GREEN SYNTHESIS CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF IRON OXIDE NANOPARTCLES FROM LEAF EXTRACT OF COCOS NUCIFERA

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Abstract

One of the newly-emerging technology we report for the synthesis of green Iron Oxide nanoparticles using leaf extract of *Cocos nucifera* as a green stabilizing and reducing agents. The green process is an eco-friendly, biocompatible, easy to handle. 1mM of Ferric Chloride as a precursor mixed with the leaf extract of 25 ml. The prepared green magnetic Iron Oxide nanoparticles are characterized by UV-Visible Spectroscopy, Fourier transform infrared spectroscopy, Scanning electron microscopy, Transmission electron microscopy, Energy dispersive X-ray spectroscopy (XEDS) and X-ray diffraction. Antibacterial efficacy of Iron Oxide nanoparticles was evaluated against gram positive bacteria *Staphylococcus aureus*(G+), *Bacillus cereus* (G+) *and gram-negative bacteria*, *Serratia marcescens* (G-), *Pseudo monas aeruginosa* (G-). Green Iron Oxide nanoparticles has shown highest shown of inhibition of about 14 mm for *Staph aureus* gram positive bacteria.

Keywords: Antibacterial, *Cocos nucifera*, Green reducing agent, Magnetic nanoparticles, Rod shape.

Introduction

Nanotechnology has been important development in the green mediated process of metal nanoparticles which are in the range between 1-100 nm and have attention due to their physical, Thermal, chemical properties[1-2]. Among the Metal Oxide nanoparticles, Iron Oxide magnetic nanoparticles are of great interest, because of their small size, large surface area to volume ratio[3], good stability, low cost, better antimicrobial activity[4], high magnetic permeability due to possess optical properties as they are small enough to confine their electrons and produce quantum effects[5]. The preparation method has a large effect on shape, size distribution[6] and surface chemistry of the particles[7] and also determined distribution and, structural defects or impurities in the particles[8]. Magnetite and maghemite are preferred in biomedicine because they are biocompatible and potentially non-toxic to humans[9]. Iron Oxide nanoparticles are used in cancer magnetic nano therapy which is based on the magneto-spin effects in free-radical reactions to

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generate oxygen radicals[10-11] and control oxidative stress in biological media under inhomogeneous electromagnetic radiation[12].

Coconut has an essential antioxidant selenium, which protects cells from oxidative steam. Coconut kernel and coconut water act as antibacterial[13], antifungal, antiviral, antioxidant, anti dermatophytic, antiparasitic, and hepatoprotective. Coconut oil is best for treating rough hair or bad scalp and hair fall. The roots of the coconut tree are used in the preparation of a dye and a mouthwash[14]. It is also used preparing a medicine for dysentery. The young leaves of coconut are ground to make a paste and it is applied on cuts to stop bleeding. Coconuts has been reported to be anthelmintic, antiseptic, astringent, bactericidal, diuretic, vermifuge, stomachic and supportive[15].

Various methods have been reported for the green synthesis of metal nanoparticles using plant extracts under mild conditions. It is an environment friendly, cost effective, less toxic green way of synthesis were developed in the preparation of nanomaterial[16]. Polyphenols, flavonoids, and tannic acid which act as reducing and stabilizing agent in the synthesis of metal nanoparticles. Iron Oxide nanoparticles are very useful in magnetic[17], electrical conductivity, and biological properties. Constituents of plants like carbohydrates, fats, flavonoids, terpenoids, polyphenols and alkaloids are capable of reducing FeCl3 to Iron Oxide nanoparticles[18]. In this present work Iron Oxide nanoparticles were prepared simple green method by using *Cocos nucifera* leaf extract. Antibacterial studies of Iron Oxide nanoparticles revealed that excellent antibacterial activity.

2. Materials and Methods

Ferric Chloride, Double distilled water, Ethanol was purchased from S A Chemicals, Tirunelveli. *Cocos nucifera* leaves were collected from local market Kanyakumari.

2.1Preparation of leaf extract and Iron Oxide nanoparticles

The leaves were collected washed with distilled water and are cut into small pieces. Take 20g of fresh leaf and 200 ml of deionized water in 500 ml beaker and it was kept at 70^0c on heating mantle and was filtered. Preparation of Iron Oxide nanoparticles was carried out using Ferric Chloride as a Precursor. Take 50ml from 1mM of $FeCl_3$ was taken in a beaker which was stirred at 70^0c on magnetic stirrer for 1 hours. Add 25ml of prepared *Cocos nucifera* leaf extract dropwise into the beaker and centrifuged the solution at 7000 rpm, green nanoparticles are washed with deionized water and ethanol and dried at 90^0c for 3 hours.

3. Characterization studies

Iron Oxide Nano particles were characterized by using UV-Visible, (FT-IR), X-Ray diffraction (XRD) analysis and Scanning Electron Microscope (SEM). 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. Antibacterial activity against gram positive and

Antifungal bacterial strains, Proteus by surface inoculation method. Antimicrobial activity is commonly evaluated using disc diffusion test. The antibacterial activity was done on various pathogenic bacteria such as *Staphylococcus aureus(G+), Bacillus cereus (G+) and gram-negative bacteria, Serratia marcescens (G-), Pseudo monas aeruginosa (G-).* Nutrient agar medium was used to cultivate bacteria. The pure cultures of bacteria were placed in each plate as control. The pure cultures of bacteria were sub cultured on nutrient agar medium. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. The plates were incubated at 37^{o}C for 24 hours. After 24 hours the diameter of the growth inhibition zones was measured.

4. Result and discussion

UV-Vis and FT-IR Spectroscopy

The optical properties of Iron Oxide nanoparticles were studied by absorption spectroscopy. It is the primary method to indicate the bio reduction of Ferric chloride solution to Iron Oxide nanoparticles. 1 mM concentration of Ferric Chloride was taken for the reduction of Iron Oxide by the leaf extract of *Cocos nucifera.* Addition of Ferric Chloride solution reacts with leaf extract of, *Cocos nucifera* the intensity of colour converted to black. The black colour indicates the presence of Iron Oxide nanoparticles due to surface Plasmon resonance phenomenon[19]. Iron Oxide nanoparticles were observed around in 450-550nm indicated magnetic particles[20]. Appearance of black colour shows that the reduction of Iron ions and formation of stable nanoparticles. The peak at 470 nm provides a convenient spectroscopic signature for the formation of Iron Oxid nanoparticles.

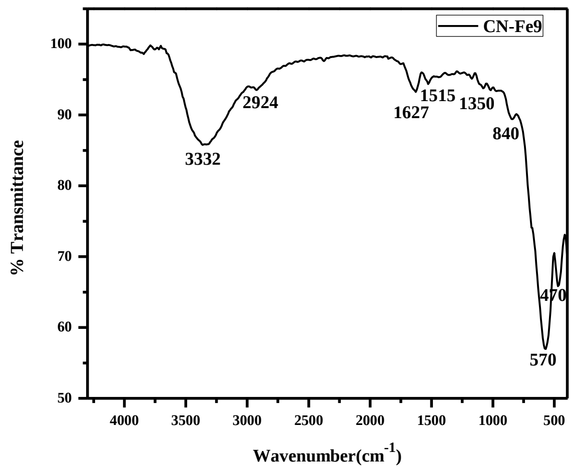

Figure 1: FT-IR spectrum of Iron Oxide nanoparticles by using CN Leaf extract

FT-IR gives the information about functional groups present in the Iron Oxide nanoparticles. A broad absorption band from 3400 to 3200 cm^{-1} due to the stretching vibration of hydroxyl group[21]. The absorption band at 2924 cm^{-1} could be assigned to aliphatic C-H stretching vibrations, and the peak at 1627 cm^{-1}corresponds to carbonyl groups from dimerized saturated aliphatic acid groups. The absorption band at 1515 cm^{-1}can be attributed to C-N stretching vibrations of amino bond of the biomass[22].

The peak at 1350 cm^{-1}can due to O-H bending vibrations of polyols such as flavonoids present in the leaf. The bands at 840 cm^{-1} regions correspond to C-H out of plane bend which are characteristic of aromatic phenols[23]. This can be attributed to the absorption of phenolic compound and Flavonoids phytochemicals on the silver nanoparticles surface which may be responsible for the reducing property.

X-Ray Diffraction and SEM Analysis

The crystallographic structure of the green magnetic nanoparticles was evaluated by X-ray diffraction studies. The peaks were recorded from 200-800 at 2 theta scale and the corresponding diffraction peaks observed at 30.67^0, 35.11^0, 42.69^0, 54.23^0, 58.23^0, 62.32^0 corresponds to the crystal lattice plane of (220), (311), (400), (422), (511) and (440). The above prominent peaks were matched with the crystalline planes of the cubic structure of metallic Iron Oxide (JCPDS file No.19-0629) which was in Fe_3O_4 (Magnetite) crystals and possess magnetic structure of Iron Oxide nanoparticles. The average crystalline size can be calculated from Scherrer equation and it was obtained at 29 nm.

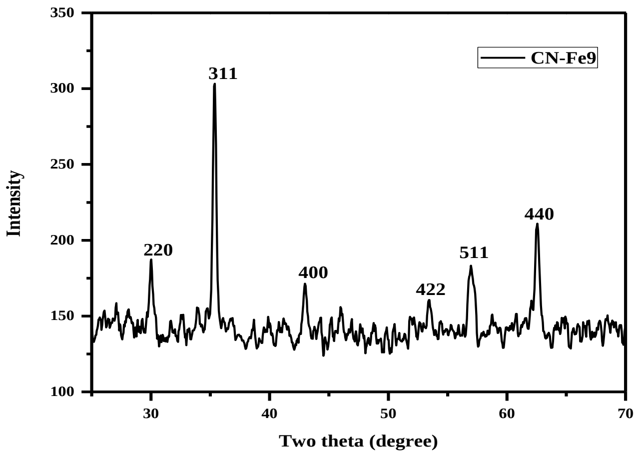

Figure 2: XRD Pattern of Iron Oxide nanoparticles synthesized from CN Leaf

SEM analysis were employed to visualize the morphology and size of the green Iron Oxide nanoparticles. SEM micrographs of Iron Oxide nanoparticles are given in figure.3 with different approbation. The SEM image also confirms that the green Fe_3O_4 magnetic nanoparticles are rod 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 20-50 nm.

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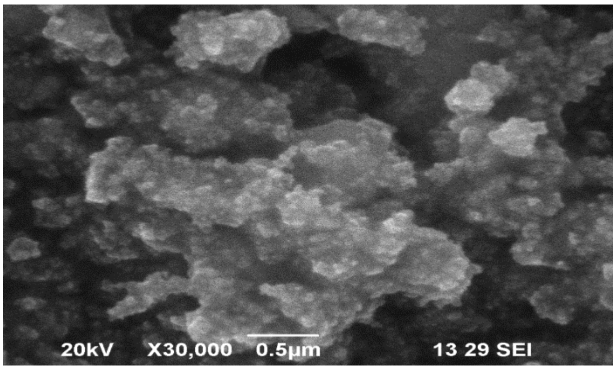

Figure 3: SEM Analysis of Iron Oxide nanoparticles synthesized from CN Leaf

EDXMA and TEM Analysis

Energy dispersive X-ray microanalysis is an analytical technique used for the elemental analysis or chemical characterization. Elemental composition of green Iron Oxide nanoparticles synthesized using *Cocos nucifera* leaf extract was determined by using EDXMA analysis[24]. The EDXMA analysis reported in Figure.4, clearly shows the presence of the K-α at 6.4 KeV due to Fe atoms present in the nanoparticles and K-α at 0.6 Kev due to O atom. The percentage of mass present under the area is 71.55% and 23.23% for Iron and Oxygen respectively.

TEM analysis was performed to measure the shapes and size of Iron Oxide nanoparticles synthesized using *Cocos nucifera* leaf extract. The sample was prepared for Transmission electron microscopy imaging by disperse drops of the green Iron Oxide nanoparticle solution on a copper grid and left to dry[25]. Figure.5 shows the TEM image of green Iron Oxide nanoparticles reveals homogeneous rod like shapes with different approbation with a uniform size range between 20-50 nm. The size distribution of nanoparticles was analysed using ImageJ 1.5J software. From analysis, the average size for Iron Oxide nanoparticles was found to be 29.4 nm.

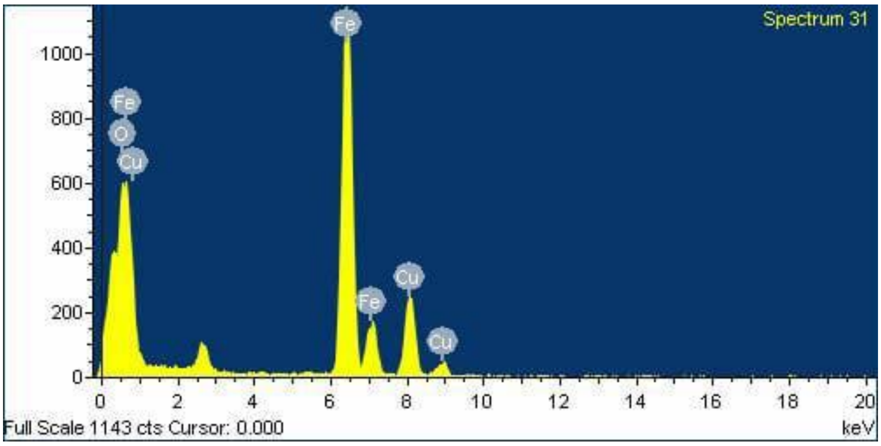

Figure 4: EDX spectrum of Iron Oxide nanoparticles synthesized from CN Leaf

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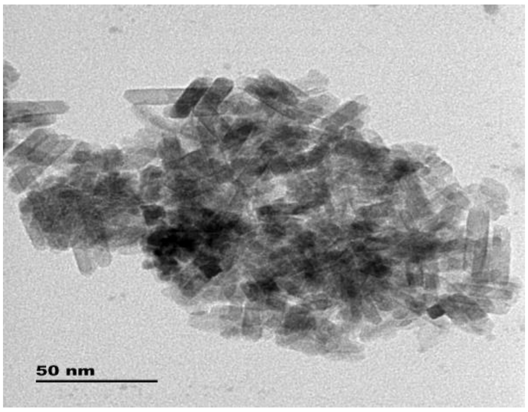

Figure 5: TEM analysis of Iron Oxide nanoparticles synthesized from CN Leaf

Antibacterial activity

Iron Oxide nanoparticles was one of the most important antibacterial substances[26]. Antibacterial activity was investigated against Antibacterial activity of Iron Oxide nanoparticles was evaluated against gram positive bacteria *Staphylococcus aureus*(G+), *Bacillus cereus* (G+) and gram-negative bacteria, *Serratia marcescens* (G-), *Pseudo monas aeruginosa* (G-).The minimum inhibitory Concentration of green synthesized Iron Oxide nanoparticles required to inhibit the growth of bacteria was documented by testing the zone with different concentration from 6 to 175 µg/ml against two gram positive and two gram negative bacteria represented above. The MIC was shown at 100 µg/ml for all tested bacterial strains, this dose was selected for further testing of antibacterial activity of Iron Oxide nanoparticles.

Table 1. Antibacterial activity of Iron Oxide nanoparticles synthesized from CN Leaf

Iron Oxide nanoparticles	*Staph aureus Gram* (+)	*Bacillus cereus Gram* (+)	*Serratia marcescens* Gram (-)	*Pseudo monas aeruginosa* Gram (-)
Control (amikacin)	**19 mm**	**15 mm**	**18 mm**	**15 mm**
Fe_3O_4	**14 mm**	**13 mm**	**13 mm**	**11 mm**
MIC	**80 µg/ml**	**90 µg/ml**	**90 µg/ml**	**100 µg/ml**

Green Iron Oxide nanoparticles has shown highest shown of inhibition of about 14 mm for *Staph aureus* followed by *Bacillus cereus* (13), *Serratia marcescens* (13), *Pseudo monas aeruginosa* (11) as shown in Table 1 and Figure 6,7,8,9 which is almost nearer to zone of inhibition of standard amikacin.

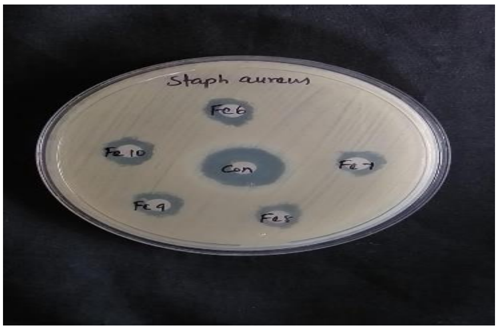

Figure 6: Sensitivity of Iron Oxide nanoparticles against *Staph aureus*

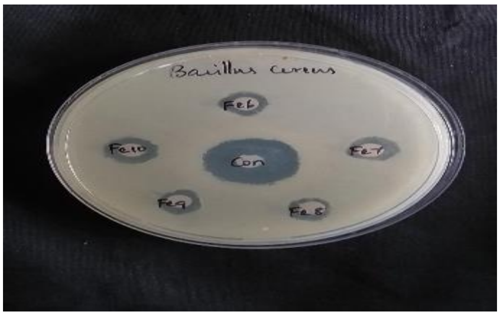

Figure 7: Sensitivity of Iron Oxide nanoparticles against *Bacillus cereus*

The maximum zone of inhibition was taking place in the bacteria *Staph aureus* Gram (+). From these results, it can be predicted that Staph aureus has shown more sensitive to Iron Oxide nanoparticles compared to other bacterial strains.

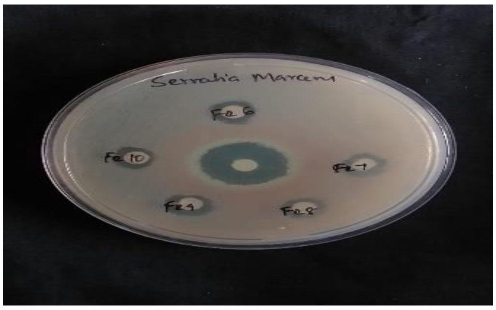

Figure 8: Sensitivity of Iron Oxide nanoparticles against *Serratia marcescens*

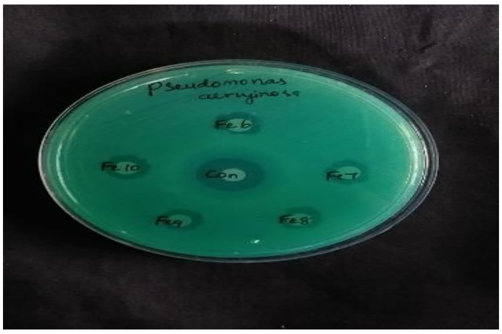

Figure 9: Sensitivity of Iron Oxide nanoparticles against *Pseudomonas aeruginosa*

The zone observed with Iron Oxide Ethanol solution 5Mm was less and *Cocos nucifera* leaf extract did not show any inhibition against bacterial strains. The positive control Amikacin was used against all four bacterial strains. The possible mechanism of Iron Oxide nanoparticles against bacterial strains can be explained by the release of Iron cations[27] from Iron Oxide

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nanoparticles, the release of Iron cations gets attached to the sulfhydryl group of bacterial enzymes due to electrostatic attraction it resulting in the enzyme inactivation which conduct to cell death[28]. It also can be interpreted on the basis of all bacterial action of Iron Oxide nanoparticles on their size, quantity and arrangement[29]. Thus, decreases in the Iron Oxide nanoparticles size can lead to an increase in ability to enter cell membrane and thus improving the antibacterial activity[30].

Conclusion

Homogeneous rod like shaped Iron Oxide nanoparticles of size 29.5 nm were synthesized by a natural, novel, reducing agent *Cocos nucifera* leaf extract. The nanoparticles were characterized by UV-visible, SEM, TEM, XRD, and FT-IR measurements. Polycrystalline nature of the nanoparticles is evident from bright circle like spots in the SAED pattern, clear rod like shape in the HR-TEM images. The green synthesized Iron Oxide nanoparticles were found to have a promising Antibacterial activity against *Staph aureus, Bacillus cereus, Serratia marcescens, Pseudo monas aeruginosa.* The maximum zone of inhibition for Fe_3O_4 were taking place in the bacterial strain *Staph aureus* as 14 mm. For all strain Iron Oxide nanoparticles are found to have high antimicrobial activity when compared to leaf extract. Hence prepared Iron Oxide nanoparticles are used in wound healing applications.

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