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BIOSYNTHESIS OF SILVER NANOPARTICLES USING LEAF EXTRACT OF *ARAUCARIA HETEROPHYLLA* AND ITS ACTIVITY AGAINST BREAST CANCER CELL LINE

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ABSTRACT

Background: Biological method of synthesis of nanoparticles using plant extract has proven to provide eco-friendly and satisfactory protocol without the utilization of toxic and costly material. This study investigates an efficient way of synthesizing an AgNPs using *Araucaria heterophylla* leaf extract and is valued for its medicinal property. **Objective:** Green synthesis of silver nanoparticle from *Araucaria heterophylla* leaf extract and its *in vitro* anticancer activity against breast cancer cell line. **Method:** Biosynthesized AgNPs were characterized using UV-Vis absorption spectroscopy, SEM, EDX, XRD and cytotoxicity of AgNPs was tested in human breast cancer cell line (MDA-MB- 231). **Results:** UV-Vis absorption spectrum of silver nanoparticles showed the appearance of absorption band at 428nm. SEM image indicated spherical shape of silver nanoparticles with particle size in the range 120- 200 nm. The EDX spectrum showed the presence of silver along with a few other elements like Na, Al, K, Cl and O. The crystalline nature of the silver nanoparticles was examined by XRD analysis for nano-crystals. Green synthesized AgNPs by *Araucaria heterophylla* leaf extract showed cytotoxicity to human breast cancer cell line (MDA-MB- 231).**Conclusion:** The present study reveals that the synthesized AgNPs from aqueous extract of *Araucaria heterophylla* provide non-toxic, eco- friendly and cost effective reducing and capping agent for bio reduction of $AgNO_3$.

KEYWORDS: *Araucaria heterophylla,* AgNPs, SEM-EDX, UV-Vis absorption spectroscopy, XRD, cytotoxicity analysis.

INTRODUCTION

Cancer is the most serious issue and health related problems in the world. It has been observed that one out of three people will suffer from some form of cancer in their lifetime. Based on the origin there are variety of cancer exist, such as thyroid, prostate, bladder cancer, kidney cancer, pancreatic, breast cancer, melanoma, leukaemia etc.[1] Breast cancer is a complicated and diverse disease. In the number of new cases diagnosed breast cancer is one of the leading types of cancer and it is the most frequent type of cancer in women.[2] The first breast cancer cell line to be established was BT-20 in 1958.[3]

Araucaria heterophylla is a large evergreen and has a single upright trunk, tiered branching habit, and a narrow pyramidal or columnar shape. Eventually reaching a height of about 80 feet, the tree possesses a rapid growth rate.[4] It is occasionally called a star pine, triangle tree or living Christmas tree, due to its symmetrical shape as a sapling, although it is not a true pine.

Over the past few years, the synthesis of metal nanoparticles is a main topic of research in modern material science. Metallic nanoparticles that have massive applications in industries are of various types, namely, Gold, Silver, Alloy, magnetic etc.**[5]** Noble metals such as gold, silver and palladium have drawn huge attention due to the wide range of new applications in various fields of industry. It is significant that the silver nanoparticles need not only the particles to be of nano-size, but also synthesis of the nanoparticles to be produced easily and at cost effective.[6] Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes.[7] Many studies have reported successful synthesis of silver nanoparticle using organisms (microorganisms and biological systems).[8]

Nanoparticles can be synthesized using different approaches including chemical, physical, and biological. Chemicals used for nanoparticles synthesis and stabilization are unsafe and lead to non-ecofriendly consequences. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as by- products. Thus, there is an increasing demand for "green nanotechnology".[9] Green synthesis of nanoparticles using plant extracts is the most appropriate method of green, ecofriendly production of nanoparticles and it also has a notable profit that the plants are widely spread, effortlessly available, and much safer to manage, and act as a source of several metabolites.[10] In present study the green synthesis of

silver nanoparticles from the *A. heterophylla* leaf extract had been carried out and characterized by UV-Vis spectra, SEM, EDX, XRD analysis. The cytotoxicity activity of synthesized AgNPs against MDA-MB- 231 breast cancer cell was determined.

MATERIALS AND METHOD

Sample collection and preparation of the plant extract

The leaves of *Araucaria heterophylla* was collected from Alandur Chennai, Tamil Nadu. The sample was transferred to lab for further processing. The aqueous plant extract was prepared by taking 2g of sample in 30ml distilled water, kept in boiling water bath for 20 minutes at 55^0 C-60^0 C and filtered using Whatmann's filter paper. The prepared extract was stored for further synthesis of nanoparticles.

Biosynthesis of silver nanoparticles

1ml of the aqueous plant extract was added to 9ml of double distilled water after which 20µl of 1mM aqueous silver nitrate ($AgNO_3$) was added. The mixture was incubated in dark for 24hrs and the colour change was observed which indicated the synthesis of silver nanoparticles.

Characterization of synthesized nanoparticles

Synthesized silver nanoparticles was confirmed by sampling the aqueous component of different time intervals and the absorption maxima was scanned by UV-Vis spectrophotometer at the wavelength of 300-700nm (UV-VIS Spectrophotometer 2202). Scanning Electron Microscopy (SEM) analysis of synthesized AgNPs was done using a Hitachi S-4500 SEM machine and EDX was carried out using the same instrument. X-ray powder diffraction (XRD) analysis was done using PAN analytical XPRT PRO,D-8, advanced Brucker instrument.

Cytotoxicity of AgNPs

The cytotoxicity of synthesized AgNPs against MDA-MB -231 cell was measured by MTT (3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide) assay. MTT assay is a colorimetric assay for assessing cell metabolic activity.[11] MDA-MB- 231 was seeded at a density of 1x 10^6 cells/well into 96 well plates. Then, the cells were treated at 37°C in 5% CO_2 environment for 24 hrs and observed for cytotoxicity using inverted microscope. MTT (5mg/mL) was added to the incubated cells then further incubated for 3- hrs in a 5% CO_2 incubator. After incubation, the MTT was discarded and 200 µL of DMSO was added in each

well and mixed gently and kept for 20 mins Purple color formation was observed. The wells were read at 595nm using 96 well plate reader (LISA microplate reader).

RESULTS AND DISCUSSION

When aqueous plant extract was added into silver nitrate solution and incubated for 24hrs, after which there was appearance of brownish color (Figure.1) which indicated the formation of AgNPs. The color change is due to excitation of the surface Plasmon vibration of silver nanoparticles.

Figure 1: *Araucaria heterophylla* after synthesis of silver nanoparticles

UV-VIS spectra analysis.

After the synthesis of silver nanoparticles the reading was taken by UV-Visible spectrophotometer. UV-Vis absorption spectrum of silver nanoparticles shows the appearance of absorption band at 428 nm, which attributed to the Surface Plasmon Resonance (SPR) response of silver nanoparticles (Figure. 2). In compared to this study carried out in the synthesized silver nanoparticle of *Calliandra haematocephala* leaf extract showed a characteristic absorption peak at the wavelength of 414nm because of SPR.[12]

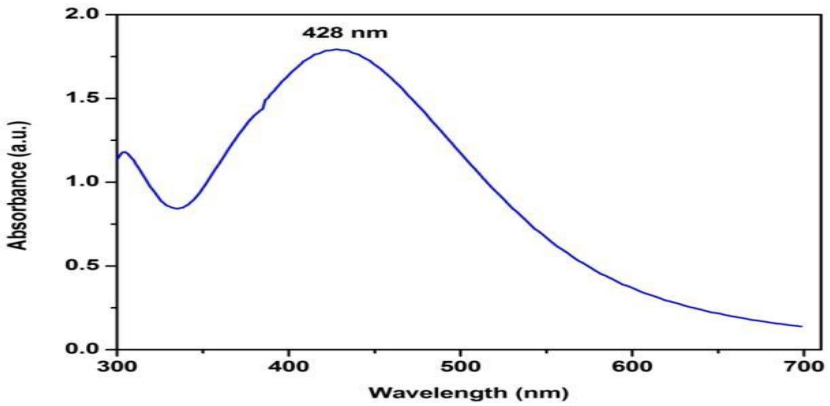

Figure- 2: UV-Vis absorption spectra effect of AgNPs synthesized from *Araucaria heterophylla* aqueous plant extract.

SEM studies

Scanning Electron Microscopy (SEM) was done for identifying the surface morphology of particles. It is observed from the SEM image that spherical shaped silver nanoparticles were formed with particle size in the range 70-90 nm. It is evident that the plant extract reduced silver ions into silver nanoparticles (Fig. 3). Devaraj *et al.*, 2014 studied spherical shaped of silver nanoparticle that was obtained with the size range of 40-70nm in *Tabernaemontana divaricate.*[13]

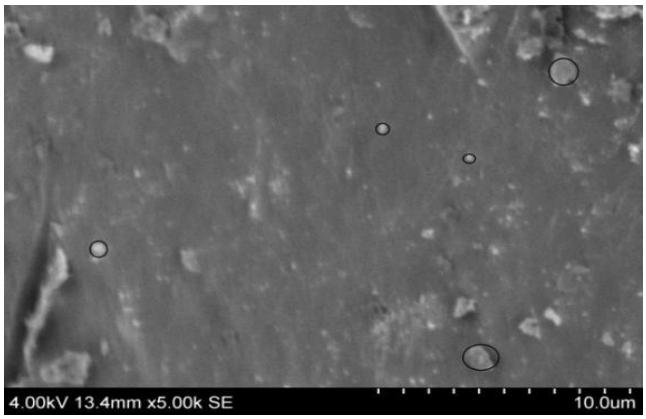

Figure 3: Topographic images of spherical shaped AgNPs by SEM analysis

EDX analysis.

Energy Dispersive X-ray Spectroscopy (EDX) is a technique that is mainly used to identify the presence of different elements in a sample. The EDX spectrum showed the presence of silver along with a few other elements like Na, Al, K, Cl and O (Fig. 4). Studies done by Singh *et al.*, 2015 on *T. catappa* and *E. officinalis* silver synthesized nanoparticle extract examined using EDX and they found the presence of inorganic compounds like potassium, calcium and magnesium along with the silver.[14]

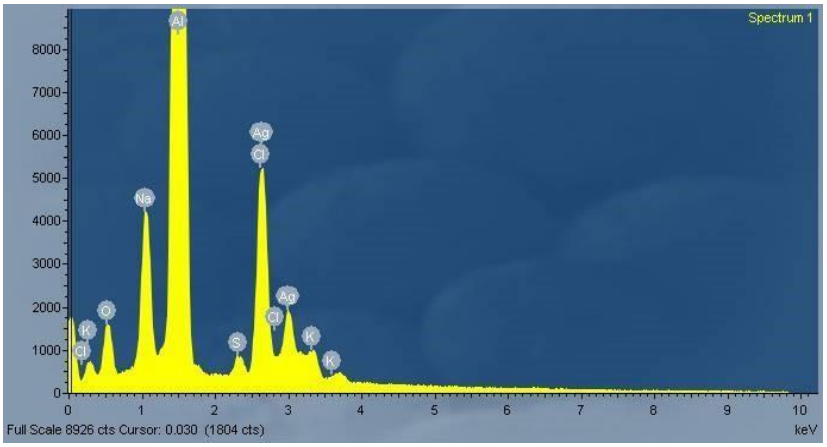

Figure- 4: Graphical representation of Energy- dispersive x-ray spectroscopy analysis.

XRD analysis

The XRD patterns of the green synthesized AgNPs using *Araucaria heterophylla* shown in (Fig.5). The characteristic peaks of silver observed at 2θ values 27.8, 38.1, 44.1 and 78.1 correspond to the (220), (111), (200) and (311), planes of the face centered cubic geometry of AgNPs, which is in agreement with the JCPDS No: 04- 0783 and 84-071. Studies done by Singh *et al.*, 2015 on *T. catappa* and *E. Officinalis* and *E. Hybrid* showed crystalline in nature and Bragg reflection observed at 2 θ values 7.9, 11.4, 17.8, 30, 32, 38, and 44° corresponding to the plane (101), (111), (200), and (220).[14] Thus the XRD pattern clearly indicates that the silver nanoparticles synthesized from *Araucaria heterophylla* are crystalline in nature.

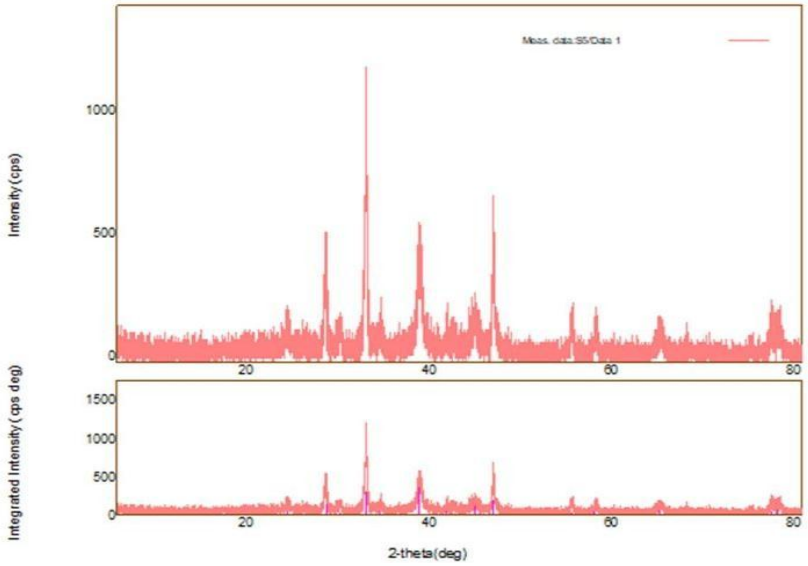

Figure 5: XRD pattern of AgNPs exhibiting crystalline structure of silver

Cytotoxic activity.

The anti-cancer activity of synthesized AgNPs from *Araucaria heterophylla* aqueous plant extract was assessed by MTT method. MDA-MB- 231 cell line was treated with different concentration of 20, 40, 60, 80, 100 µg/ml and incubation for 24hr showed percentage of cell viability (Graph) of the cells in a dose dependent manner (Fig.6) the inhibiting concentration IC_{50} value of 75.29 µg/ml. This result is an agreement with a study carried out by Babu *et al.*, 2014, who reported the anticancer effect of AgNPs synthesized from *D. inoxia* leaf extract on human breast cancer cell line MCF 7. Thus the cytotoxic study reveals the morphological changes are a consequence of characteristic, molecular and biochemical events occurring in an apoptotic cell.[15]

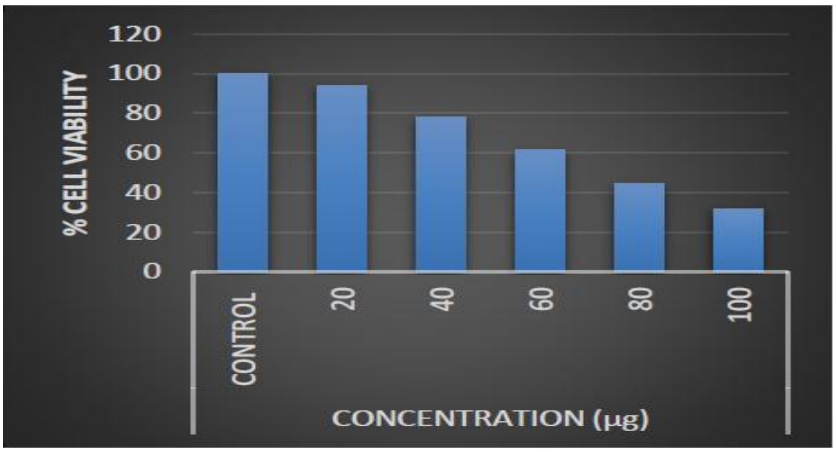

Figure-6: Cytotoxic activity of *A. heterophylla* synthesized AgNPs against MDA- MB-231 cell line.

CONCLUSION

The present study reveals that the synthesized AgNPs from aqueous extract of *Araucaria heterophylla* provide non-toxic, eco-friendly and cost effective reducing and capping agent for bio reduction of $AgNO_3$. The anti-cancer activity of synthesized AgNPs from *Araucaria heterophylla* aqueous plant extract was assessed by MTT assay with IC50 value of 75.29µg/ml. Hence the present study reveals that the AgNPs synthesized from *A. heterophylla* can be used against challenging diseases such as cancer. Isolation of compound from *A. heterophylla* inhibiting cancer would provide a way to drug discovery in the near future.

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