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Biogenic synthesis of iron nanoparticles using *Amaranthus dubius* leaf extract as a reducing agent

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ABSTRACT

The biogenic synthesis of nanosize powder is of great interest due to its multifunctional applications and easy and eco-friendly process. The biosynthesis of nanosize iron particles using *Amaranthus dubius* leaf aqueous extracts that are reduced from ferric chloride was investigated. *A. dubius* aqueous leaf extraction process parameters were optimized based on the antioxidant capacity for attaining efficient extract. The leaf extract contains a rich source of amaranthine and phenolic compounds that are used as reducing agents. The effects of different process parameters like pH, temperature, concentration of leaf extract and time on the nanoparticle synthesis were examined. The synthesised nanoparticle size, morphology, crystallinity, composition, and stability were investigated. Results showed that *A. dubius* extract mediated nanoparticles are in spherical shape with a cubic phase structure and a diameter range from 43 to 220 nm with less aggregation. *A. dubius* and sodium borohydride mediated iron nanoparticles for decolouration efficiency against methylene orange and antioxidant against 1, 1-diphenyl-2-picrylhydrazyl were studied.

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1. Introduction

The effective and efficient synthesis of nanoparticles (Nps) with properties like high monodispersity and biocompatibility is needed for a wide range of application from environment to biomedics [1]. Various techniques applied to the synthesis of metallic Nps like chemical [2,3], physical [4,5], and laser ablation [6], electric arc discharge [7] and microbial methods [8] were already reported by many investigators. These methods require expensive instruments, high-energy [9], release of inimical chemicals [10,11], and culture of cell [12] and wasteful purifications [13]. The biogenic synthesis of Nps has been considered as a rapid, clean, nontoxic, cost-effective and environmental friendly compared to other conventional techniques [10,14,15]. Several investigations reported that constituents of various herbs, spices and plants have high levels of powerful antioxidant compounds such as polyphenols, reducing sugars, nitrogenous bases, and amino acids [16]. These compounds are used as reducing [17] and capping agents for the synthesis of Nps [16,18]. The plant extracts used for Np synthesis could be advantageous by eliminating the elaborate process like maintaining cell cultures [17, 19], recovery steps, lesser reaction time [12] and it does not produce any hazardous waste [20]. Praveen et al. reported that plant leaf extract implicated Nps can be suitably scaled up for large scale production; besides, the process can be economically viable [21]. The plant extract-mediated metal and metal oxide Nps are quite stable and no visible changes were observed even after a month [19]. The biogenic synthesis of Nps using various plant extracts like *Dodonaea viscosa* [22], *Camellia sinensis* [23], *Sargassum muticum* [24], *Ipomea carnea* [25], *Mukia maderaspatana* [26] *Mangifera indica*, *Syzygium aromaticum*, *Rosa indica*, *Murraya koenigii* and *Azadirachta indica* [27] were investigated. Bashir et al. synthesised Fe_2O_3 Nps using *C. sinensis* plant extract and tested its catalytic performance by decolourization of aqueous dye solution [23,28]. Albergaria et al. utilized 26 different tree leaf extractions for the production of zero-valent iron Nps and tested an antioxidant capacity for dried leaf extract [29].

Iron nanoparticles (FeNps) have unique characteristics like catalytic activity [28] and optical [29], electronic [30] and antibacterial properties [31]. Thus, it has a wide range of applications, including cosmetics [32], biomedicine [33], bioremediation [34], clinical [35] materials and engineering [36]. Due to its enhanced properties and multifunctional application it highly encourages us to choose FeNp synthesis. The conventional synthesis of FeNps used a variety of organic solvents and reducing agents like sodium borohydride ($NaBH_4$) [27], hydrazine, sodium dodecyl sulphate, etc [22,37]. These reducing agents pose great risks to the environment; it also creates harmful by-products to human health [37, 38]. In spite of this difficulty, high amounts of organic compounds in the plant extracts are used for the reduction process; it stabilizes Nps by preventing agglomeration [39,40] and Nps produced from biogenic synthesis compared to the traditional methods that demonstrate less toxicity [17,28]. The problems associated with conventional methods initiate us to synthesise biocompatible FeNps using leaf extracts.

The aqueous extract of *Amaranthus* species leaves was used for synthesising Nps, because the species has many remedial properties that are cheap and easily available [41]. *Amaranthus dubius* leaf extract

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contains various photochemicals like amaranthine, isoamaranthine, phenols, flavonoids, and lysine that are used as reducing agents [42,43]. The economical and eco-friendly leaf extract from *A. dubius* is an alternative reducing agent for synthesising FeNps.

In this study, biosynthesis of nanosized iron particles reduced from $FeCl_3$ using *A. dubius* leaves aqueous extract was investigated. The extraction process parameters like temperature, time and mass of leaves were optimized in order to achieve a higher antioxidant capacity. For synthesising FeNps, various operating parameters like pH, time, concentration ratio of leaf extract and $FeCl_3$ were optimized. The Nps were characterized using a UV–vis spectrophotometer, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Particle size analyzer (PSA) and scanning electron microscopy (SEM). The catalytic activity of both $NaBH_4$ (BFeNps) and *A. dubius* (DFeNps) mediated Nps were tested through the decolourization efficiency of methyl orange (MO) by UV irradiation and antioxidant capacity by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay. The effect of initial MO concentration and FeNps loading on decolourization was also investigated.

2. Materials and methods

2.1. Preparation of leaf extract

Fresh *A. dubius* leaves were collected and thoroughly washed with deionized water, then chopped into small pieces. Twenty grammes of leaves were heated with 100 mL distilled water at 50 °C for 45 min. The supernatant was filtered through a Whatmann filter paper to yield the leaf extract and stored at 4 °C for further use. The reducing capacity of *A. dubius* leaf extract was evaluated using DPPH radical scavenging activity. The experiments were performed at different temperatures from 40 to 70 °C, contact time of 20 to 120 min, and volume ratio of leaf mass (5 to 25 g):water (100 mL) for attaining an optimized condition with a higher antioxidant capacity of leaf extract.

2.2. Synthesis of $NaBH_4$ and A. dubius mediated Fe nanoparticles

The BFeNps were carried out as a control experiment. 100 mL of 0.5 M $FeCl_3$ was placed in an Erlenmeyer flask and maintained at 60 ± 1 °C with continuous stirring using a magnetic stirrer. 50 mL of 0.2 M $NaBH_4$ solution was added drop by drop using a burette. The colour changes from brown to colourless solution with a black precipitate of Nps confirm the formation of BFeNps. The Np synthesis was also carried out using 40 mL of leaf extract and 50 mL of 0.5 M $FeCl_3$ solution. The leaf extract (pH 6) was added drop wise to the $FeCl_3$ solution with continuous stirring for 90 min. The solution pH was adjusted using 0.1 N HCl and 0.1 N NaOH. The yellowish solution was turned into a blackish green colour with the formation of DFeNps as black precipitate [28]. Both precipitates of BFeNps and DFeNps were collected and washed with absolute ethanol; finally FeNps were dried in an oven at 60 °C for 180 min. The Np samples were stored in sealed bottles under dry condition. The effect of pH, temperature, time, concentration of leaf extracts and $FeCl_3$ ratio were studied for synthesis of DFeNps with conditions of pH varied from 4 to 9, concentration ratio of 1:5 to 5:5, temperature from 25 to 60 °C and time from 15 to 120 min for attaining a higher yield of Nps.

2.3. Analytical techniques

The absorbance spectra of the samples were measured within a wavelength range of 200–800 nm using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan). The functional group of leaf extract and Nps were recorded by a PerkinElmer FTIR spectrophotometer (Massachusetts, USA) over the spectral range of 400–4000 cm^{-1}. The morphologies of the Nps were analyzed using SEM (Tecson, Czech Republic). The XRD data were obtained by Rigaku Ultima III XRD using step scan technique and with Cu-K α radiation (1.500 Å, 40 kV, 30 mA). The PSD and zeta potential were measured by SZ-100 Nanopartica (Hiroba USA).

2.4. Photocatalytic activity

The photocatalytic activity of DFeNps and BFeNps was performed by decolourization of MO solution under UV irradiation. The experiments were carried out in a cylindrical double jacket vessel that is equipped with a magnetic stirrer under UV light of 20 W. Twenty milligrammes of Nps were added to 500 mL of 20 ppm aqueous MO solution. The solution was continuously exposed to a UV light maintained at 37 ± 1 °C with continuous stirring (100 rpm) for 6 h. The samples were collected at a regular interval of time and analyzed using a UV–visible spectrophotometer with a wavelength range of 200–900 nm. The photocatalytic decolouration efficiency of the Nps for MO solution was calculated using the following formula:

$$\text{Colour removal } (\%) = \frac{C_0 - C_t}{C_0} \times 100$$

where C_o and C_t are the absorbance of the initial concentration and after time 't' of MO solution, respectively.

3. Results and discussion

3.1. Leaf extract optimization

In the aqueous leaf extraction process, temperature, time and leaf mass played a major role in getting an efficient extract. The effect of leaf mass on the antioxidant activity of extract is shown in Fig. 1. For the increasing mass of leaves from 5 to 20 g per 100 mL of water which increases the percentage of antioxidant activity from 34.2 to 94.9%, respectively. Pablo et al. also reported that the chemical score of the essential amino acids of *A. dubius* leaf extract was 92.83% [44]. Hence the increasing mass of the leaves increase the concentrations of several organic substances in the leaf extract; therefore, the antioxidant activity was increased. The effect of leaf extraction process temperatures, which varies from 40 to 70 °C was investigated. Fig. 2 shows that the antioxidant activity increases along with temperature from 40 to 50 °C. In the case of temperature above 50 °C, it shows that the inhibitory rate decreases from 94.9 to 52% with an increasing temperature from 50 to 70 °C. *A. dubius* leaf extraction heating at 50 °C conferred scavenging activities on DPPH radical higher with an inhibitory rate of 94.9%. By increasing the temperature above 50 °C, it can degrade the active

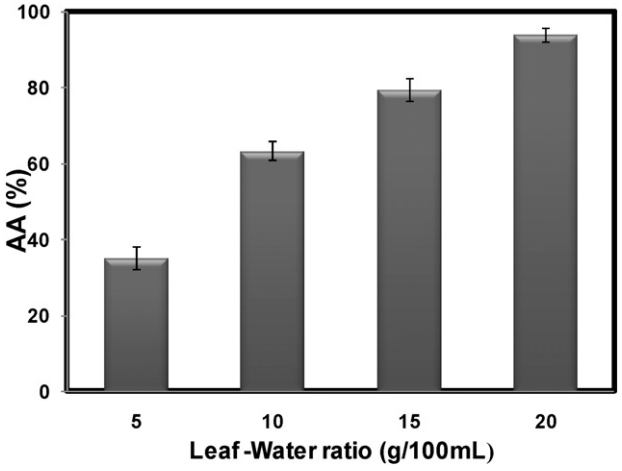

Fig. 1. The effect of different leaf masses: water volume ratios on percentage of antioxidant activity for leaf extract. (conditions: temperature: 50 °C, Time: 45 min).

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