Anticancer Activity of Magnetic Iron Oxide Nanoparticles Synthesized Using Anacardium Occidentale Leaf Extract

A. Ashlin Rose [1], Dr.K. R Sheeja [2]

[1]*Research Scholar, Women's Christian College, Nagercoil-629001, Tamilnadu, India*

Affiliated to Manonmaniam Sundaranar University Abishekapatti, Tirunelveli 627012, Tamil Nadu, India

[2] *Assistant Professor, Department of Chemistry, Women's Christian College, Nagercoil-629001, Tamilnadu India.*

Abstract-Iron Oxide nanoparticles were synthesized using extract of Anacardium Occidentale leaves which act as reducing as well as capping agents, and Ferric Chloride as a precursor. The Iron Oxide nanoparticles have been characterized using UV-Visible, FT-IR, SEM, TEM, EDAX and X-Ray diffraction studies. Anticancer activity of Iron Oxide nanoparticles was carried out against breast cancer cell (MDA-MB-231) and Skin cancer (SKMEL) cell lines. MTT assay identified significant anticancer activity of the nanoparticles against both cell lines. The result has shown that the Iron nanoparticles are crystalline with sizes ranging from 15 to 20 nm. Dose dependant reduction in cell viability were observed in breast cancer cell (MDA-MB-231) and Skin cancer (SKMEL) cells with different concentrations of the Iron Oxide nanoparticles. Viability percentage were reducing significantly and the IC_{50} value of Iron Oxide nanoparticles against Skin cancer cells were found to exhibit 35.35 µg/ml. This present study revealed that the successful green synthesis and characterization of Iron Oxide nanoparticles having excellent anticancer activities.

Keywords: Anacardium Occidentale, Anticancer, Green approach, Magnetic nanoparticles.

I.INTRODUCTION

Nanotechnology is a sub-classification of technology in colloidal science, biology, physics, chemistry and other scientific fields and involves the study of phenomena and manipulation of material at the nanoscale, in essence an extension of existing sciences into the nanoscale [1]. Cancer is one of the major public health burdens in the world. Cancer develops because of mutation in genes that regulate normal cell cycle and cell division, thereby resulting in uncontrolled division and proliferation of cells [2]. Nanotechnology has an assemblage of applications in the biomedical field. Green synthesis was useful and pertinent due to an environment friendly, cost effective, less toxic and sustainable [3]. Polyphenols, flavonoids, and tannic acid which act as reducing and stabilizing agent in the synthesis of metal nanoparticles [4].

The Cashew (Anacardium Occidentale) is a tree in the family of the flowering plant Anacardiaceae[5]. Cashew tree has been used medically in many ways. The bark, leaves and shell oil of the plant are used medicinally to treat different ailments [6]. The shell oil around the presence of bioactive ingredients in the cashew the nut is used medically and has industrial applications in plastics and resin industries for its phenol content. In Brazil it is also used to treat diabetes, muscular ability, urinary disorders and asthma. Cashew gum is used in pharmaceuticals and as substitute for gum Arabic. Leaves are carbohydrate, tannins, saponins, resins, alkaloids and flavonoids. The oil obtained from nut shell is industrially important and has very good antibacterial activity [7].

The Biocompatibility of Fe_3O_4 metal nanoparticles makes them suitable for biomedical applications, such as cellular therapy, tissue repair and drug delivery [8]. In this present work Iron Oxide nanoparticles were synthesized simple green method by using Anacardium Occidentale leaf extract. These prepared Iron Oxide nanoparticles were characterized and evaluated Anticancer studies. Anticancer studies of Iron Oxide nanoparticles prepared from leaf extract were evaluated by MTT assay method.

II MATERIALS AND METHODS

A. *Preparation of leaf extract from Anacardium Occidentale*

Ferric Chloride, Double distilled water, Ethanol was purchased from S A Chemicals, Tirunelveli. Anacardium Occidentale leaves were collected from Nagercoil.The leaves were collected washed with distilled water and are cut into small pieces. Take 25g of fresh leaf and 100 ml of deionized water in 250 ml beaker and it was kept at 70^0c on magnetic stirrer for 10 minutes. The extract was filtered and taken for further uses.

B. *Preparation of green Iron Oxide nanoparticles*

Preparation of Iron Oxide nanoparticles was carried out using Ferric Chloride as a Precursor. Take 50ml from 0.1mM of $FeCl_3$ was taken in a beaker. This setup was stirred at 70^0c on magnetic stirrer for 1 hours. Add 50ml of prepared Anacardium Occidentale leaf extract dropwise into the beaker. The solution allowed to cool and centrifuged 7000 rpm, Nanoparticles are washed with deionized water and are allowed to dry oven at 90^0c for 3 hours.

C. *Characterization of Iron Oxide nanoparticles*

Iron Oxide Nano particles were characterized by using UV-Visible, FT-IR, X-Ray diffraction analysis SEM, TEM and EDAX. The functional group present in Iron Oxide nanoparticles were determined by using FT-IR spectroscopy. XRD is used to study phase composition of a sample and crystal structure. Scanning Electron Microscopy is used to determine particle size and distribution. Anticancer activity against breast cancer cell (MDA-MB-231) and Skin cancer (SKMEL) cells were evaluated by MTT assay method.

IV. RESULT AND DISCUSSION

A. *UV-Vis and FT-IR Spectroscopy*

Absorption spectroscopy revealed that information about optical properties of Iron Oxide nanoparticles. It is the primary method to indicate the bio reduction of Ferric chloride solution to Iron Oxide nanoparticles. 0.1 mM concentration of Ferric Chloride was taken for the reduction of Iron Oxide by the leaf extract of Anacardium Occidentale. Addition of Ferric Chloride solution reacts with leaf extract of, Anacardium Occidentale the intensity of colour converted to black [9], which indicates the presence of Iron Oxide nanoparticles. Iron Oxide nanoparticles were observed in 490 nm.

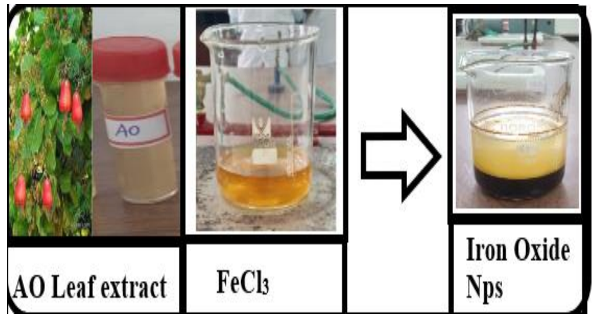

Figure 1: Preparation Iron Oxide Nanoparticles

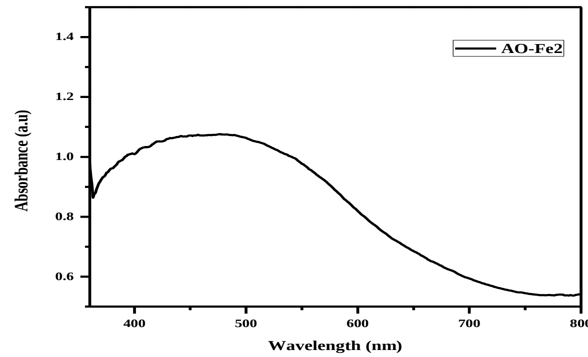

Figure 2: UV-Visible spectrum of Iron Oxide Nanoparticles

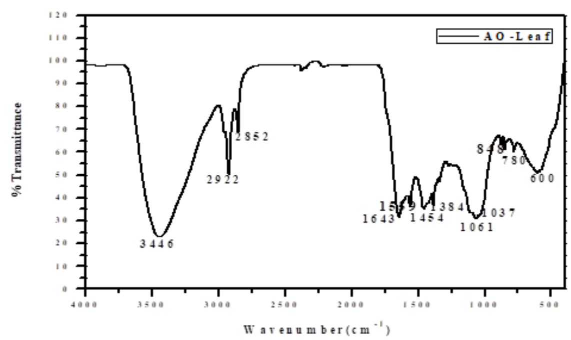

Figure 3: FT-IR spectrum of Anacardium Occidentale Leaf extract

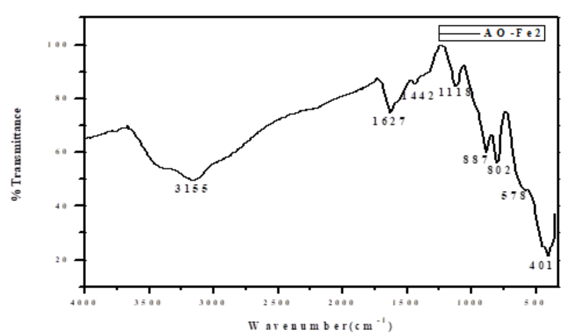

Figure 4: FT-IR spectrum of Iron Oxide nanoparticles by using AO Leaf extract

FT-IR gives the information about functional groups present in the Iron Oxide nanoparticles. Anacardium Occidentale leaf contain the following functional groups (figure3).1643 cm^{-1} due to C=O stretching of (amide) and 1061 cm^{-1} (C-N stretching vibrations of aliphatic amines) appears as most intense one.1037 cm^{-1} and 3446 cm^{-1} hydrogen bonded C-O stretching and O-H stretching (broad) of alcohols and phenols.2922 cm^{-1} (medium) O-H stretching of carboxylic acid.2852 cm^{-1} (medium) H-C=O: C-H stretching of aldehydes.1384 cm^{-1} due to C-N stretching mode of aromatic amine.1454 cm^{-1} (medium) due to C-C stretching (in-ring) of aromatic compound.600 cm^{-1} (broad) due to OH out of plane deformation of Ar-OH in phenols.848 cm^{-1} due to C-Cl stretching of halo compound. (sharp)1559 cm^{-1} and 780 cm^{-1} due to stretching vibration of -C-C- in aromatic rings

Iron Oxide nanoparticles prepared from Anacardium Occidentale leaf extract contain the following functional groups. The peak at 3155 cm^{-1}can due to O-H stretching vibrations of alcohols and phenols.

The bands at 1627cm^{-1} region correspond to C-H bending overtone band of aromatic compound. 887 cm^{-1} due to aromatic C-H bending band. 578 cm^{-1} and 401 cm^{-1} Stretching mode of Fe-O, which indicated the formation of Iron Oxide nanoparticles [10].

B. *X-Ray Diffraction and SEM Analysis*

The peaks were recorded from 200-800 at 2 theta scale and the diffraction peaks observed at 29.63^0, 35.03^0, 42.68^0, 56.67^0, 62.31^0 corresponds to the crystal lattice plane of (220), (311), (400), (422), (511) and (440). The prominent peaks are observed above, which was in Fe_3O_4 crystals and possess magnetic structure of Iron Oxide nanoparticles [10].

SEM analysis were employed to visualize the size and shape of the nanoparticles. SEM micrographs of Iron Oxide nanoparticles are given in figure.6 with different approbation. It was observed that the particles are spherical in shape with a uniform size about 20-50 nm. The particle size attained from SEM images is well correlated with the particle size resolute from XRD using confer to the Scherrer formula and the average of the synthesized nanoparticles was in the range of 20 nm.

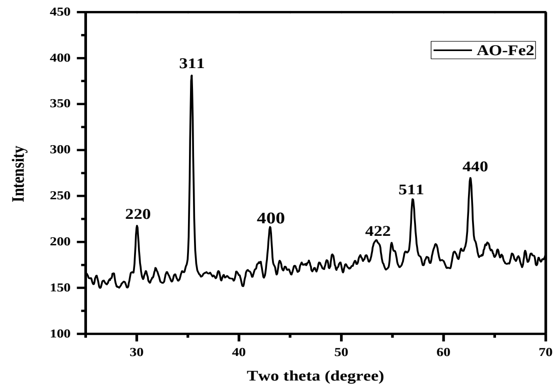

Figure 5: XRD Analysis of Iron Oxide nanoparticles

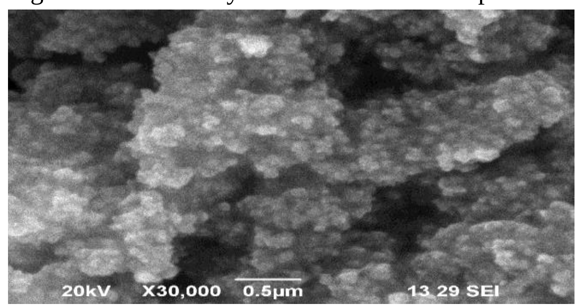

Figure 6: SEM Analysis of Iron Oxide nanoparticles

C. *EDAX and TEM Analysis*

Elemental composition of Iron Oxide nanoparticles synthesized using Anacardium Occidentale extract was determined by using EDAX analysis. It was observed that the weight percentage of Iron is 74.04% and Oxygen is 20.75%. TEM analysis was employed to measure nanoparticle size and distribution which is shown in figure.3 with different approbation. It was observed that the particles are spherical in shape with a uniform size about 20 nm.

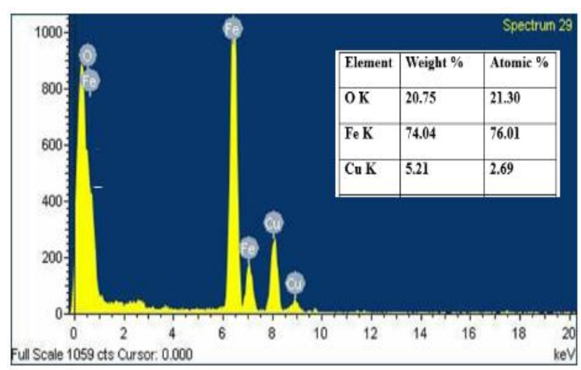

Figure 7: EDX spectrum of Iron Oxide nanoparticles

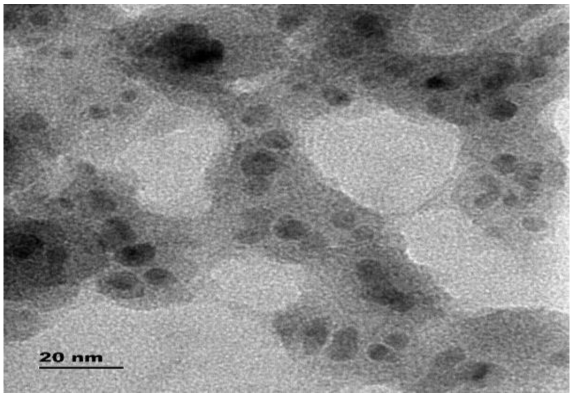

Figure 8: TEM images of Iron Oxide nanoparticles

D. *Anticancer activity*

Nano biomaterials proposed for biomedical applications are expected to be inert and biocompatible with cells, and blood components. Metallic nanoparticles are known to extremely interact with cells and intracellular macromolecules leading to produce of reactive oxygen species [11]. Anticancer of Iron Oxide nanoparticles against breast cancer cell (MDA-MB-231) and Skin cancer (SKMEL) cell lines was studied using colorimetric MTT assay.

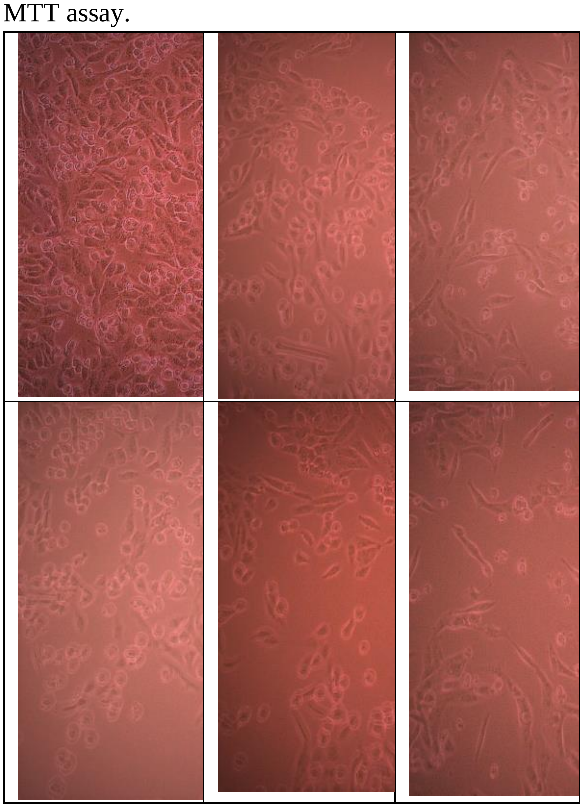

Figure 9: Anticancer Activity of Iron Oxide Nanoparticles against breast cancer cell

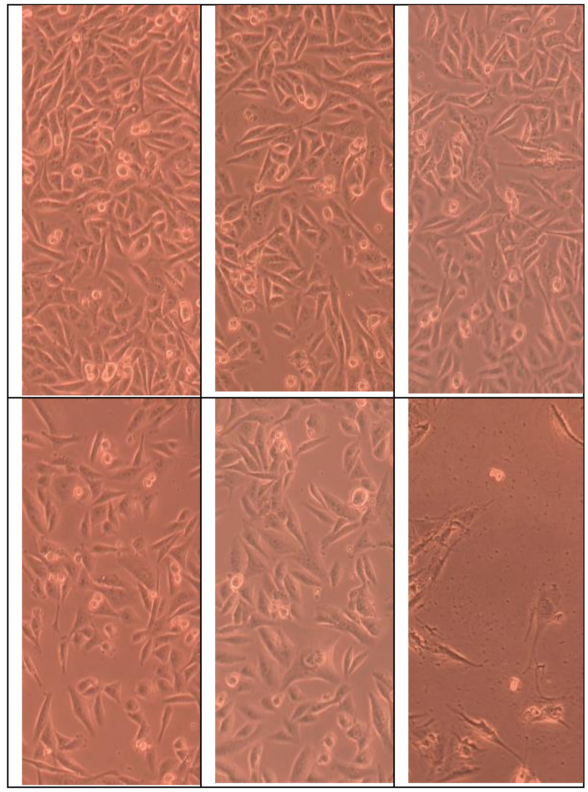

Figure 10: Anticancer Activity of Iron Oxide Nanoparticles against melanoma cancer cell

Breast cancer cell and Skin cancer were treated with Iron Oxide nanoparticles at variable concentrations of 6.25, 12.5, 25, 50, 100 µg/mL and evaluated for cell viability after 24 hours shown in (Figure 9 and 10). Concentration of breast cancer cell increases from 6.25 to 100 µg/mL the viability of cell gradually decreases from 82.74 to 22.85. Concentration of skin cancer cell increases from 6.25 to 100 µg/mL the viability of cell gradually decreases from 76.50 to 20.45.

Table 1: Viability percentage of Iron Oxide Nanoparticles against breast cancer cell

Concentration µg/ml	Percentage viability	IC 50
6.25	82.74	
12.5	65.50	
25	52.52	
50	37.78	
100	22.85	42.75

Table 2: Viability percentage of Iron Oxide Nanoparticles against skin cancer cell

Concentration µg/ml	Percentage viability	IC 50
6.25	76.50	
12.5	65.75	
25	45.52	
50	32.38	
100	20.45	35.35

This decreasing viability indicates Iron Oxide nanoparticles have the capacity to kill breast cancer cell (MDA-MB-231) and Skin cancer (SKMEL) cell lines. Present study investigated the anticancer IC_{50}= 42.75 of the synthesized Fe_3O_4 nanoparticles against breast cancer cell and the anticancer IC_{50}= 35.35 of the synthesized Fe_3O_4 nanoparticles against skin cancer cell. These studies highlight the potential of using metallic nanoparticles for efficient targeted chemotherapy. Thus, decreases in the Iron Oxide nanoparticles size can lead to an increase in ability to enter cell membrane and thus improving the anticancer activity [12].

V. CONCLUSION

The green synthesis of Iron Oxide nanoparticles has been achieved using the bio reducing agent Anacardium Occidentale leaf extract. TEM revealed that the particles are spherical in shape with a uniform size about 20 nm. Concentration of breast cancer cell increases from 6.25 to 100 µg/mL the viability of cell gradually decreases from 82.74 to 22.85. Concentration of skin cancer cell increases from 6.25 to 100 µg/mL the viability of cell gradually decreases from 76.50 to 20.45. This decreasing viability indicates Iron Oxide nanoparticles have the capacity to kill breast cancer cell (MDA-MB-231) and Skin cancer (SKMEL) cell lines. Hence, Lower IC_{50} means more potent the drug. This study investigated the anticancer IC_{50}= 42.75 of the synthesized Fe_3O_4 nanoparticles against breast cancer cell and the anticancer IC_{50}= 35.35 of the synthesized Fe_3O_4 nanoparticles against skin cancer cell.

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