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BIOGENIC SYNTHESIS OF Fe_3O_4 NANOPARTICLES USING *TRIDAX PROCUMBENS* LEAF EXTRACT AND ITS ANTIBACTERIAL ACTIVITY ON *PSEUDOMONAS AERUGINOSA*

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Iron oxide nanoparticles (Fe_3O_4) were rapidly synthesized by reduction of ferric chloride solution with Tridax procumbens leaf extract containing carbohydrates as a major component which act as reducing agent. The results indicated that water-soluble carbohydrates which have aldehyde groups may cause the formation of Fe_3O_4 nanoparticles. The purification process of the Fe_3O_4 product does not require any expensive methods, since solid product is obtained from the reaction in liquid phase. The antibacterial effect of Fe_3O_4 nanoparticles against *Pseudomonas aeruginosa* was investigated as a model for Gram-negative bacteria. Bacteriological tests were performed in Potato Dextrose Agar (PDA) medium on solid agar plates and in liquid systems supplemented with different concentrations of nanosized Fe_3O_4 particles. These particles were shown to be an effective bactericide. The resulting Fe_3O_4 nanoparticles were characterized by X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), UV-VIS absorption and Fourier-transform infrared (FTIR) spectroscopy.

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1. Introduction

In recent years, various nanomaterials have been extensively pursued for their catalytic, optical, electrical, mechanical and magnetic properties which are quite different from those of their bulk counterparts [1]. Metal oxides have important applications such as magnetic storage media, solar energy transformation, electronics, catalysis and biological applications like imaging, and delivery [2]. Iron oxides are used extensively due to the development of preparation technology of nanometer powders.

Magnetite (Fe_3O_4) is a common magnetic iron oxide that has a cubic inverse spinel structure with oxygen forming fcc closed packing and Fe cations occupying interstitial tetrahedral sites and octahedral sites [3]. The electrons can hop between Fe^{2+} and Fe^{3+} ions in the octahedral sites at room temperature rendering magnetite an important class of half-metallic materials [4].

Magnetite (Fe_3O_4) is one of the iron oxides with a non-normal spinel structure which can offer great potential applications in different fields such as recording materials [5], photocatalysis [6], ferro fluid technology, magneto caloric refrigeration [7], drug delivery [8], pigment [9], magnetic storage, magnetic ink printing, microwave absorption, biosensors, bioseparation, in-vivo drug delivery, immune magnetic array, magnetic resonance imaging contrast agents, hyper thermia treatment of cancer [10–13] etc.

Various chemical synthetic routes have been employed to produce magnetite nanoparticles with desired physical and chemical properties such as coprecipitation of aqueous ferrous (Fe^{2+}) and ferric (Fe^{3+}) salt solution by the addition of a base [14], microemulsion

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technique [15], hydrothermal synthesis [16], sonochemical approach [17], non aqueous route [18] and thermal decomposition of organic iron precursor like $Fe(cup)_3$ [19], $Fe(CO)_5$ [20] or $Fe(acac)_3$ [21], sol-gel technique [22], γ-ray radiation [23], thermal decomposition of organic metals [24], microwave plasma synthesis [25], thermal decomposition of alkaline solution of Fe^{3+} chelate in the presence of hydrazine [26], the reduction of hematite by CO at high temperature with good size and morphology control [27].

Developing a facile and green method for synthesizing superparamagnetic Fe_3O_4 is of importance and still a challenge for materials scientists. Over the past two decades, there have been increased emphases on the topic of green chemistry and chemical processes. Utilization of non toxic chemicals, environmentally benign solvents, and renewable materials are some of the key issues that merit important consideration in a green synthetic strategy. The *Tridax procumbens* leaves possesses biomolecules such as carbohydrates, proteins and lipids [28], which could be used as reducing agent to react with ferric ions and as scaffolds to direct the formation of Fe_3O_4 NPs in solution. To the best of our knowledge, the use of plant extracts at room temperature for the green synthesis of Fe_3O_4 nanoparticles has not been reported.

Sundaram Ravikumar [29] reported that the various nanoparticles *viz.,* Al_2O_3, Fe_3O_4, CeO_2, ZrO_2 and MgO were subjected to evaluate its antibacterial potential against ophthalmic pathogens such as *Pseudomonas aeruginosa, Acinetobacter* sp., *Klebsiella pneumoniae, E. coli, Streptococcus viridans* and *Streptococcus pyogenes*. Among the nanoparticles, Fe_3O_4 showed maximum activity against *Pseudomonas aeruginosa.* The reactive oxygen species (ROS) generated by Fe_3O_4 nanoparticles could kill bacteria without harming non-bacterial cells [30]. Specifically, Pareta et al cultured osteoblasts (bone-forming cells) with Fe_3O_4 nanoparticle and found that cell density was greatly enhanced in the presence of Fe_3O_4 nanoparticles compared with cells cultured without nanoparticles [31].

2. Experimental details

2.1 Materials

Ferric chloride ($FeCl_3$) was purchased from Merck and was used without further purification. *Pseudomonas aeruginosa* (ATCC 9027) was purchased from Institute of Microbial Technology (Chandigarh, India). The components of the Potato Dextrose Agar (PDA) medium used in growing and maintaining the bacterial cultures were supplied by Projen Laboratories (Chennai, India).

2 .2 Preparation of *Tridax procumbens* leaf extract

About 20g of fresh taxonomically authenticated healthy leaves of *Tridax procumbens* were collected, washed thoroughly with double distilled water, cut in to fine pieces and boiled with 100mL double distilled water in Erlenmeyer flask for 8-10 min. The extract was cooled to room temperature and filtered through Whattman filter paper (No.42).

2.3 Preparation of Ferric chloride solution

8.1g of Ferric chloride was dissolved in 50 ml deionized water and stirred for 15 minutes.

2.4 Preparation of Fe_3O_4 Nanoparticles

In a typical experiment, 10 ml of Ferric chloride solution was mixed with 10 ml of the Tridax procumbens leaf extract in a beaker. After 10 minutes, the colour of the solution changed from brown to black indicating the formation of iron oxides nanoparticles. The solid product was filtered and washed with ethanol and then dried at room temperature.

2.5 FTIR spectral analysis

FTIR spectra were performed and recorded with a Fourier-Transform infrared spectrophotometer of type Nicolet 870 between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1}.

2.6 SEM analysis

The morphologies and compositions of the Fe_3O_4 nanoparticles were examined by Scanning Electron Microscopy (SEM) using a LEO 1455 VP equipped with energy dispersive.

2.7 X-ray diffraction studies

X-Ray Diffraction (XRD) patterns were recorded with a Philips analytical X-ray diffractometer using CuKα radiation (λ= 1.5406 Å).

2.8 Antibacterial assay

The Fe_3O_4 nanoparticles synthesized using *Tridax procumbens* was tested for antibacterial activity by agar well-diffusion method against *Pseudomonas aeruginosa*. The pure bacterial culture was subcultured on nutrient agar and Potato Dextrose Agar (PDA). Wells of 10 mm diameter were made on nutrient agar and PDA plates using gel puncture. The strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Using a micropipette, different concentrations of the sample of nanoparticles solution (10 µl, 20 µl, 30 µl and 40 µl) was poured onto each well on the plates. After incubation at 37°C for 24 hours, the different level of zone of inhibition of bacteria was measured.

3. Results and discussion

The addition of Ferric chloride solution to the plant extract containing carbohydrates as a major component which have aldehyde group may cause the partial reduction of Fe^{3+} to form Fe_3O_4. The possible reduction mechanism leading to the formation of Fe_3O_4 only from the single iron precursor, $FeCl_3$, is proposed in the following equations.

$$Fe^{3+} + 3H_2O \longrightarrow Fe(OH)_3 + 3H^+ \quad (1)$$

$$Fe(OH)_3 + R\text{-}CHO \longrightarrow Fe_3O_4 + R\text{-}COOH \quad (2)$$

First, $FeCl_3$ hydrolyzes to form ferric hydroxide and releases H^+ ions in the proper pH value and temperature. After that, ferric hydroxide is partially reduced by the plant extract containing carbohydrates (glucose) to form Fe_3O_4, while aldehyde group is oxidized to corresponding acid.

The representative absorption peaks in FT-IR spectra of nanoparticles located mainly at 3436cm^{-1}, 1362cm^{-1}, 1611cm^{-1} , 1113cm^{-1} and 587cm^{-1} in the region 400cm^{-1}-4000cm^{-1} shown in Fig.1. The peaks at 3436$cm^{-1}$and 1611cm^{-1} are the characteristic bands of hydrogen bonded OH groups present in the aqueous phase. The peaks at 1362cm^{-1} and 1113cm^{-1} indicate the presence of (-COO-) carboxylate ions responsible for formation of Fe_3O_4 nanoparticles and C-O-C stretching frequency. The peak at 587cm^{-1} indicates that Fe-O vibration of Fe_3O_4 nanoparticles [32].

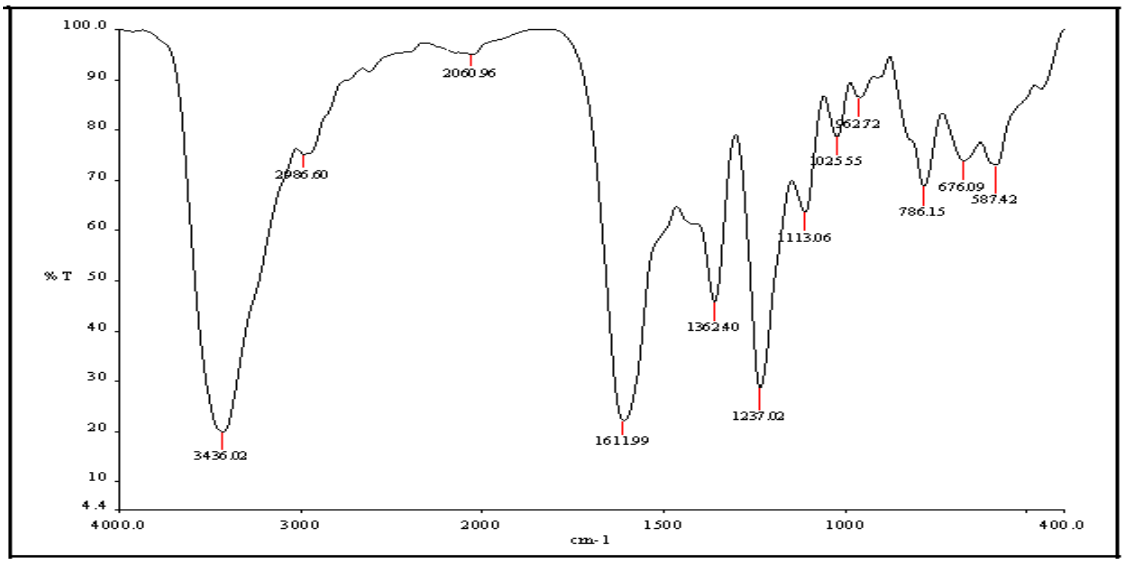

Fig.1 FT-IR spectrum of Fe_3O_4 nanoparticles synthesized using Tridax procumbens leaf extract

The X- ray diffraction patterns obtained for the Fe_3O_4 nanoparticles synthesized using Tridax procumbens leaf extract is shown in Fig. 2. The XRD spectrum contains four peaks that are clearly distinguishable. All of them can be perfectly indexed to crystalline Fe_3O_4 not only in their peak positions, but also in their relative intensities. The peaks with 2θ values of 29.1°, 35.4°, 56.7° and 61.2° correspond to the crystal planes of (200), (311), (511) and (440) of crystalline Fe_3O_4 respectively. The crystallite sizes can be estimated using Scherrer's formula $D = K\lambda/\beta\cos\theta$ where the constant K is taken to be 0.94, λ is the wavelength of X-ray and β and θ are the half width of the peak and the Bragg angle respectively. Using the equation, the crystallite sizes found to be in the range of 80-100nm.

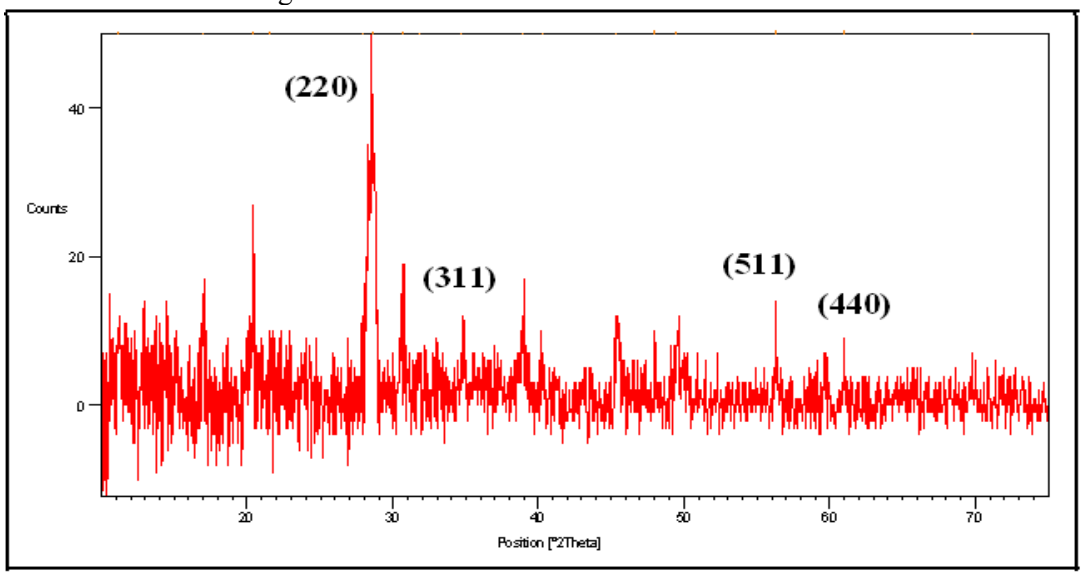

Fig. 2 XRD pattern of Fe_3O_4 nanoparticles synthesized using Tridax procumbens leaf extract

The morphological studies of synthesized Fe_3O_4 nanoparticles were analyzed by scanning electron microscopy as shown in Fig.3- a, b, c & d. It was identified that shapes of the Fe_3O_4 nanoparticles appeared like irregular sphere shapes with rough surfaces. All the nanoparticles were well separated and no agglomeration was noticed. The XRD pattern suggested that the unassigned peaks may indicate the crystallization of bio-organic phase present in the extract which was also observed from SEM micrographs.

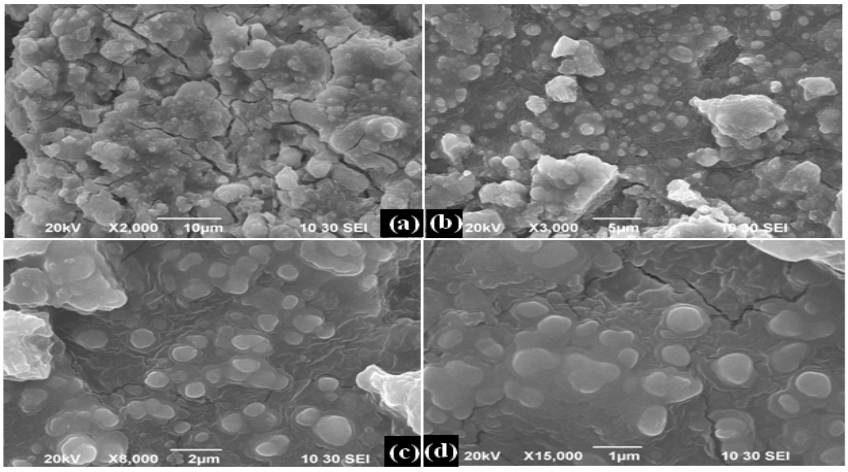

Fig.3 (a, b, c & d). SEM micrographs of Fe_3O_4 nanoparticles synthesized using Tridax procumbens leaf extract at different magnification levels

The antibacterial activity of *Tridax procumbens* mediated Fe_3O_4 nanoparticles was performed against *Pseudomonas aeruginosa* using agar well diffusion method. The mean of three replicates of zone of inhibition (mm) around well with *Tridax procumbens* mediated Fe_3O_4 nanoparticles is presented in the Table 1.

Table 1. Zone of inhibition (mm) of Tridax procumbens mediated Fe_3O_4 nanoparticles (µl)

Test organism	Concentratio of nanoparticles(µl)			
	10 µl	20 µl	30 µl	40 µl
Pseudomonas aeruginosa	1.0	1.6	1.8	2.0

The Figure 4 shows the zone of inhibition of bacterial growth on agar plates as a function of the different concentrations of Fe_3O_4 nanoparticles. It was observed from the images a, b, c, d. The growth of bacteria was inhibited gradually with increase in concentration of Fe_3O_4 nanoparticles. incre Results clearly demonstrate that newly synthesized Fe_3O_4 nanoparticles could promise s antimicrobial agent for *Pseudomonas aeruginosa.*

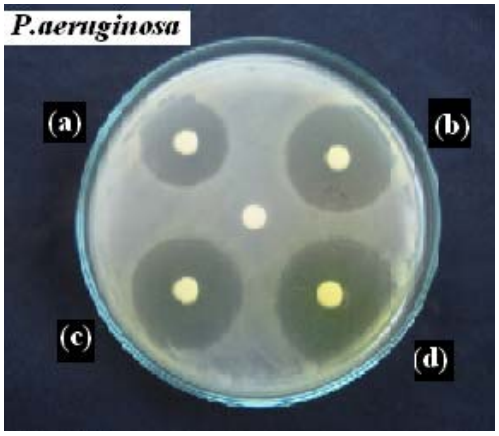

Fig. 4(a, b, c, d). Representative images of agar plates containing Fe_3O_4 nanoparticle impregnated disks and appearances of inhibitory zones of Pseudomonas aeruginosa with different concentrations.

4. Conclusion

A critical need in the field of nanotechnology is the development of reliable and eco-friendly processes for synthesis of metal oxide nanoparticles. Here, we have reported a simple, eco friendly and low-cost approach for preparation of Fe_3O_4 nanoparticles by reduction of ferric chloride solution with a green method using *Tridax procumbens* leaf extract as the reducing agent. The characteristics of the obtained Fe_3O_4 nanoparticles were studied using FTIR, XRD and SEM techniques. The antibacterial effect of Fe_3O_4 nanoparticles against *Pseudomonas aeruginosa* was investigated as a modal for Gram-negative bacteria by agar well-diffusion method.

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