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CHARECTERIZATION OF GREEN SYNTHESIZED SILVER NANO PARTICLES FROM LEAF EXTRACT OF Erythroxylum monogynum BY USING SCANNING ELECTRON MICROSCOPE, FOURIER TRANSFORM INFRARED SPECTROSCOPY AND STUDY OF CELL VIABILITY OF MCF-7 BREAST CANCER CELL LINES BY USING THE SILVER NANO PARTICLES

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ABSTRACT Silver nanoparticles are prepared from the leaf extract of Erythroxylum monogynum by using biological method with the diamine silver solution.Later they are charecterized by using scanning elctron microscope and Fourier transform infrared spectroscopy. These silver nanoparticles are ustilised to test the viability of the MCF-7 cancer cell lines.Cell viability test can be used to know the number of cells which are are living after they exposed to any toxic or influencing materials or particles. MTT assay is used for this purpose.This viability test also indicates the efficiency of the toxicity of particles or materials taken.The decline growth of MCF-7 cell lines indicates the effectiveness of the silver nanopartcicles.

KEYWORDS : Erythroxylum monogynum, Silver nanoparticles, Scanning elctron microscope, Fourier-transform infrared spectroscopy, MCF-7 Cell lines, MTT Assay

INTRODUCTION :

Silver nanoparticles [AgNPs] are tiny particles which ranges from the 1nm to 100 nm.They are biologically produced from the selected plants with incubation of silver compounds. *Erythroxylum monogynum* is a small plant which usually found in hilly areas and contains great number of medicinal properties.The leaves of *Erythroxylum monogynum* was collected and shade dried and powered.Silver nano particles formation can be seen after 24 hours incubation with diamine silver solution. Later these formed silver nanoparticles are charecterized with Scanning electron microscope (SEM) and fourier transform infrared spectroscopy. Scanning Electron Microscopy (SEM) contains a great role in research from 1962 of its invension and gradually exeeds its roots into multiple branches of biology. (Mc Comb D *et al*.,1975 ; Yamada RS *et al*.,1983 ; Saleh IM *et al*.,2003) .Silver nano particles are known to contain a lot of applications in various fields with thier physical ,chemical and biological properties. They are claimed to contain anti bacterial, antiviral, anticancer properties.The silver nanoparticles which are prepared from the leaf extract of *Erythroxylum monogynum* tested for the anti cancer properties. For this the effectiveness of silver nano particles utilised in MCF-7 breast cancer cell lines. MTT assay was used to know the concentration of cells survived after the application of AgNPs.

MATERIALS AND METHODS :

Scanning Electron Microscope (SEM) :

Recently, the area of nano science contains a driving force in the improvement of the various high-resolution techniques of microscopy for to investigate much regarding the nano materials by utilising a beam of electrons with more energy on the very fine scale in order to probe objects (Pawley, J 1997 ; Wang, Z.L,2000 ; Yao, H et al., 2007). For SEM analysis our samples were performed JEOL 840 with Resolution at 20kV: 10nm which is located in material science, IISC, Bangalore. The sample thin films were prepared on a copper grid which is coated with carbon by just putting a very little amount of the sample on grid, extra solution was taken out by using a blotting paper and then the film on the SEM grid was allowed to dry by keeping it under a mercury lamp for five min. The instrument used was a JEOL 840 which have the Resolution of 20kV: 10nm.

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) :

FTIR is having great capacity to provide reproducibility, accuracy, and also a gives signal to noise ratio.On applying this spectroscopy, it is possible to sense very small absorbance alterations in the order of 10^{-3} which favours to do different spectroscopy, where it is possible to differentiate small bands of absorption.They are residues which are functionally active from the large absorption background of total protein (Gerwert,1999 ;Jung .C .,2000; Kim, S.*et al*.,2001; Mantele, W.G.*et al* 1988; Vogel R.*et al*., 2000, Wharton, C.W,2000 ; Zscherp, C.*et al*., 2001).

FTIR spectroscopy is usually used to know whether biomolecules are utilised in the production of of nanoparticles, which are frequently used in the laboratory and industrial research (Shang, L *et al*., 2007). Later the FTIR also been distributed to the study of material or particles of nanosized as covalently grafted molecules onto silver, nano tubes of carbon,gold nano particles and graphene, or reactions which took place between occurring between substrate and the enzyme in the the process of catalysis (Baudot, C *et al*.,2010, ; Barth, A *et al*.,. 2002).

Later more , it is the technique of non-invasive. Finally, the benefits of FTIR spectrometers when compared with the dispersive ones are the strong signal, data collection, less sample heat-up (Kumar, S *et al*.,2010), large signal to noise ratio. The advanced FTIR method known as attenuated total reflection which is shortly denoted as ATR-FTIR spectroscopy (Goormaghtigh E *et al*.,1999, 1422 ; Harrick, N.J etal., 1974 ; Hind, A.R *et al*., 2001).By using ATR-FTIR,we can predict the chemical chrecteristic features on the surface of polymer, and it is easy to prepare sample preparation when compared with the traditional FTIR (Johal, M.S., 2011 ; Kazarian, S.G *et al*., 2006 ; Liu, H *et al*., 2007 ; Acosta, E.J *et al*.,2005 ; Demathieu, C 1999). Recognition of unique types of chemical bonds or functional groups depended on their specific absorption signatures is possible by the infrared spectroscopy. Chemical bond stretching and bending is possible by energy absorption .In the present study, FTIR spectra were recorded for leaf dried biomass of *Erythroxylum monogynum*. Sample was prepared by drying the biomass at 60°C; solid biomass of *Erythroxylum monogynum* was milled with 0.8% potassium bromide (KBr) to form a very fine powder. This powder was then compressed into a thin pellet which can be analyzed.

Human breast cancer cell lines (MCF-7)

MCF-7 were grown in DMEM-HG supplemented with 10% heat-inactivated FBS, 2% Penicillin-Streptomycin and 2.5 µg/mL Amphotericin-B solution(All from HiMedia Labs, Mumbai, India); and incubated at 37°C in a humidified atmosphere of 95% air, 5% CO_2 for 24-48 hrs. Following 24-48 hrs of incubation period, the adherent cells were detached using Trypsin-EDTA solution 1X/0.25% (HiMedia Labs, Mumbai, India). Cell count was carried out using the Luna automated cell counter (Logos Biosystems, India) based on trypan blue dye exclusion method.

MTT Assay

Cell viability assay is one of the vital steps in analysing the cellular response to toxic compounds, playing fundamental roles in determining the cell death andsurvival rates and assessing the metabolic activity. In our study, MTT assay method was applied to determine the inhibitory activity of AgNPs against the mammalian cell lines of MCF-7.under normoxia and hypoxia conditions. MTT is a blue color dye which was reduced into purple colored formazan product by the action of mitochondrial succinate dehydrogenase enzyme. The amount of purple colored formazan is directly related to the percentage of cell viability both under normoxia and hypoxia conditions.

Normoxia is the normal atmospheric conditions, where hypoxia literally means "low oxygen," but is defined as a deficiency in the amount of oxygen that reaches the tissues of the body. Hypoxic regions can develop as a tumor grows beyond the ability of its blood supply to deliver oxygen to the full extent of the tumor, exacerbated by vascular spasm or compression caused by increased interstitial fluid pressure. The hypoxia inducible factors (HIFs) are the key mediators of the cellular response to hypoxia. Hypoxia plays an important role in normal development and disease progression, including the growth of solid tumors

RESULTS :

The SEM image of the sample was shown in the figure from the SEM experiment showed silver nano particles was synthesized from the *Erythroxylum monogynum.* From the results morphology of Silver nano particles is more clearly seen, the shape of particles are circular, poly-dispersed and the size ranged between 15- 20nm.

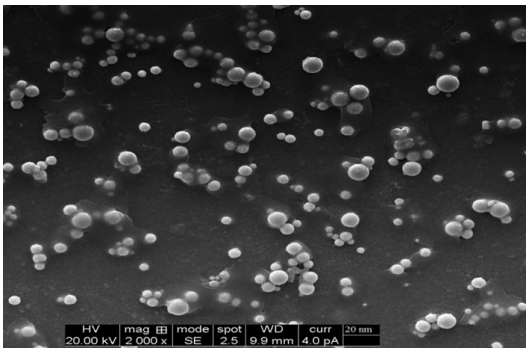

Figure 1: Scanning electron microscope image of synthesized silver nano particles

The FTIR measurement can also be utilised to study the presence of a protein molecule in the solution, as the FTIR spectra in the 1400–1700 cm–1 region provides information about the presence of "C=O" and "N-H" groups (Senapati *et al.,* 2005). The linkages of amide bond between the amino acid residues in the polypeptides as well as proteins processes to well to the electro magnetic spectrum infrared region.The band positions in the spectra of FTIR for the proteins acts as as the secondary indicators for the changes that took place conformationally for the secondary structures of the proteins.

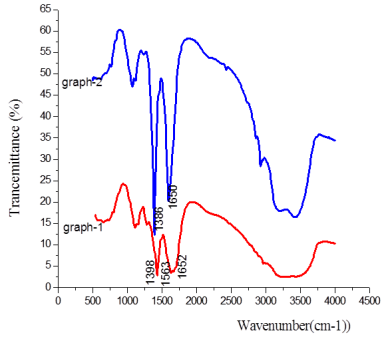

Figure 2
Graph 1 : FTIR graph of *Erythroxylum monogynum* leaf extract
Graph 2 : FTIR graph of AgNPs of *Errythroxulum monogynum* leaf extract

Cell Viability

In vitro Cytotoxicity of AgNPs in MCF-7, cell were done in dose-dependent manner ranging the concentration of AgNPs from 25 to 125µg/ml. The MTT dye was yellowish in colour before incubation shown .MTT was turned into purple colour after 24 hours of incubation shown.The intensity of purple color was decreased in the wells with the increased concentration of AgNPs. Decreased color intensity was the indication of increased death of cells. In other words increase of AgNPs concentration the viability decreased in cell lines shown in dose-dependent curves. From the dose-dependent curves, it was found that the synthesized AgNPs were more toxic towards the MCF-7 both under normoxia and hypoxia conditions.

Figure 3
96-well micro titre plate containing silver nano particles treated cancerous cell lines and MTT showing yellow colour before incubation

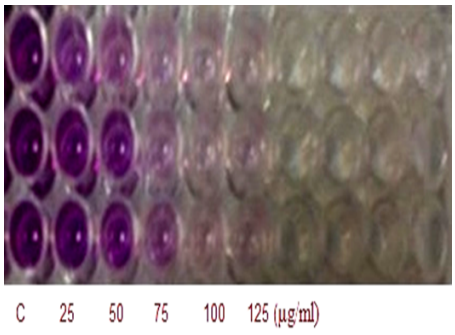

Figure 4
96-well micro titre plate containing AgNPs treated cancerous cell lines and MTT after incubation showing decreased purple color intensity with increasing concentration of Silver nanoparticles

Dose-dependent curve

Table 1 : AgNPs Vs MCF-7- Normoxia

OD 570nm	Untreated cells /Control	AgNPs µg/ml				
		25	50	75	100	125
1	0.855	1.001	0.967	0.636	0.558	0.212
2	0.834	0.907	0.732	0.454	0.403	0.167
Mean OD	0.8456	0.954	0.8495	0.5454	0.485	0.1945
% of viability	100	101	87.95	49.87	42.9	4.61

Table 2 : AgNPs Vs MCF-7- Hyoxia

OD 570nm	Untreated cells/ Control	AgNPs (Silver nanoparticles)				
		25	50	75	100	125
1	0.946	1.138	0.867	0.578	0.503	0.744
2	1.044	1.003	0.82	0.612	0.472	0.168
Mean OD	0.9944	1.072	0.843	0.594	0.4774	0.356
% of viability	100	107	82.76	54.44	43.13	37.68

CONCLUSION :

The silver nanoparticles prepared from the *Erythroxylum monogynum* are known to have circular, poly-dispersed and the size ranged between 15- 20nm with the help of Scanning electron microscope and by using the FTIR the struture of proteins are determined and also it was concluded that, the synthesized AgNPs were inducing Cytotoxicity in cell lines even under hypoxic conditions. This indicated that AgNPs were suggested to use in treatment of solid tumors.

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