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Effect of Green synthesized iron nanoparticles by *Azardirachta Indica* in different proportions on antibacterial activity

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ABSTRACT

Green synthesis of iron nanoparticles has accumulated an ultimate interest over the last few years to their distinctive properties, applicable in various fields of science and technology. In the present study, synthesis of iron nanoparticles using *Azadirachta Indica* leaf extract is performed. Different proportions of precursor (ferrous sulphate) to leaf extract were prepared by varying the volume quantity of leaf extract from 1:1 to 1:5. Their morphology, structure and size distribution is confirmed by Scanning Electron Microscope along with X-ray energy dispersive spectroscopy and Fourier Transform Infrared spectroscopy analysis. Presence of accountable polyphenols such as total phenolic content by Folin-Ciocalteau(FC) method (20 mg per gram of leaf extract) and gallic *acid(*0.280 mg/g)*, caffeic* acid(0.278 mg/g) & *catechin*(0.532 mg/g) for synthesis conciliation and were quantified by High Performance Liquid Chromatography. Its performance is evaluated on treatment of petrochemical refinery waste water to assess Chemical Oxygen Demand(COD) and nitrate removal for different proportions. The performance of COD removal (77%) and nitrate removal(74%) for 1:5 on 5th day is observed to be efficient. To further study this effect, size of FeNPs formed and concentration of polyphenols were taken into consideration. Antibacterial activity of synthesized iron nanoparticles from AI leaf extract on bacteria (*Escherichia Coli*, *Pseudomonas Aeruginosa*, and *Staphylococcus Aureus*) is accomplished by well diffusion method. Size of FeNPs achieved for 1:2 ratio was 98–200 nm and for all proportions varying between 120 and 600 nm due to agglomeration enhanced bacterial decay. It was portrayed that there was an increase in the inhibition zone as the proportions increased from 1:2 to 1:5. Hence for the contact time of 48 h, 1:5 found to be effective in inhibiting more number of bacterial cells compared to other proportions.

1. Introduction

Eco-friendly solutions are gaining popularity in this contemporary world. The end product should be environmental friendly and the process must be sustainable are the key things that are anticipated. Ability of Iron nanoparticles (FeNPs) in treating water is demonstrated since 1990s due to its interesting behaviour like presence of high reactive sites, adsorption phenomenon and availability of more surface area. Synthesis of FeNPs by various physical (Baskoutas et al., 2008; Tosco et al., 2014; Bang and Sunlick, 2007) and chemical (Kassaee et al., 2011; Zhang 2003) approaches end up with several limitations interms of high energy, temperature and pressure requirement, discharge of toxic by-products and also expensive. In order to address the limitations, green approach of FeNPs (Kumar et al., 2013; Huang et al., 2014; Machado et al., 2013) came into existence recently to restrain the ecosystem with available naturally biodegradable matter for its production. The degradation of any organic compounds by green approach (plant extracts) is mainly due to the presence of polyphenols in the biodegradable material used for treatment purpose. Enormous research is carried out on wide range of plant extracts and its chemical constituents.

Meticulously, analysis of chemical constituents in *Azardirachta Indica* (AI) is carried out by Hossain et al. (2013). Many bioactive components were isolated and identified from AI extracts such as *azadirachtin, salannin, meliantriaol* and *nimbin*. Also the other tri and tetra cyclic compounds were isolated. It was evident from the literature that polyphenols in AI was acting as good antifungal agent (Mahmoud et al., 2011), antioxidant capacity (Prieto et al., 1999; Hossain et al., 2013) and antimicrobial activity (Alves et al., 2009). Literature clearly states that polyphenols present in AI leaves is quantified in the context of pharmacological aspect but not in the circumstance of waste water treatment. It is well known fact that High Performance Liquid Chromatography (HPLC) is used for quantification of polyphenols. Nour et al. (2012) presented HPLC-DAD analysis to enumerate polyphenols

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like gallic acid, caffeic acid, salicylic acid and also for flavonoids in walnut leaves. Analogous to this, Wang et al. (2000) and Zuo et al. (2002) accomplished for tea catechins, caffeine and gallic acid which are the preferred polyphenols applicable to contaminant remediation. With the mentioned background, gallic acid, caffeic acid and catechin is favored to analyze in orientation with waste water treatment in the present study. Though presence of polyphenols is accountable to act as capping agent but still question of microbial contamination arise because the base material involved is biodegradable.

Interaction of nanoparticles with the biodegradable material results in new nanomaterial which will possess specific properties under control viz. size, surface chemistry, roughness (Singh et al., 2011). These inorganic compounds such as metal oxides have high affinity towards antimicrobial agent. Mechanism between iron oxide nanoparticle-bacteria interface is explored by Arakha et al. (2015) for studying antimicrobial activity. The produced iron nanoparticle will hold positive surface potential to have better surface for bacterial attachment thereby shoots up the interaction at the interface which in turn enhance production of reactive oxygen species (ROS) in the culture media.

Similar other studies by Lee et al. (2008) and Sneha et al. (2014) studied the antibacterial activity of zero-valent iron nanoparticles (nano-Fe^0) and silver nanoparticles on *E. coli* and *P. Aeruginosa*. Jones et al. (2008) illustrated the effect of nanoparticles of ZnO on *Staphylococcus Aureus* and showed that it holds significant antibacterial activity compared to other metal oxide (MgO, TiO_2, CuO,Al_2O_3) nanoparticles. Green synthesis of ZnO nanoparticles utilizing the biocomponents of powder extract of dry ginger rhizome. Proficiency of antimicrobial activity by ZnO nanoparticles was demonstrated against pathogenic organisms like *Staphylococcus Aureus*, *Penicillium Notatum* (Janaki et al., 2015). Still wide research is in progress to investigate the antimicrobial activities of other leaf extracts and its practical application in the field of drug delivery, water treatment & its purification and contaminant remediation.

Till date, very limited studies were available for contaminant remediation in the area of waste water treatment applying FeNPs. Recently, Devatha et al. (2016) demonstrated the applicability of green synthesis of iron nanoparticles using different leaf extracts (*Mangifera Indica, Murraya Koenigii, Azardirachta Indica and Magnolia Champaca)* for domestic wastewater treatment. Similar studies were reported by Luo et al. (2015) for dye removal using grape leaf extract and Wang et al. (2014a, 2014b) for treating swine wastewater using eucalyptus leaf extract. Literature discloses that application of green synthesis of FeNPs in various fields is gaining more importance in last few years due to the benefit of environmental friendliness and cost effective approach. Consequently, an attempt is made to study by green approach keeping in view to vary only the leaf extract proportion but not the nanoparticle volume.

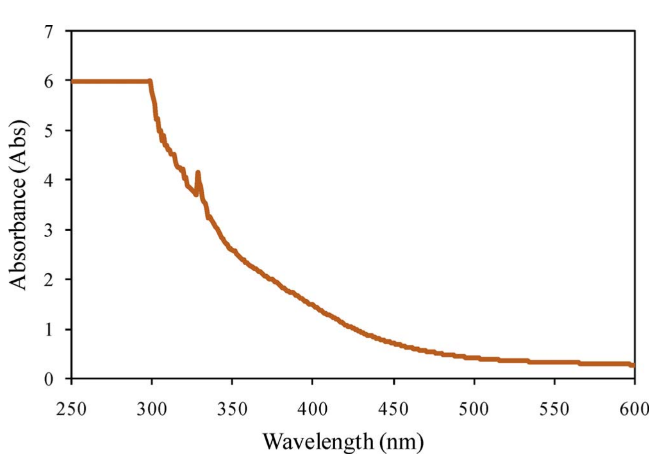

Fig. 1. UV spectrum for *Azadiracta indica* Iron Nanoparticles (AI-FeNPs).

Therefore the aim of the present study is worked out as following 1. Green synthesis of FeNPs from AI leaves in different proportions(1:2 to 1:5, Precursor: Leaf extract) and its effect is studied based on size of AI-FeNPs formed and concentration of polyhenols. 2. Quantitative estimation of polyphenols like total phenolic content, gallic acid, caffeic acid and flavonoids like catechin and is selected based on the preferred polyphenols which persuade antimicrobial activity and also due to its capping performance. and 3. Applicability of synthesized AI-FeNPs on petroleum refinery waste water (PRW).4. Antibacterial activity of synthesized iron nanoparticles for all mentioned ratios was carried out against *Escherichia Coli* (*E. Coli:* ATCC-25922), *Pseudomonas Aeruginosa* (*P. Aeruginosa:* ATCC − 27853), and *Staphylococcus Aureus* (*S. Aureus:* ATCC − 29213). AI leaves were opted in the present study due to presence of polyphenols and its abundant availability in India.

2. Experimental work

2.1. Materials and methods

2.1.1. Preparation of leaf extract

AI leaves were thoroughly washed with deionised water to remove dust particles and sun dried to remove moisture content and these leaves were cut into small pieces and later grinded to make it into powder form. Further, 15 mg of this powder was mixed with 250 ml of water in a 500 ml flask and kept for water bath at constant temperature of 80 ° Celsius. Later this was vacuum filtered through whatman no 2 filter paper (Wang et al., 2014a, 2014b).

2.1.2. Preparation of precursor

Ferrous sulphate heptahydrate was used as precursor for synthesizing iron nanoparticles solution. $FeSO_4.7H_2O$ of concentration 0.1 M was prepared using de-ionized water for the experiments.

2.2. Synthesis of FeNPs

In order to synthesis the nanoparticles leaf extract was mixed with metal salt of 0.1 M FeSO4. In the ratio of 1:5 (Example- 10 ml of leaf extract with 2 ml of metal salt solution), 1:4, 1:3, 1:2 and 1:1 separately. It was kept in refrigerator for further use.

2.3. Characterization of FeNPs

Characterization of prepared AI-FeNPs was done by UV Visible spectrophotometer to observe the peak wavelength, SEM-EDS (Scanning electron microscope equipped with X-ray Energy Dispersive Spectrometer EDS) to study the morphology and Fourier Transform Infrared Spectroscopy (FTIR-400S SHIMADZU) to identify the responsible functional groups.

2.4. Gallic acid, caffeic acid, and catechin estimation using high performance liquid chromatography (HPLC)

Quantitative analysis of the gallic acid, Caffeic acid and catechin present in aqueous extracts of AI leaves is performed by HPLC − DAD with an Agilent 1100 Series HPLC system with diode array detector and injection valve with 20-μL sample loop. Compounds were separated on a 4.6 mm × 250 mm, i.e., 5-μm pore size Zorbax SB RP-C18 column protected by a guard column containing the same packing. The mobile phase was HPLC-grade acetonitrile (component A) and water (component B). The flow rate maintained was 1.0 ml min^{-1}. Sample volume injected was 10 μl. Methodology is developed to estimate the determination of mentioned polyphenols.

2.5. Total phenolic content

Phenolics include simple phenols, phenolic acids (benzoic and