Production and characterization of Green Iron Oxide nanoparticles with antifungal properties against fungal pathogens

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ABSTRACT

In this study, iron oxide nanoparticles were synthesized through the green chemistry method. It involved the use of plant extract for synthesis of metal oxide nanomaterials. Aqueous extract of Tridax procumbens was employed for the production of iron oxide nanoparticles. T. procumbens is a weed and it has good medicinal properties. The green chemistry approach provides a simple, cost-effective and eco-innovative method to the synthesis of nanomaterials as compared to the other alternative methods. Phase, crystal, surface chemistry, size, shape and element composition of nanoparticles were assessed and determined by various techniques like Fourier transform infrared spectroscopy (FTIR), X-Ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy dispersion X-Ray (EDX). In addition, the antifungal properties of the biosynthesized nanoparticles were investigated against plant fungal pathogens. Spherical-shaped nanoparticles with a size of 26 ± 5 nm were obtained using the extract of T. procumbens. Bio-molecules from T. procumbens served as reducing, capping and stabilizing agents for the fabrication of nanoparticles. The highest zone of inhibition was obtained in S. rolfsii (16.5 ± 0.5) at 50 µg/ml of concentration of TFeONPs. Biosynthesized TFeONPs were capable of inhibiting the growth of fungal pathogens such as S. rolfsii and F. oxysporum and they might be employed as antifungal agents in agriculture to control fungal infections.

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INTRODUCTION

Nanotechnology is a growing innovative technology for manipulation, creation and application of nanomaterials. It has the combination of new ideas and knowledge from physics, material science, chemistry and biological sciences, has been applied in various research areas such as biomedical products, catalysis, energy, photocatalysis, electrochemical products, optics etc. (Song and Kim, 2009; Rajasekharreddy and Rani, 2014). Nanomaterials have been produced by different types of traditional methods such as physical and chemical science. Not with standing the fact that these production methods are innovative, they have some drawbacks like high usage of hazardous chemicals and high electric power in addition to controlling the formation of aggregation and crystal growth is very difficult in these methods (Izadiyan et al., 2018). Green approach for synthesis of metal oxide and metallic composite is an inexpensive, non-toxic and environment-friendly technique.
Several plants and their extracts have been employed for the production of metal oxide nanomaterials. Secondary metabolites from plant extracts play an important role during the formation of nanomaterials by serving as capping, reducing and stabilizing agents (Rajiv et al., 2013; Prasad, 2014).

In the recent years, iron oxide nanoparticles have attracted the attention of researchers because of their interesting properties such as high stability, catalytic activity, magnetic and electrochemical power, crystal properties, photo-catalysis, thermal conductivity etc. Jagathesan and Rajiv (2018).

Several plants namely Artemisia vulgaris (Wang et al., 2014), Eichhonia crassipes (Jagathesan and Rajiv, 2018), Syzygium cumini (Venkateswarlu et al., 2014), Jatropha gossypifolia (Karkuzhali, 2015), Lagernaria siceraria (Kanagasubbulakshmi and Kadirvelu, 2017), Couroupita guianensis (Sathishkumar et al., 2018), Ficus carica (Demirezen, 2019), Albizia lebbeck (Bharadwaj et al., 2016), Phyllanthus niruri (Kumar and Prem, 2018), Lantana camara (Rajiv et al., 2017), Platanus orientalis (Devi et al., 2019) have been employed for the green synthesis of iron oxide nanoparticles. Devi et al. (2019) reported the production and characterization of iron oxide nanoparticles by green chemistry approach and concluded that iron oxide nanoparticles show excellent antifungal properties against fungal pathogens (Aspergillus niger and Mucor piriformis).

Sclerotinum rolfsii and Fusarium oxysporum are major plant pathogens for oil seed crops. Groundnut (Arachis hypogaea), a major oil crop, is mostly infected by S. rolfsii that causes diseases like sclerotic wilt, rott in root, pod and stem (Chohan, 1974; Mehan et al., 1995; TH et al., 2016). F. oxysporum is a plant fungal pathogen whose habitat is soil and is known to cause Fusarium wilt in A. hypogaea and other oil seed crops (Rajeswari, 2014).

The aquatic weed (E. crassipes) has been utilized in the synthesis of iron oxide nanoparticles and also to assess the antibacterial activity against water-borne pathogens (Jagathesan and Rajiv, 2018). 

The aim is to explore the synthesis and characterization of iron oxide nanoparticles with its
antifungal potent using the extract of weed (*T. procumbens*) serving capping, reducing and stabilizing agent at suitable ambience.

**MATERIALS AND METHODS**

*T. procumbens* was acquired from the lands in and around Karpagam Academy of Higher Education, Coimbatore, India (Figure 1). It was then authenticated by Botanical Survey of India, Coimbatore. 99% pure reagents, chemicals and solvents were obtained from sigma-aldrich chemicals, India.

Plant pathogens namely, *S. rolfsii* and *F. oxysporum* were collected from the Department of Plant Pathology, Tamil Nadu Agriculture University, Coimbatore, India.

**Production and characterization of TFeONPs**
Ten grams of fresh and healthy whole plant (T. procumbens) was taken and washed in tap water, and was further rinsed with de-ionized water. Plant samples were mashed into fine powder with the help of mortar and pestle using de-ionized water. 100 mL of crude extract was kept in water bath under 80°C for 30 min. Next, the crude extract was filtered, and the filtrate was kept at 4°C in store for use in the production of NPs.

FeCl₃ was used as precursor materials for synthesis of TFeONPs. A 1:1 (v/v) of 0.1 M of FeCl₃ and 50 mL of plant extract was taken in a sterile conical flask and kept in water bath for 30 min at 80°C. Then, 20 mL of 0.2 M NaOH was mixed to the reaction mixture under the stirring condition of 60°C for 30 min. Finally, the end product (black colour) precipitate was obtained.

The pellets were collected with the help of centrifugation method and washed with 80% ethanol followed by de-ionized water. The precipitate was then dried in hot air oven after which the fine powder was collected and stored in clean bottles for further analysis (Rajiv et al., 2017; Jagathesan and Rajiv, 2018).

The properties of synthesized TFeONPs such as...
crystalline nature, surface chemistry, size, shape, purity, elemental compositions and stability were analyzed by XRD, FT-IR, SEM, EDX and Zeta potential analyzer.

**Assessment of antifungal properties**

Antifungal properties of the synthesized TFeONPs were assessed using soil-borne fungal pathogens following well diffusion method (Bauer et al., 1966). The pathogenic fungi were grown in potato dextrose broth. Potato Dextrose Agar (PDA) was prepared and 200 µL of broth culture was spread on it. After 10 min, wells of 5 mm were made on agar and Nanosolution of TFeONPs (25 and 50 µg/mL) was added.

Fluconazole was used as a positive control and a concentration of 10 µg/mL was employed. Plant extract was used as negative control. After the incubation of three days at 30°C, the zone of inhibition was measured, and the results are presented as mean with standard error.

**RESULTS AND DISCUSSION**

**Analysis of crystalline nature**

TFeONPs were synthesized using *T. Procumbens* extract and were proved by XRD analysis (Figure 2). The lattice planes of (220), (311) and (411) were obtained at 2θ: 30.47°, 35.75° and 43.51° respectively. The spectra were compared to JCPDS file (01-088-0315), which clearly revealed the formation of spinel single-phase with a cubic structure. The characteristic peaks show that the produced TFeONPs were crystalline in nature. The mean size of particles was deliberate by Debye-Scherer’s formula. Mean size of TFeONPs was found to be about 26 nm. Several earlier reports showed similar XRD pattern for green synthesized nanoparticles (Yew et al., 2016; Jagathesan and Rajiv, 2018).

**Analysis of surface chemistry**

FT-IR spectra of the as-synthesized nanoparticles and plant extract are given in Figure 3 A & B. It confirms that the functional groups are present on the surface of nanoparticles and are involved in the formation of nanoparticles. The IR peaks observed at 3448, 1647, 1639, 1566 and 1381 cm⁻¹ are referring to the N-H bending, C=O stretching of amine and amide groups respectively. The peaks of 592 and 848 cm⁻¹ refer to metal oxide group. This confirmed that the bioactive compounds from plant extracts were actively involved in the formation of TFeONPs. The FT-IR results are comparable to those determined in the previous investigations (Kanagasubbulakshmi and Kadirvelu, 2017; Kumar and Prem, 2018).

**Morphology analysis**

The surface morphology of TFeONPs is shown in Figure 4. SEM images showed mono-dispersed distribution with spherical shape with less aggregation. The aggregation may be due to a large surface area and a tendency to stick. Our result is in agreement with Karkuzhali (2015), where they produced spherical-shaped nanoparticles using Jatropha plants which was confirmed by SEM analysis.

**Elemental analysis**

Figure 5 determines the presence of various elements like iron and oxygen in as-synthesized nanoparticles. The EDX spectra determined the occurrence of iron (46.18%) and oxide (27.11%) and 26.71% of other elements were measured from the EDX analysis. Badni et al. (2016) performed the production of iron oxide nanoparticles using Roman nettle, and investigated the analysis of elemental compositions by EDX analysis, where they concluded that nanoparticles have 61.2% of iron, 17.2% of oxygen and 21.6% of other elements.

**Zeta potential analysis**

Dynamic light-scattering has been utilized to find out the surface zeta potential of the as-synthesized nanoparticles. It is used to determine the stability and surface charge of the nanomaterials. According to Figure 6, the zeta potential for TFeONPs was -27.9 mV. In addition, the TFeONPs was negatively charged on their surface. The values of zeta potential between -20 to -30 mV indicated that particles were moderately stable (Ardani et al., 2017). Hence, the result clearly reveals that as-biosynthesized TFeONPs were moderately stable in nature.

**Analysis of antifungal properties**

Figure 7 and Figure 8 represent the antifungal activity of as-synthesized TFeONPs. Plant extracts did not show any antifungal activity against the used plant fungal pathogens. The highest zone was obtained in *S. rolfsii*(16.5 ± 0.5 mm) at 50 µg/mL concentration of TFeONPs, which was compared to standard antibiotic. *F. oxysporum* shows less zone of inhibition against the TFeONPs. The inhibition of fungal growth occurred due the destruction of cell membranes inducing the reactive oxygen, which caused cell death. This analysis proves the antifungal properties of TFeONPs.

**CONCLUSION**

Green synthesis of nanoparticles using plants is considered a biological, non-hazardous and eco-friendly method. The present investigation, which is a first attempt was used to determine a green
chemistry technique for yield of iron oxide nanoparticles with antifungal properties by means of weed plant. The synthesized TFeONPs were spherical-shaped with size of 26 ± 5 nm. TFeONPs have an extremely potent antifungal properties, which inhibit the growth of plant fungal pathogens and can be utilized for the management of *F. oxysporum* and *S.*

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