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# *Ocimum sanctum* leaf extract mediated green synthesis of iron oxide nanoparticles: spectroscopic and microscopic studies

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**ABSTRACT**

An eco-friendly green synthesis of iron oxide nanoparticles using leaf extract of *Ocimum sanctum* was investigated. UV-visible spectra showed the maximum absorbance of 285 and 324 nm due to the excitation of surface plasmon vibrations in the iron oxide nanoparticles formation. FTIR spectrum exhibited the characteristic band at 618 $cm^{-1}$ which indicated the Fe-O stretching of $Fe_2O_3$ nanoparticles. The XRD spectrum showed three different diffraction peaks corresponding to the crystal planes of crystalline $Fe_2O_3$. The sharp peaks indicated the crystallinity and purity of iron oxide nanoparticles. The average particle size of the synthesized iron oxide nanoparticles was estimated to be 47 nm using the Scherrer equation. The formation of $Fe_2O_3$ nanoparticles as well as their morphological dimensions in the SEM study revealed that the particles were aggregated. Transmission electron microscope image of iron oxide nanoparticles showed that the nanoparticles size was below 20 nm.

**Keywords**

Green synthesis, Iron oxide nanoparticles, *Ocimum sanctum*, Spectroscopic studies, Microscopic studies

## 1. INTRODUCTION

Iron oxide nanoparticles have attracted intensive research interest because of their important applications in cancer therapy, drug delivery, magnetic resonance imaging (MRI) and wastewater treatment (Vicky *et al*., 2010). The biosynthesis of iron oxide nanoparticles of different sizes and shapes has been reported using bacteria (Yeary *et al*., 2005), fungi (Roh *et al*., 2006) and plant extract (Senthil *et al*., 2012). Green synthesis of nanoparticles is very cost effective, environment friendly and non-toxic. The bioreduction of metal by combinations of biomolecules found in plant extract, resulting in the formation of metal NPs has been extensively reviewed (Iravani *et al*., 2011). The synthesis of nanoparticles using plant extract could be advantageous over other biological processes since it eliminates the elaborate process of maintaining cell cultures.

*Ocimum sanctum* (Tulsi) an Indian origin is considered as Holy basil in India. *Ocimum sanctum* belongs to the family Lamiaceae, which is well known for its medical use. In Ayurveda, the Indian system of medicine since ancient times, *Ocimum sanctum* attributes several medicinal properties (Kirtikar *et al*., 1991). The plant has been widely acknowledged for the treatment of coryza, hay asthma, bowel complaints, worm infestations and kidney stones in traditional medicine (Bauer *et al*., 1966). Hence it is also termed as the Queen of Herbs. *Ocimum sanctum* remains as an active area of scientific research for both human nutritional needs and therapeutic applications (Sood *et al*., 2005). *Ocimum sanctum* leaf broth mediated synthesis of silver (Garima *et al*., 2011; Mallikarjunaa *et al*., 2011), copper (Vasudev *et al*., 2013) and platinum (Soundarrajan *et* al., 2012) nanoparticles has been reported. To the best of my knowledge, the use of *Ocimum sanctum* leaf extract for the biosynthesis of iron oxide nanoparticles has not yet been reported. Hence, the present study reports on the biosynthesis of iron oxide nanoparticles using *Ocimum sanctum* leaf extract. The synthesized nanoparticles were characterized using UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray Diffraction (XRD), scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

## 2. MATERIALS AND METHODS

**2.1 Reagents and Chemicals:** For the synthesis of iron oxide nanoparticles, *Ocimum sanctum* (commonly called as Krishna Tulsi) leaf extract was used as the reducing agent. Ferrous sulphate ($FeSO_4$) was used as precursor. MilliQ water was used throughout the experiment.

**2.2 Preparation of *Ocimum sanctum* Leaf Extract:** About 20 g of fresh and healthy leaves of *Ocimum sanctum* were collected, washed thoroughly with MilliQ water, cut into fine pieces and boiled with 200 mL MilliQ water in Erlenmeyer flask at 80°C for 10-15min. The extract was cooled at room temperature and filtered using Whatman filter paper and stored at 4°C for further experiments.

**2.3 Green Synthesis of Iron Oxide Nanoparticles:** The various volumes of leaf extract including 1.0, 2.0, 3.0, 4.0 and 5.0 mL were added to the constant volume of ferrous sulphate solution at different pH conditions (2.0, 3.0, 5.0, 8.0 and 9.0). The optimization of physiochemical parameters including volume of leaf extract and pH was done using UV-visible spectrophotometer (Jasco V650) in the range of 200–800 nm. During the synthesis of iron oxide nanoparticles, both the aqueous leaf extract and the precursor salt solution were mixed in 1:5 proportions. After the addition of leaf extract to the salt solution, the color changed from colorless to black. The reaction mixture was centrifuged at 10,000 rpm for 15 min. The supernatant was discarded and the pellets were repeatedly washed with MilliQ water and dried for the evaporation of aqueous phase in hot air oven.

**2.4 Characterization of Iron Oxide Nanoparticles:** The black colored solid precipitate obtained was characterized for the bioreduction of $Fe^{2+}$ ions using Fourier transform infrared spectroscopy (Perkin Elmer RX1) and X-ray diffraction (Philips PW 1880). Morphology and size distribution of iron oxide nanoparticles were performed using scanning electron microscopy (JEOL JSM 5610) and transmission electron microscopy (Technai T20; Philips) respectively.

## 3. RESULTS AND DISCUSSION

**3.1 UV-visible Analysis of Iron Oxide Nanoparticles:** UV-visible spectroscopy is most widely used technique to investigate the optical properties of the particles. The color change from colorless to black (Fig.1) indicated the formation of iron oxide nanoparticles. UV-visible spectroscopy analysis was done in the range of 200-800 nm and the maximum absorbance was observed at 285 and 324 nm regions for the formation of iron oxide nanoparticles due to the excitation of surface plasmon vibrations. The lambda

maxima of synthesized iron oxide nanoparticles were quite similar to those reported for $Fe_2O_3$ (Klacanova *et al*., 2013). The UV-visible spectra was observed (Fig. 2(a)) for different volume of extract (1.0, 2.0, 3.0, 4.0 and 5.0 mL) with 1.0 mM of $FeSO_4$ solution. The results depicted that 5.0 mL of leaf extract was considered as the optimum volume. The pH was varied as 2.0, 3.0, 5.0, 8.0 and 9.0 (Fig.2 (b)) and the optimum pH was found to be 5.0. As seen in figures, the decrease in intensity of peak at 324 nm may be due to the oxidation of zero valent iron to iron oxide nanoparticles. Similar observation was reported earlier (Guo *et al*., 2001).

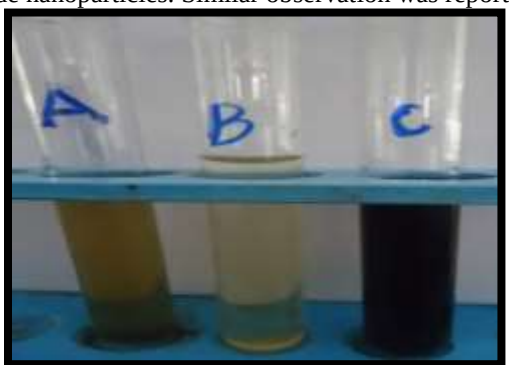


**Fig.1 Visual observations of A - Leaf extract, B - Ferrous Sulphate and C- Iron oxide Nanoparticles**

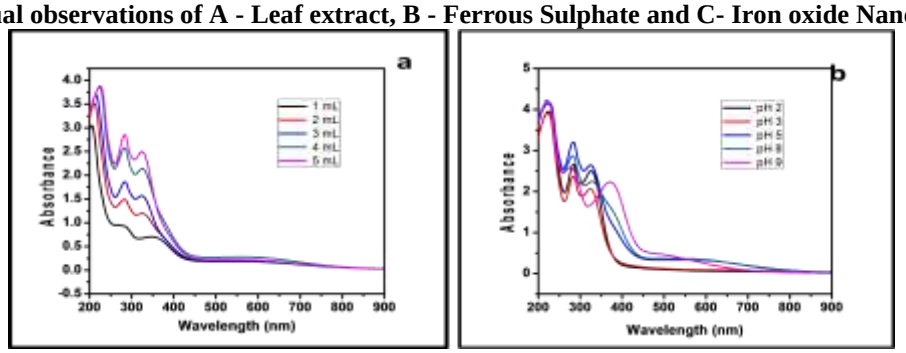


**Fig. 2 (a) UV-Visible spectra of iron oxide nanoparticles at 1 mM ferrous sulphate with different volume of extract, (b) UV-visible spectra of iron oxide nanoparticles at different pH**

**3.2 FTIR Analysis of Synthesized Iron Oxide Nanoparticles:** FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of Fe ions and capping of the reduced iron oxide nanoparticles. The *Ocimum sanctum* leaf extract, ferrous sulphate and the synthesized iron oxide nanoparticles were analyzed using FTIR. The FTIR spectrum of ferrous sulphate (Fig. 3(a)) represents the major absorption bands. The peaks 3369 and 1096 $cm^{-1}$ represents the characteristic band of O-H group and sulphate groups respectively. The band 617 $cm^{-1}$ represents the Fe-O stretching. The FTIR spectrum of *Ocimum sanctum* leaf extract (Fig. 3(b)) represents the major absorption bands 3428 and 1630 $cm^{-1}$ corresponding to O-H group of phenol compounds and N-H bending of amide group respectively. The band 2926 $cm^{-1}$ indicates the presence of carboxylic acid groups and 2361 $cm^{-1}$ corresponds to the N-H / C-O stretching vibration. The bands 1411 and 1035 $cm^{-1}$ is assigned to the C-C stretching and C-N stretching of aliphatic amines respectively. The band 666 $cm^{-1}$ represents the characteristics of C-H bending vibration. The FTIR spectrum of synthesized iron oxide nanoparticles (Fig. 3(c)) represents the shift in peak from 3428 to 3403 $cm^{-1}$, 2361 to 2365 $cm^{-1}$ and 1630 to 1613 $cm^{-1}$ in comparison with the FTIR spectrum of leaf extract. The band 618 $cm^{-1}$ indicated the Fe-O stretching of $Fe_2O_3$ nanoparticles, as reported earlier (Gotic *et al.*, 2009) which indicated the formation of iron oxide nanoparticles. Based on these results, the presence of phenolic compounds and proteins were believed to be responsible for the formation and stabilization of synthesized iron oxide nanoparticles.

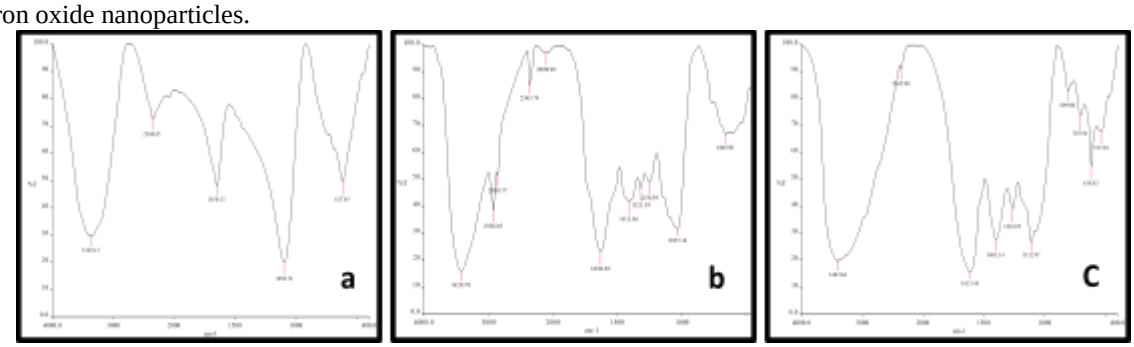


**Fig. 3 FTIR spectra of (a) Ferrous sulphate, (b) *Ocimum sanctum* leaf extract and (c) Synthesized Iron oxide nanoparticles**

**3.3 XRD Analysis of Synthesized Iron Oxide Nanoparticles:** The average size, the crystalline nature of the particles and quality of compounds were determined by X-ray Diffraction (XRD) spectrum with CuKα radiation λ=1.504A° over a wide range of Bragg angles 20°≤2θ≤90° using X-ray Diffraction patterns (Fig. 4). The XRD spectrum showed different diffraction peaks at 32.15°, 44.7°,

56.15° and 75.5° corresponding to the crystal planes of (221), (410), (510) and (622) of crystalline $Fe_2O_3$. The obtained data matched with the Joint Committee on Powder Diffraction Standards (JCPDS) File No. (39-1346). The size of the synthesized iron oxide nanoparticles using the Scherrer equation ($d = k\lambda / \beta \cos\theta$ by determining the width of the Braggs reflection, where k is the Scherrer constant (0.54), λ is the wavelength of X ray (1.54 A°), β is the half width of the peak and θ is the Bragg angle) was in the range from 35 to 60 nm and the average size was estimated to be 47 nm.

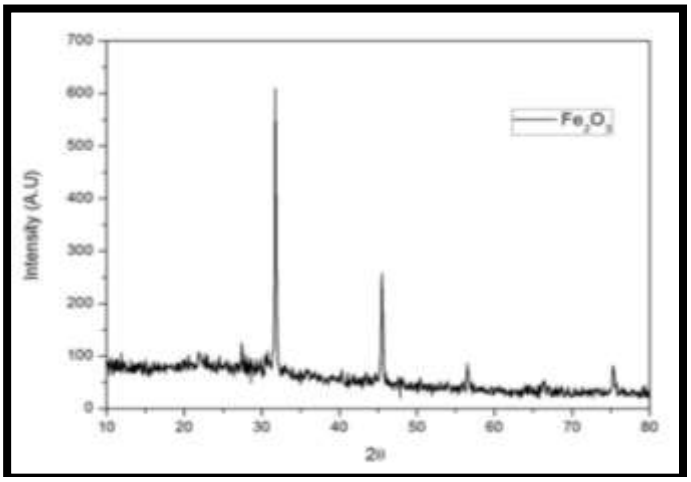


**Fig.4 XRD pattern of synthesized iron oxide nanoparticles**

**3.4 SEM and TEM Analysis of Synthesized Iron Oxide Nanoparticles:** The powdered sample was analyzed for the structure and morphology of the synthesized iron oxide nanoparticles using SEM at different magnification levels including 10µm and 50µm (Fig.5). SEM images revealed that the synthesized iron oxide nanoparticles were aggregated as irregular sphere shapes with rough surfaces. The morphology of the nanoparticles mostly appeared to be a porous and spongy. However, to obtain a clear size, shape and structural image of the nanoparticles the samples were analyzed using Transmission Electron Microscopy (Fig.6). Transmission electron microscope image reveals the size of the synthesized iron oxide nanoparticles to be less than 20 nm.

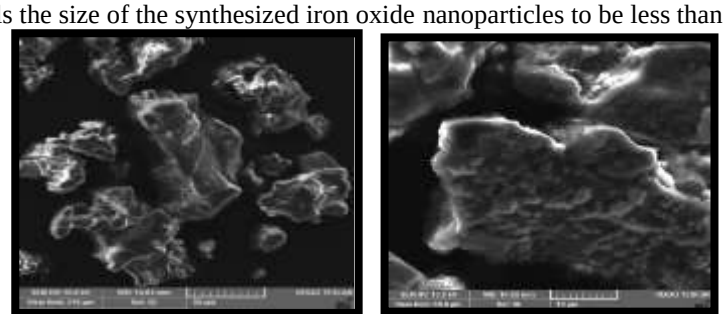

**Fig.5 SEM images of synthesized iron oxide nanoparticles**

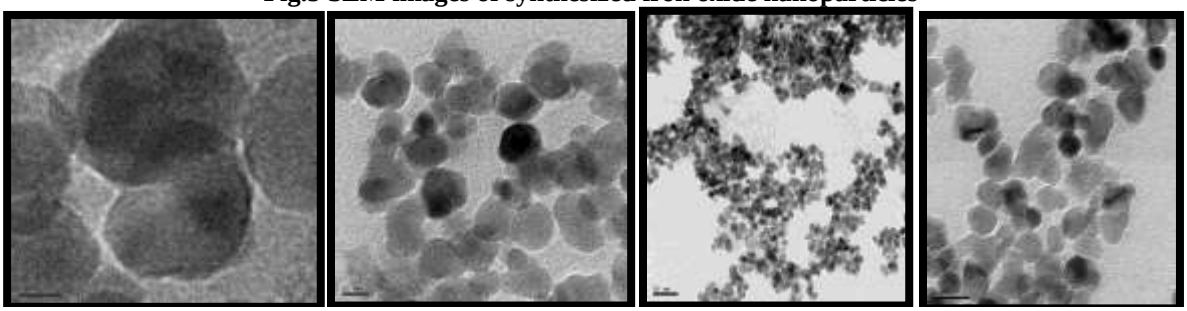

**Fig.6 TEM images of synthesized iron oxide nanoparticles**

## 4. CONCLUSIONS

The rapid biological synthesis of iron oxide nanoparticles using leaf broth of *Ocimum sanctum* provides an environment friendly, simple and efficient route. Fourier transform infrared spectroscopy indicated that the phenolic compounds and proteins may be responsible for the reduction of ferrous ions. The XRD spectrum matched with the JCPDS File No. (39-1346) and the average size was estimated to be 47 nm. SEM micrographs at different magnification levels showed that the synthesized iron oxide nanoparticles were in the form of irregular shapes in aggregated form. TEM analysis confirms that the size of the iron oxide nanoparticles was found to be less than 20 nm. Thus, the green synthesis using *Ocimum sanctum* leaf extracts can be economic and effective method for the synthesis of iron oxide nanoparticles.

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