Research Article

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# Green Route Synthesis and Preliminary Characterization of Iron Oxide Nanoparticles using Leaf Extract of *Ocimum tenuiflorum*

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*Green route synthesis of iron oxide nanoparticles (IONPs) was successfully carried out using leaf extract of Ocimum tenuiflorum (Ram Tulsi variety). The synthesis method employed was found to be easy and made use of simple precursors. The IONPs thus formed were magnetic in nature and easily recovered from the solution. Preliminary characterization of the synthesized IONPs was carried out using UV-Visible spectroscopy. The spectrum exhibited characteristic absorption peak at 282 nm corresponding to magnetite ($Fe_3O_4$). FTIR spectrum showed formation of IONPs with distinct absorption bands assigned to the Fe-O bond stretching. XRD spectrum confirmed formation of magnetite along with α-$Fe_2O_3$. Further studies using TEM indicated presence of nanospheres and nanoellipsoids. Nanospheres were found to range from 3-5 nm and nanoellipsoids were of the dimensions of 100-200 nm in length and 20-30 nm in diameter. The dye degradation capacity of the synthesized IONPs revealed high reactivity and efficiency of dye removal showing their potential as nanocatalysts. The synthesis method reported here was found to generate two different phases of IONPs viz. magnetite ($Fe_3O_4$) and hematite (α-$Fe_2O_3$) both of which possess important biomedical and environmental applications.*

**Keywords:** *Ocimum tenuiflorum*, Green Route Synthesis, Magnetite, Iron Oxide Nanoparticles, Nanocatalyst

## Introduction

Nanotechnology has become a hot-spot of interdisciplinary research in the recent years to discover and explore the immense potential of metallic nanoparticles. These nanomaterials are highly promising and possess unique properties which make them suitable for the development of newer and innovative applications in different fields such as electronics, engineering and material sciences, biomedical, besides environmental remediation and other biotechnological applications [1-3]. These nanoparticles possess extremely small size and large surface area to volume ratio, which influences their physicochemical, thermal, mechanical as well as magnetic properties [4]. The size and shape of the particles can be tuned under controlled conditions of temperature, pH, precursor concentration etc. depending on the application [5].

Chemical or physical methods for synthesis of different types of metallic nanoparticles are currently being used by researchers around the globe. These procedures mostly involve hazardous chemicals and organic solvents that are toxic, corrosive and flammable, in addition to use of specialized equipments and high energy. Moreover, the by-products post nanoparticles synthesis may pose a severe disposal problem as these harmful substances cannot be released freely in the environment [6-8].

Metallic nanoparticles especially iron oxide nanoparticles (IONPs) have received tremendous attention due to the wide range of promising applications in different arenas. They have been studied for diverse applications such as degradation of organic dyes, in heavy oil viscosity treatment, removal of toxic pollutants and wastewater treatment [6, 9-12]. Major therapeutic applications include drug delivery, magnetic hyperthermia, magnetic resonance imaging (MRI) etc. which make these nanoparticles ideal candidates in biomedical field [13-17]. Reportedly, biologically synthesized IONPs are more superior to the chemical counterparts owing to their extremely small size and being biocompatible [18].

Biosynthesis of IONPs can be carried out either using microbes or plants. The only constraint in their large scale synthesis involving microbes especially magnetotactic bacteria, is due to the exhaustively challenging culturing conditions as they are fastidious in their growth behavior [19]. Some of the strains could be pathogenic and may involve a risk associated with handling such cultures [20]. They also need regular maintenance under aseptic conditions therefore requiring trained personnel. Further, controlled parameters such as temperature, pH and/or other factors may be required for their optimum cellular growth. All these necessities contribute to additional scaling up cost and is time consuming [20-22].

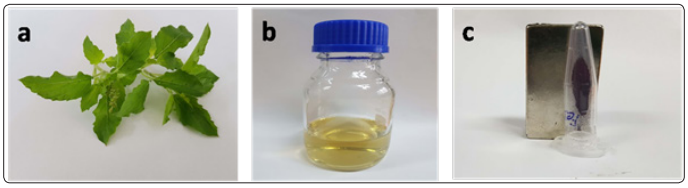
The alternate green route synthesis method involving plant tissue or plant extract is advantageous in being easy and simple, low on cost, uses safe, ecofriendly raw materials and is pollution free. The entire process can be designed to be reproducible, sustainable and scalable [23-27]. Moreover, plant extracts can be obtained readily and the synthesis can be directed towards production of desirable nanoparticles in bulk quantities [5, 28-30].

However, studies on IONPs synthesis using green route method are limited and currently only a few reports are available. Therefore, the present study was aimed at synthesizing IONPs using plant biomaterial. *Ocimum tenuiflorum* (Tulsi) synonymically known as *Ocimum sanctum* belongs to the family *Lamiaceae*. This holy basil is known for its medicinal and therapeutic properties since ancient times. It is being consumed in the form of herbal tea and used in a variety of food products, ayurvedic medicines, perfumes etc. [31, 32]. Here, we report the synthesis of IONPs using the leaf extract of *Ocimum tenuiflorum* (Ram Tulsi variety) which acts as a reducing and capping agent.

## Materials and Methods

### Preparation and synthesis of IONPs using *Ocimum tenuiflorum* leaf extract

Ferric chloride hexahydrate ($FeCl_3.6H_2O$), ferrous chloride tetrahydrate ($FeCl_2.4H_2O$) and sodium hydroxide (NaOH) of analytical grade (Hi Media) were used for the study. All solutions were prepared using sterile deionised water (DW). Fresh *Ocimum tenuiflorum* leaves were procured from a local garden (Figure 1a). The leaves were washed with running tap water to remove any adhering dust particles and rinsed with distilled water. Approximately 10 g (wet weight) of leaves were suspended in 200 ml of sterile DW and boiled for 10 min. After boiling, the colour of the aqueous solution changed from colourless to yellow. The resulting leaf extract was allowed to cool at room temperature and decanted. The aqueous extract was filtered using Whatman No. 1 filter paper and centrifuged at 5000 rpm for 10 min to sediment any residual particulate plant material. This aqueous supernatant of *Ocimum tenuiflorum* leaf extract was used as the source of reducing and capping agent for the synthesis of IONPs (Figure 1b). For the synthesis of IONPs, 1 mole of $FeCl_2.4H_2O$ and 2 moles of $FeCl_3.6H_2O$ were dissolved in 100 ml sterile DW and heated at 80 °C under constant stirring conditions. To this solution, 5 ml aqueous leaf extract of *Ocimum tenuiflorum* was added followed by dropwise addition of NaOH solution [33, 34]. Continuous stirring was carried out during the entire synthesis process to ensure uniform mixing. The solution was found to immediately form reddish brown magnetic IONPs that could be easily separated using a magnet. On cooling, the solution was subjected to centrifugation at 5000 rpm for 10 min. The supernatant was decanted and the pellet containing synthesized IONPs was washed three times with sterile DW and dried overnight at 80 °C. These magnetic nanoparticles (Figure 1c) were then used for the characterization studies.




**Fugure 1:** (a) Fresh leaves of *Ocimum tenuiflorum*, (b) Aqueous leaf extract of *Ocimum tenuiflorum* and (c) Magnetic IONPs synthesized using *Ocimum tenuiflorum* leaf extract.

### Characterization of the synthesized IONPs

The UV-Visible absorption spectrum of the synthesized IONPs was measured from a wavelength range of 200 to 800 nm using Shimadzu spectrophotometer (UV-1800, Japan). Fourier Transform Infra Red (FTIR) spectrum of the synthesized IONPs was obtained over a scan range from 4000 to 450 $cm^{-1}$ using the FTIR instrument (8201 PC, Shimadzu, Japan). The X-Ray diffraction pattern was determined using X-Ray Diffractometer (Rigaku, Miniflex II, Japan) for phase identification at values of $2\theta = 20°$ to 80°. Microstructural and morphological studies of the synthesized IONPs were carried out using Transmission Electron Microscope (TEM) (Phillips CM200). The synthesized IONPs were also studied with respect to their capacity to degrade two commonly used dyes, Malachite green (MG) and Bromothymol blue (BTB). The experiment was carried out in triplicate at room temperature under shaking conditions at 150 rpm. The IONPs (10 mg) were incubated with 8 ml of the respective dye solution and the absorbance was recorded after every 1 h interval for 6 h. MG was used at a concentration of 200 mg/L and BTB at 50 mg/L and the OD was determined at 617 nm and 431 nm, respectively. The dye degradation efficiency was then calculated using the formula,

$$R\ (\%) = [(C_0 - C_t) / C_0] \times 100$$

Where, R (%) represented the percentage of dye degradation efficiency (removal efficiency), $C_0$ and $C_t$ (mg/L) represented the dye concentration at initial and at time't' respectively [35].

## Results and Discussion

The synthesis of reddish brown IONPs occurred within a few minutes and was indicated by a visible colour change due to the surface plasmon resonance phenomenon characteristic of IONPs. This was confirmed spectrophotometrically using UV-Visible and FTIR spectroscopy. A prominent peak due to magnetite formation was observed at 282 nm (Figure 2). Synthesis of magnetite nanoparticles that absorb at wavelengths corresponding to 294 nm and 233 nm has been reported [27, 33]. The minor differences in the absorption maxima of the obtained wavelengths reported by various authors have been suggested to be due to the binding of the different biomolecules from the leaf extract to the surface of the nanoparticles, their aggregation state and valency [36, 37]. Presence of another broad spectrum peak was visible at 428 nm. These two peaks could be attributed to two different phases of synthesized IONPs. The broadness of the peaks may be associated to different sizes as well as agglomeration of the nanoparticles [36].

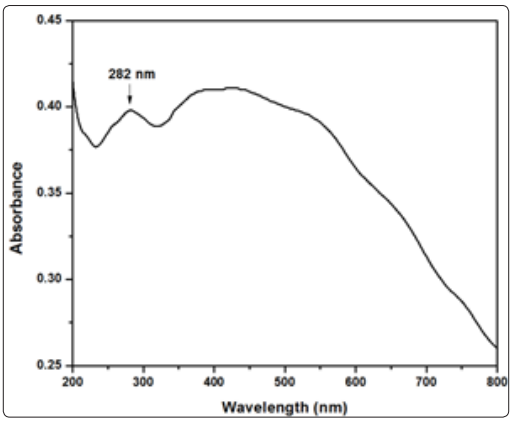



**Figure 2:** UV-Visible spectrum of the synthesized IONPs using *Ocimum tenuiflorum* leaf extract.

Results of FTIR analysis revealed formation of IONPs using *Ocimum tenuiflorum* leaf extract (Figure 3). The FTIR spectra of the as-synthesized IONPs showed the presence of two absorption peaks at 610 and 438 $cm^{-1}$. Vibrations of Fe-O bond indicating formation of IONPs at around 618 and 625 $cm^{-1}$ have been demonstrated in earlier reports [26, 27]. It has been suggested that the organic moities present on the surface of IONPs have a strong influence on the FTIR signals [38]. When the as-prepared IONPs powder was dried further at 400 °C and used for the analysis, there was a shift in the peak wavelength. In this case, two prominent absorption bands were observed at 542 and 461 $cm^{-1}$ signifying Fe-O bond stretching. Presence of characteristic peaks at wavelengths between 400 to 600 $cm^{-1}$ has been reported to confirm the existence of magnetite in the synthesized IONPs powder [39]. The other peaks could be assigned to the O-H stretching and bending vibrations of the water molecules, C-H stretching, C=C aromatic bonds, N-H stretching and bending vibrations of $NH_2$ groups. These groups could be possibly associated with organic moieties present in *Ocimum tenuiflorum* leaf extract and responsible for the synthesis and stabilization of IONPs [26]. Therefore, this data confirmed the formation of IONPs using a bioreduction method involving *Ocimum tenuiflorum* leaf extract.

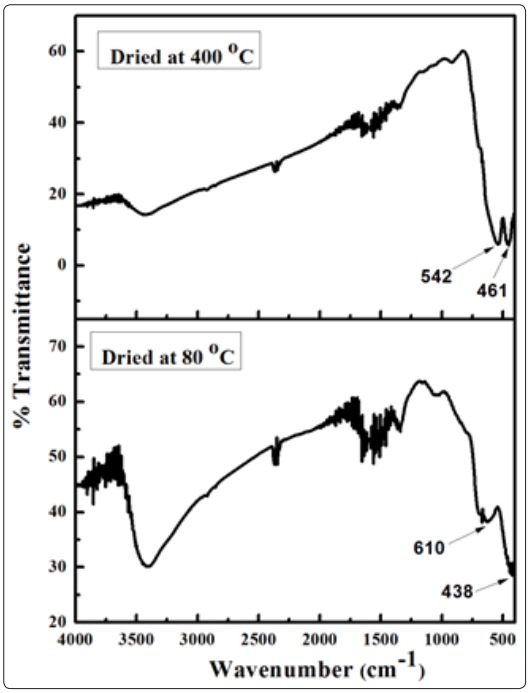



**Figure 3:** FTIR spectra of synthesized IONPs using *Ocimum tenuiflorum* leaf extract dried at 80 °C and 400 °C.

XRD data was used to confirm the crystal structure of the synthesized IONPs dried at 80 °C and 400 °C. The XRD peaks of the IONPs powder dried at 400°C revealed a clear phase transition when compared to that dried at 80°C (Figure 4a). The diffraction peaks matched the standard $Fe_3O_4$ peaks (JCPDS card No: 89-0691) and were detected at 2θ values of 35.641°, 54.168° and 62.485°. These peaks were assigned to the lattice planes (311), (422) and (440), respectively [14]. The other peaks at 2θ value 24.045°, 33.122°, 40.8°, 49.503° and 64.125° were attributed to the lattice planes (012), (104), (113), (024) and (300), respectively of α-$Fe_2O_3$ phase (Figure 4b). Similar 2θ values have been reported by other authors for the diffraction peaks of α-$Fe_2O_3$ synthesized using green routes indicating crystalline nature of these nanoparticles [28, 40].

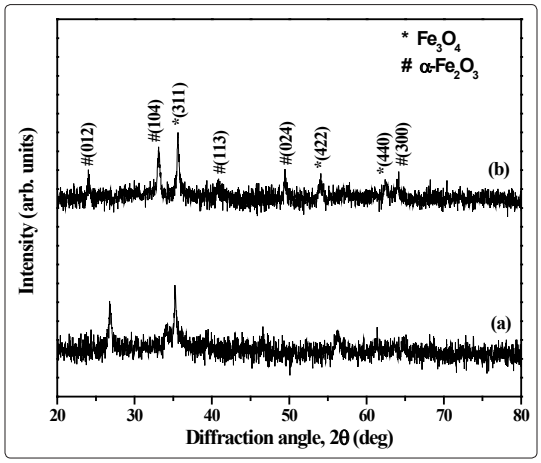



**Figure 4:** XRD patterns for synthesized IONPs using *Ocimum tenuiflorum* leaf extract dried at (a) 80 °C and (b) 400 °C

TEM micrographs of synthesized IONPs indicated presence of nanospheres as well as nanoellipsoidal particles (Figure 5). The nanospheres were found to be in the range of 3-5 nm and were mostly agglomerated. The tendency of these particles to agglomerate could be attributed to the magnetic property of IONPs as well as the relatively large surface area to volume ratio leading to high surface energy [20, 41]. The surface energy is reported to be minimized when IONPs form aggregates [41]. The nanoellipsoids varied in size and ranged between 100-200 nm in length and 20-30 nm in breadth. These bigger particles could be due to the formation of α-$Fe_2O_3$. Reports on the synthesis of α-$Fe_2O_3$ have revealed presence of ellipsoidal geometry of these particles [42].

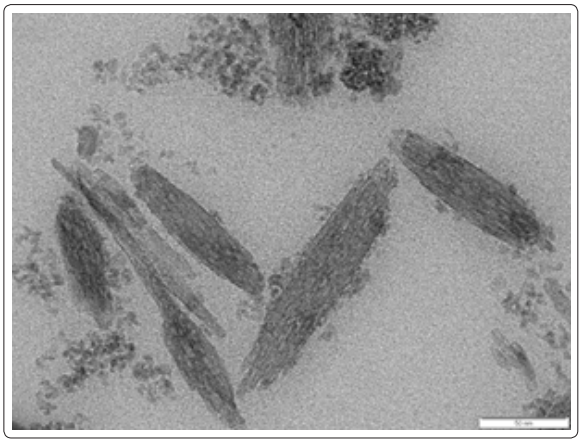


**Figure 5:** Transmission Electron Micrograph (TEM) of the synthesized IONPs.

It was observed that the removal efficiency of Malachite green (MG) used at an initial concentration of 200 mg/L was 77.42% at 2 h of incubation with IONPs (Figure 6). The dye degradation capacity using IONPs synthesized from oolong tea extract has been reported previously [43]. The data revealed 75.5% removal of MG in 1 h of incubation period when used at an initial concentration of 50 mg/L of the dye [43]. The second dye, Bromothymol blue (BTB) was tested for its removal efficiency at an initial concentration of 50 mg/L. The removal efficiency was estimated to be 64.19 % at 2 h of incubation with IONPs.

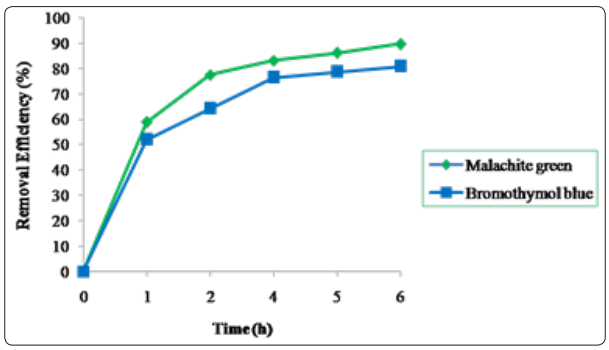



**Figure 6:** Percentage removal efficiency of the dyes, Malachite green (200 mg/L) and Bromothymol blue (50 mg/L) respectively, using synthesized IONPs

Dye removal efficiency of BTB has been studied using IONPs synthesized from Sorghum bran extract, green tea extract (*Camellia sinensis*) and Tie Guanyin Tea extract [25, 44, 45]. IONPs synthesized by the respective green routes when used as a catalyst, enhanced the reaction rate, however, the assays incorporated $H_2O_2$ as the free radical oxidant [25, 44, 45]. In the present study, the IONPs synthesized using *Ocimum tenuiflorum* leaf extract were able to efficiently degrade BTB without the requirement of $H_2O_2$, thereby eliminating the need for introducing another contaminant in the degradation process. Moreover, since the synthesized IONPs are magnetic in nature, this nanocatalyst can be easily retrieved and recycled making the entire process cost effective. Therefore it is envisaged that these nanopaticles possess vast potential for applications in textile, leather, food and cosmetic industries which generate large amount of wastes containing dyes that pose a major disposal challenge and a big environmental threat [46].

## Conclusions

This is the first report on the biosynthesis of IONPs from leaf extract of *Ocimum tenuiflorum* (Ram Tulsi variety). In this study an attempt was made to synthesize magnetic IONPs using leaf extract of *Ocimum tenuiflorum* as a reductant and capping agent. These magnetic nanoparticles are likely to find a wide range of potential applications, as such IONPs are approved for medical and food applications by the Food and Drug administration. The synthesized IONPs in this study revealed the presence of α-$Fe_2O_3$ along with magnetite which could be used for an array of commercial applications after further characterization and optimization of synthesis process for large scale production of these nanoparticles. Overall, it can be inferred that this green route synthesis method is not only cost effective and easy but also has the potential towards scaling up for different biomedical as well as environmental applications.

## Conflict of Interests

The authors declare no conflict of interests.

## Acknowledgements

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