**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

**GREEN SYNTHESIS OF GOLD NANOPARTICLES FROM *Plectranthus amboinicus*
FOR INHIBITION OF CERVICAL CANCER CELL LINE-HELA**

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Received 9th Dec. 2019; Revised 6th Jan. 2020; Accepted 8th Feb. 2020; Available online 1st Aug. 2020

<https://doi.org/10.31032/IJBPAS/2020/9.8.5152>

ABSTRACT

Background: Gold nanoparticles (AuNPs) act as efficient carriers for drug delivery applications. Because they allow controlled intracellular release. AuNPs are preferred because of their high biocompatibility, and ease of synthesis. Present work reveals the capability of green synthesis of gold nanoparticles by the leaves of pharmacologically important plant *Plectranthus amboinicus*. Cervical Cancer is one among the commonly occurring diseases in women. A potential drug with anticancer property was the need of the hour. **Aim:** To synthesize and characterize gold nanoparticles from *Plectranthus amboinicus* and study its ability to inhibit cervical cancer using cell line HeLa. **Materials and Methods:** Synthesis was done using aqueous method since it was rapid and cost effective. The synthesized gold nanoparticles were characterized by using UV-vis spectroscopy, FTIR, SEM analysis. **Results:** The results of SEM showed that the nanoparticles are in spherical shape. FTIR confirmed the presence of characteristic peaks of gold nanoparticle. **Conclusion:** Thus, the synthesized gold nanoparticles were tested for their anticancer potential for cervical cancer

cell line HeLa and the result was determined to be positive using MTT assay and AO/EB staining.

Keywords: Gold Nanoparticles, Green synthesis, HeLa, *Plectranthus amboinicus*

INTRODUCTION

Nanoparticles have several applications in many areas such as electronics, optics, medical sciences, drug delivery and fabrication of prosthetics due to its smaller particle size. The recent developments in nanotechnology have paved a wide way of research by intersecting with many branches of science and forming impact on all lives.

Metallic Nanoparticles can be synthesized and modified with various chemical functional groups which allow them to be conjugated with antibodies, ligands, and drugs of interest and thus opening a wide range of applications in medicine and targeted drug delivery. Integration of green chemistry principles with nanotechnology is one of the best developments done. The inspiration for green chemistry comes from nature through yeast, fungi, bacteria and plant extracts in the synthesis of biocompatible metal nanoparticles. Things considered for green method include, solvent choice, benign reducing agent, and the use of a non-toxic material for nanoparticle synthesis.

Green synthesis of nanoparticles differs from chemical synthesis by use of water instead of toxic organic solvents as water is an environmentally benign solvent. Gold

nanoparticles have been considered as important because of its unique Surface Plasmon resonance (SPR) and their applications in biomedical science including drug delivery, tissue/tumor imaging and photothermal therapy [1].

Plectranthus is a genus with more than 350 species in the family Lamiaceae grown as ornamental, edible or as medicine. *Plectranthus amboinicus* is a species having therapeutic applications. It has a lot of medicinal properties like antimicrobial, antibacterial, antifungal, antiviral and anticancerous properties [2].

Cervical cancer is a public health problem worldwide [3]. It is the most frequently occurring cancer which causes high mortality rate in women [4, 5]. Hence this paper discusses the effects of gold nanoparticles synthesized from *Plectranthus amboinicus* on an important cervical cancer cell line, HeLa.

MATERIALS AND METHODS

Plant Collection:

The plant was collected in and around Chengalpattu and was authenticated by Dr. S. Jayaraman, Director of Plant and Anatomy Research Centre, West Tambaram, Chennai.

Chemicals:

Chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), Minimal Essential Media (MEM), Phosphate buffered Saline (PBS), Preparation of Leaves Extract Methylthiazolyl Diphenyl- Tetrazolium Bromide (MTT), Dimethyl Sulfoxide (DMSO), Acridine Orange (AO), Ethidium Bromide (EB), HeLa cell line [6].

Plant Extract:

Leaves of *P. ambionicus* were washed thoroughly with water and were shade dried for about 10 days. The dried leaves were finely powdered and stored for preparation of leaf extract. 10 grams of powdered leaves was mixed with 100ml of deionized water and boiled for 30 minutes at 60°C and was filtered. The filtrate was centrifuged at 4°C for 10 minutes at 1000 rpm to obtain aqueous extract of the plant [7].

Synthesis of Gold Nanoparticles:

70ml of aqueous extract of plant was mixed with 30ml of 2mM aqueous $\text{AuCl}_4 \cdot 3\text{H}_2\text{O}$ solution and kept at room temperature. The immediate change in color of the solution from pale yellow to dark purple indicated the synthesis of gold nanoparticles in association with plant extract.

RESULTS AND DISCUSSION**UV-Vis Spectra Analysis:**

UV-vis spectra recorded at different time intervals for the reaction with aqueous chloroauric acid solution showed an initial

increase in the absorbance, which later decreased with higher incubation period and became constant giving a maximum absorbance at 530 nm. The appearance of the lower color confirms the formation of gold nanoparticles in the reaction mixture and efficient reduction of the Au^{3+} to Au^0 [8, 9]. Colored solution allowed measuring the absorbance against distinct wavelength to confirm the formation of AuNPs (Figure 1).

FTIR Analysis

The FTIR spectra of *Plectranthus amboinicus* leaf extract and AuNPs. The spectrum of leaves extract showed peaks at 3406, 2918, 2851, 2343, 2315, 2112, 1621, 1424, 1319, 1282, 1259, 1198, 1153, 1101, 1063, 1034, 894, 708 and 626cm^{-1} . These peaks confirmed the presence of Monomeric alcohols, Phenols, Carboxylic Acids, Alkanes, Nitriles, Phenyl Rings, Amines, Nitro Compounds, Alcohols, Ethers and Esters (Figure 2).

The spectrum of gold nanoparticles showed peaks at 3373, 2950, 2916, 2848, 2775, 2354, 1844, 1595, 1399, 1386, 1281, 1066, 776, 706, 632 and 595cm^{-1} . Characteristic peaks of gold nanoparticles synthesized by reduction of Au^{3+} ions by *Plectranthus amboinicus* leaves extract were found. The peaks of gold nanoparticles confirmed the presence of Amines, Carboxylic Acids, Alkynes, Nitro

Compounds and Phenyl Ring Substitutes [10] (Figure 3).

Scanning Electron Microscopy:

Scanning electron microscopy (SEM) was used to confirm the production of AuNPs and examine the morphology of the obtained AuNPs. SEM images were recorded at magnifications from 5.3 mm to 10.9 mm. An accelerating voltage of 15 kV was used. The synthesized nanoparticles were of Spherical shape and of sized from 80 to 100 nm (Figure 4).

Energy Dispersive X-ray:

The elemental composition of the synthesized AuNPs was determined by energy dispersive X-ray analysis (EDX). This analysis of the synthesized nanoparticles revealed strong peaks of Au confirming the formation of AuNPs. The freeze-dried gold nanoparticles were mounted on specimen stubs with double-sided taps, coated with copper in a sputter coater. Peak belonging to impurity was not detected.

The results indicated that the reaction product was composed of highly pure gold nanoparticles. EDAX spectrometry also confirmed the presence of gold with no other contaminants. The optical adsorption peak was observed at approximately 2keV, which

is typical for the adsorption of gold nanocrystallites because of SPR (Figure 5).

MTT Assay:

The *invitro* anti-cancerous property of the generated Gold Nano particles was analyzed by MTT assay on the cell lines of HeLa. The cell viability was found to be at 42.9% for 500µg/ml the least value at high concentration of AuNP. And at 92.13% for the least concentration of AuNP at 25µg/ml. Similarly, Niranjana et al., reported the *invitro* anticancer activity of AgNP's using *Cardiospermum halicacabum* extract against A-549 cells [11] (Figure 6).

AO/EB Staining:

Both live and dead cells are stained by a vital dye called Acridine Orange. Cells that have lost membrane integrity are stained by Ethidium Bromide. Live cells will appear uniformly green. Early apoptotic cells will stain green. Late apoptotic cells will also incorporate ethidium bromide and therefore stain orange in contrast to necrotic cells [12]. Necrotic cells stain orange, but have a nuclear morphology resembling that of viable cells, with no condensed chromatin. The results of AO/EB staining are in the **Figure 7 and Figure 8** at 250 µg/ml of plant extract and Gold nanoparticles respectively.

Figure 1: Result of UV Spectroscopy

Figure 2: FTIR spectra of plant

Figure 3: FTIR spectra of Gold nanoparticles

Figure 4: SEM micrographs of the synthesized gold nanoparticles

Figure 5: EDAX spectrum of gold nanoparticles

Figure 6: MTT Assay of Gold Nanoparticles

Figure 7: Cells treated with 250 µg/ml of plant extract

Figure 8: Cells treated with 250 µg/ml of AuNPs

CONCLUSION

A simple and cost-effective method was adopted to synthesize gold nanoparticles from a medicinally important plant leaves called *Plectranthus amboinicus*. The obtained gold nanoparticles were characterized by UV-vis spectroscopy, SEM and EDAX to prove that the synthesis was correctly achieved. It also had ability to inhibit cervical cancer cells which was confirmed by the MTT assay and AO/EB staining of HeLa cell line.

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