepared and free of chloramphenicol due to pure cultures. The pureed cultured was transferred by sterile needle in the PDA slant and kept at 4°C until used.

Molecular characterization of endophyte fungus

Genomic DNA was isolated using FavoPrep fungi/ yeast Genomic DNA Extraction Mini Kit (Cat. No.: FAFYG001) following the manufacture instructions [22]. The TIS region were amplified using oligonucleotide primer ITS1 (5-TCCGTAGGTGAACCTGCGG-3) and ITS4 (5-TCCTCCGCTTATTGATATGC-3). The GDNA and PCR products were separated into agarose gel stained with ethidium at the concentrations (1µl, 1.5%) respectively. PCR products were send for sequencing and compared with the sequences deposited in the public database.

### Biosynthesis of gold nanoparticles

Endophyte fungus was inoculated in a media containing 3.0g malt extract, 3.0g yeast extract, 5.0g peptone and 10 g D-glucose in 1000 ml distilled water [23] at 27°C for 10-14 days with agitation at 120 rpm/min. Then the mat was filtered by filter paper. The mat of fungus was washed with distilled water to remove media remnants and suspended in 100 ml deionized water for 72 hours of incubation with dark and shaking condition. The mycelium was separated by vacuum device. Free fungal cell filtrate was collected and mix gradually with 100 ml of 2Mm gold salt (HAuCL4.4H2O).

### Characterization of synthesized gold nanoparticles

#### UV-Vis spectroscopy

The optical characteristics of biosynthesis AuNPs were determined by Au+3reduction in sample, which confirmed by UV-vis spectral analysis from (200-800) nm using a Dual Beam UV-1800 spectrophotometer instrument (Shimadzu, Japan) using D.w was utilized as the blank [18] .This was processed at the Department of Biology /College of science/University of Missan.

### X-Ray diffraction analysis

An AuNPS powder was obtained and characterized by x-ray diffraction (XRD) using X`Pert High with Cu Kal radiation ƛ=1.54060 and 40 KV voltage at (measurement temperature of 25°C.

### Tem Examination

The shape and size of gold nanoparticles were determined by Transmission Electron Microscopy examination according to magnification TEM micrographs.

Determination antioxidant activities of gold nanoparticles

The DPPH radical scavenging activity of gold nanoparticles was determined as previously described [24]. Briefly,1ml of AuNPs of each concentration (800,400,200,100) in DMSO has been added to 1ml of DPPH (0.004 mg dissolved in 100 ml methanol) solution then have been put mixtures in dark place for 30 min.Ascorbic acid solution was used in same concentration as positive control and DPPH+DMSO as negative control. Using the uv-vis spectrophotometer, the absorbance of the blank and all other test solution were recorded at 517 nm. The percentage of DPPH scavenging (RSA %) was estimated using the equation:

% scavenging of DPPH= [(A0 –A1)/A0] X 100

Where A0=absorbance of the negative control and A1=absorbance of the AuNPs.

## 3. Result and Discussion

### Isolation of entophytic fungus

From the surface sterilized leaves segment of Sanna fumigatus plant, the endophyte fungus started to grow from cut ends of leaf segment after 3 days and appreciable growth was observed after 7 days' finger (1)

*Fig (1) endophyte fungus from surface-sterilized leaf segment of*

*Sanna surattensis*

### ITS rRNA sequencing

By comparing nitrogen base sequence for DNA amplified product (pcr product) from endophyte fungus isolated with already available data in (NCBI) using the program (Blast) the results were proven that the isolate came from the fungus Aspergillus fumigatus strain Zbf-R10 ,the percentage of congruence with the isolates of the fungus A.fumigatus was 100%.The Polymerase chain reaction (PCR) technique, which is well-known for accurate diagnosing fungus and other living organisms the reliance on molecular diagnostics was to get rid of the negative results based on phenotypic features [25].

### Synthesis and characterization of nanoparticle

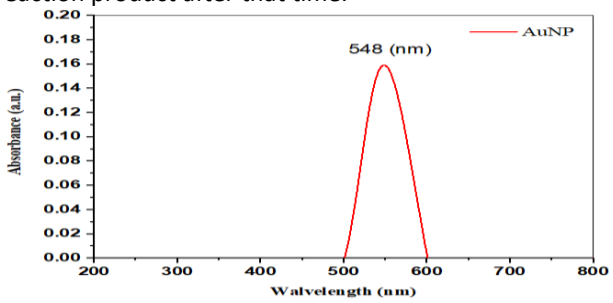
Initial signs of the production of gold nanoparticles were aching in the reaction solution`s color from yellow to dark

brown [26].(fig.2).In contrast to times previously reported in studies to several days for the extracellular biosynthesis of gold nanoparticles, the color change was observed in the process biosynthesis of gold nanoparticles 24 hours after mixing the filtrate with gold salt solution [27, 28].The color of the solution changed as a result of the solution's plasmon surface resonance (PSR) in gold nanoparticles being excited [29].

*Fig (2) The chang color to dark brown of endophytic fungi A.fumigatus filtrate after treating with 2 Mm HAuCL4*

## VIS-Vis spectra

The biosynthesis of gold nanoparticles was also measured using UV-Vis spectroscopy, the UV-vis spectrum was taken when the aqueous reaction mixtures color changed. The absorption peak was recorded at 548 nm fig (3) and colloidal gold exhibits this feature [30]. The reaction reached equilibrium at around 24 hours because there was no discernible change in the UV-Vis spectra of the reaction product after that time.




*UV-Visible spectroscopy of AuNPs of endophyte fungus A.fumigatus.Fig (3)*

## XR diffraction

The crystalline nature of the gold nanoparticles can be determined by measuring the X-Ray diffraction over the entire spectrum of 2ᵒ values from 10° to 80°.The diffraction pattern showed four Bragg reflections (111),(200),(220) and (311) angle interview standards (JCPDS) gold file NO(00-004-0784),respectively, at 33°,44°,65° and 79°Fig (4).This outcome is compatible with the qualities of bulk gold that have been described ,as well as the properties of gold nanocrystals [31, 32].The findings demonstrate the preferential formation of gold nanocrystals by filtrate from and their strong Bragg reflection (111).

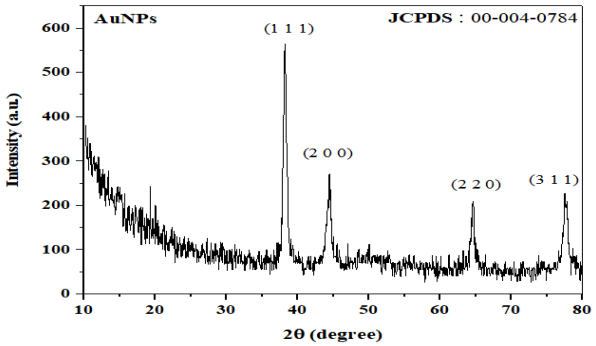



*Fig (4) X-Ray diffraction of synthesized AuNPs analysis diffraction standard (JCPDS) gold file NO (00-004-0784)*

## TEM analysis

Through TEM measurements was obtained information about the size distribution and shape of AuNPs synthesized by extracellular filtrate of a .fumigatus .The AuNPs are not scattered and most of them are present in spherical Fig (5). The TEM image shows the particles are in the size range of 13 to 40 nm. The average dimeter is estimated to be 23 nm fig (6).

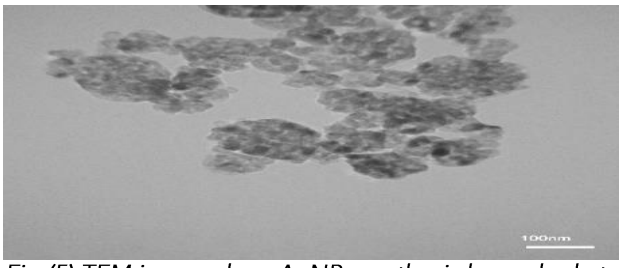


*Fig (5) TEM image show AuNPs synthesis by endophyte fungus A.fumigatus*

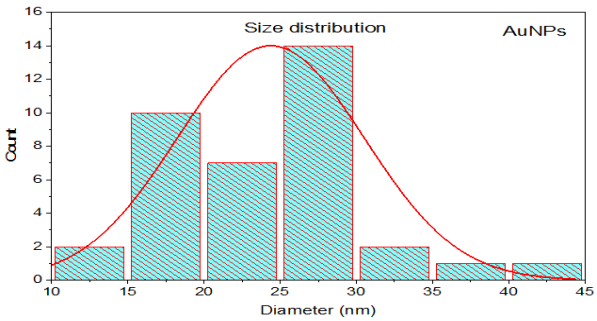



*Fig (6) Size distribution of AuNPs synthesis by endophyte fungus A.fumigatus*

## Antioxidant activity

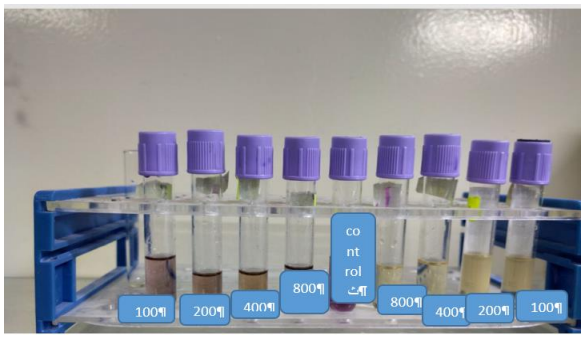



*Fig (7) the change color of DPPH by AuNPs synthesized by endophyte fungus A.fumigatus and ascorbic acid.*

By using a 1, 1-dipheny 1-2-picrylhydrazyl (DPPH) radical scavenging assay, the antioxidant activity of

biosynthesized AuNPs was assessed. The assay measured the decrease in DPPH solution absorbance at 517 nm with various concentration (800,400.200.100) µg/ml. The maximum antioxidant percentage of biosynthesis AuNPs was 71% at 800µg/ml concentration, compared to ascorbic acid 92% at the same concentration. The results stated that the biosynthesized AuNPs`ability to scavenge DPPH radicals increased with concentration Fig(7).The reduction of DPPH ,which is made up of stable free radical molecule occurs when they get hydrogen or electron from AuNPs [28, 29].The percentage of inhibition of free radical scavenging activity is shows by color change of DPPH from purple to yellow due to reduction by ascorbic acid and AuNPs Fig(8).Endo fungal extract-produced AuNPs exhibit dose-dependent suppression of free radicals. The greater the concentration, the more both AuNPs and ascorbic acid are inhabited, with the lowest percentage concentration being 100 µm/ml being 62% and the highest concentration being 800µg/ml being 71% active. The findings show that the blocking properties of gold nanoparticles were responsible for the rise in deradical activity. Previous studies confirm that gold nanoparticles that were synthesized using endophyte fungi have an antioxidant because they contain an amino group [14, 33].

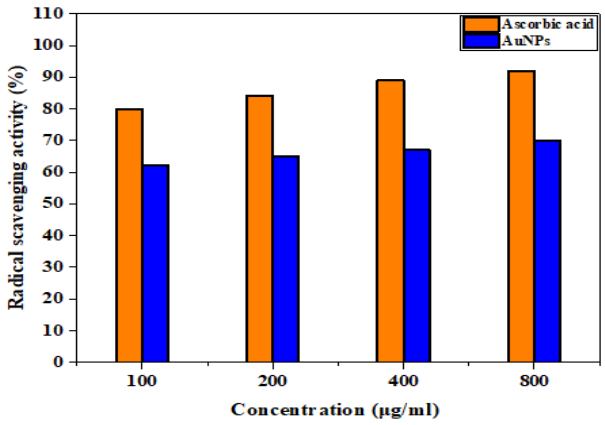



*Fig (8) percentage of radical scavenging by AuNPs and ascorbic acid.*

## 4. Conclusion

The present study is an attempt at the synthesis of gold nanoparticles from endophytic fungi, which were identified by DNA extraction.The morphology and crystal structure of the AuNPs were determined by uv- vis,XRD and TEM.These result suggest that the biosynthesized AuNPs may serve as effective alternative antioxidant agents.

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