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Application of *Gymnema sylvestre* Leaves Extract for Iron Nanoparticles Synthesis and Antibacterial Activity Evaluation

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Asian Journal of Organic & Medicinal Chemistry

Volume: 7 Year: 2022
Issue: 4 Month: October–December
pp: 299–303
DOI: https://doi.org/10.14233/ajomc.2022.AJOMC-P406

Received: 9 November 2022
Accepted: 20 December 2022
Published: 14 January 2023

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ABSTRACT

In this research study, iron nanoparticles (FeNPs) were synthesized using *Gymnema sylvestre* (Asclepiadaceae) leaves extract. Iron(III) ions present in ammonium ferrous sulphate hexahydrate were reduced to FeNPs by phytoconstituents in *Gymnema sylvestre* leaves extract. The characteristics of biosynthesized FeNPs were studied by using UV-visible absorption spectroscopy (UV-vis), transmission electron microscopy (TEM), particle size analyzer (PSA) and Fourier transform infrared spectroscopy (FTIR). The typical absorption peak was found to lie within 650-700 nm due to the excitation of surface plasmon vibration in FeNPs. The spherical structures of FeNPs were found in the range of 8-40 nm with average particle size of 405.8 nm. The antibacterial study of the biosynthesized FeNPs was performed against Gram-positive (*B. subtilis*) bacteria and Gram-negative (*E. coli*) bacteria by the agar well dispersion technique. The zone of inhibition of antibacterial activity of FeNPs against *B. subtilis* and *E. coli* bacteria was found to be 27.5 and 29 mm, respectively.

KEYWORDS

Nanotechnology, Iron nanoparticles, *Gymnema sylvestre.*

INTRODUCTION

Nano is the latest technology for designing materials at the nano range (1-100 nm) [1]. Due to increased pollution and toxicity in nature, material science researchers are primarily interested in nanotechnology so that they can invent eco-friendly production methods. The aim of this study is to enhance the yield of desirable nanoparticles and decrease the subsequent contribution of pollutants [2]. In addition, iron nanoparticles have drawn significant attention among researchers because of their unusual physico-chemical and magnetic properties. These iron nanoparticles (FeNPs) have a valuable potential to be applied as biosensors, magnetic resonance imaging (MRI), food preservation, smart-drug delivery, magnetic storage media, ferrofluids, catalysis, hyperthermic cancer treatments and cell sorting [3]. The application of these iron nanoparticles in all fields entails major requirements because iron nanoparticles must be in nano range, which is a pre-requisite for preserving these specific magnetic and physico-chemical properties [4]. The literature survey shows that some of the studies on physical and chemical methods such as sonochemical methods [5], hydrothermal processes [6], electrochemical routes [7], micro-emulsion techniques [8] and co-precipitation methods [9] of iron nanoparticles are available. However, the chemical synthetic method contribute to the presence of certain toxic chemicals in medical applications may have an adverse effect [10]. The biosynthesis of iron nanoparticles using biogenic/green method especially phytoconstituents has drawn considerable interest now-a-days due to its facileness, cost-effectiveness and ecological benignity [11-15].

The use of phytoconstituent for synthesizing iron nanoparticles offers various benefits to the environment and human beings because it doesn't use harmful chemical reagents [16]. *Gymnema sylvestre* (Asclepiadaceae) is an herbaceous plant found in the tropical rain forests area of Sri Lanka, central and southern India, commonly known as Gurmar. *G. sylvestre* leaves are known for various medicinal uses such as antidiabetic, hypolipidemic, stomachic, diuretic, astringent and tonic [17]. The main phytoconstituents of *G. sylvestre* are gymnema saponins and gymnemic acids, which are responsible for their effective therapeutic value. Anthraquinones, hentriacontane, butyric acid pentatriacontane, stigma sterol, formic acid, β-amyrin related glycosides, resins, tartaric acid, lupeol, flavones, phytin and calcium oxalate are other phytoconstituent. *G. sylvestre* has a special molecular mechanism of the gene, which is responsible for its medicinal properties [18,19]. The occurrence of triterpene saponins (gymnema saponins, gymnemic and gurmarin acids) is responsible for its antidiabetic and anticancer activity [20-22]. The effective anti-arthritic activity of the leave is due to the nature of saponin glycosides, triterpenoids and steroids. The strong quality of the plant's hydroalcoholic extract contributes to the antidental caries [23]. The presence of gymnemic acid in *G. sylvestre* shows significant antihyperlipidemic, antibacterial and antimicrobial effects [24-27]. The bioactive constituents of *G. sylvestre* named saponins and tannins are responsible for the anti-inflammatory activity [28]. *G. sylvestre* has also shown remarkable immune-stimulatory activity on human neutrophils. The plant of *G. sylvestre* also exhibits pharmaceutical and medicinal significance in the treatment of leukoderma, bronchitis, cardiopathy, jaundice, renal and vesical calculi, Parkinsonism (nervous system disorder), asthma, amenorrhea, constipation dyspepsia and conjunctivitis [29]. To our best of information, no single report has been published on the *G. sylvestre* mediated production of iron nanoparticle and have been employed for antibacterial evaluation.

EXPERIMENTAL

Gymnema sylvestre leaves (powder form) were purchased from the local market of Hisar city, India. Ammonium ferrous sulphate hexahydrate was supplied by Himedia and dioctyl sodium sulphosuccinate was supplied by Sigma-Aldrich, USA. Double de-ionized water was used throughout the experiments. All of chemicals and solvents used were of analytical (AR) grade.

Biogenic synthesis of iron nanoparticles

Preparation of *Gymnema sylvestre* extract: *Gymnema sylvestre* fine powder (7.5 g) was dissolved in 150 mL of boiled double distilled water (DDW), stirred for 45 to 60 min and then centrifuged. The supernatant was collected and then preserved for synthesis of iron nanoparticles experiments.

Biogenic synthesis of iron nanoparticles: Dioctyl sodium sulphosuccinate (8 mL) solution was added dropwise in 10 mL ammonium ferrous sulphate hexahydrate solution under stirring for 30 min. After 30 min of proper dissolution of above solution, 5 mL extract of *G. sylvestre* solution was added dropwise. Following the addition, the reaction blend colour gradually changed to a blackish-brown colloidal suspension, indicating that Fe^{3+} ions were reduced into FeO nanoparticles by the contribution of phytochemicals of leaves extract of *G. sylvestre*.

For complete reduction of Fe^{3+} ions into FeO nanoparticles, the samples were stirred overnight at 28 ºC. At 2000 rpm for 20 min, the colloidal suspension of the reaction blend was centrifuged and formerly Whatman filter was used to collect FeNPs from the reaction mixture. The FeNPs collected were washed using ethanol and double deionized water and then dried for 2 h at 120 ºC.

Characterization: The biosynthesized iron nanoparticles (FeNPs) were categorized with the support of a UV-vis spectrophotometer (Shimadzu UV-2450 Sr. No. A10834701961). The dimension of nanoparticle was principally investigated by using active light scattering instrument. The nano ranged size of iron nanoparticles (FeNPs) were established by transmission electron microscopy (Hitachi H7650). The phytoconstituents existing in *G. sylvestre* and its possible contribution in the production of iron nanoparticles (FeNPs) was resolute by FTIR (spectrum BX Perkin Elmer spectrum BX II).

Antibacterial activity: The antibacterial action of biosynthesized FeNPs was evaluated against *Escherichia coli* MTCC 040 (Gram-negative) and *Bacillus subtilis* MTCC 441 (Gram-positive) by agar well diffusion method. Nutrient agar was employed as a substrate for the development of bacteria taken from the petri plates. Four wells were prepared in each Petri plate by well cutter. A typical well filled with streptomycin was allocated. Three well-loaded with 200 µL stock solution of 5.0 mL, 10.0 mL and 15.0 mL extract prepared iron nanoparticles (FeNPs) and the agar plates were incubated at 36 ºC for 24 h. The zone of inhibition around the well was measured after the incubation.

RESULTS AND DISCUSSION

Iron(III) ions present in ammonium ferrous sulphate hexahydrate were reduced to FeO nanoparticles by phytoconstituents present in the extract of *Gymnema sylvestre* leaves. Reduction was accompanied by an immediate colour turn from yellowish-brown into blackish-brown. This may be an indication of the formation of iron nanoparticles.

UV-visible studies: Absorption peaks at the wavelengths between 650-700 nm are usually the characteristic of iron nanoparticles in this region. The UV-vis spectrum was recorded in the wavelength range of 200-700 nm for the biosynthesized iron nanoparticles. The biosynthesized iron nanoparticles from *G. sylvestre* leaves extract showed a single surface plasmon resonance (SPR) peak at 660 nm, which indicated the formation of iron nanoparticles. The observed colour change was the result of the excitation of SPR of iron nanoparticles. The band was observed only after the addition of extracts as UV readings of the samples taken prior to the addition of extract did not show any visible peak *i.e.*, UV-visible spectrum of ferrous sulphate 0.015 M solution, which suggests that nanoparticles (NPs) were not formed before the addition of extracts [30]. Therefore, the absorbance spectrum of the blackish-brown iron nanoparticles solution prepared with the green method revealed a surface plasmon absorption band at 660 nm, indicating the formation of iron nanoparticles (FeNPs), which are similar to the various UV-visible spectrum characteristics of metallic iron nanoparticles by the different researchers [31].

Particle size distribution studies: The particle size of iron nanoparticles biosynthesized using *G. sylvestre* leaves extract was evaluated and the average particle size was found to be 405.8 nm (Fig. 1). It was inferred that the particle size of the biosynthesized nanoparticles was within the favourable size range. Also, the sharp peaks indicated the highly stable nature of the biosynthesized products. Hence, iron nanoparticles average size was found to be 405.8 nm. The two peaks observed in this result, which revealed the size of 406.8 and 536.2 nm iron nanoparticles (FeNPs) and the peak strength found to be 96.4% and 3.6%, respectively, which are very similar to the particles size of metallic iron nanoparticles as reported by a different researchers [31].

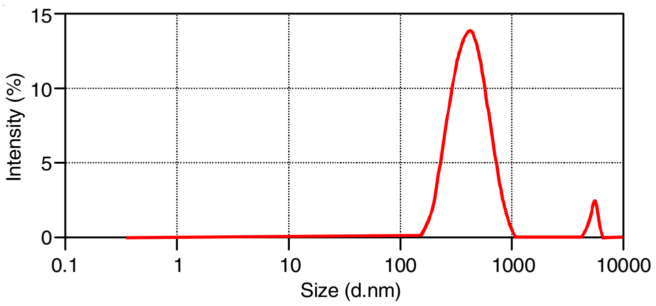

Fig. 1. Particle size distributions of iron nanoparticles prepared using extract of *G. sylvestre* leaves

TEM studies: The TEM images (Fig. 2) of biosynthesized iron nanoparticles using *G. sylvestre* leaves extract revealed the spherical structures in the range of 8 to 40 nm, which is due to agglomeration of iron nanoparticles [32].

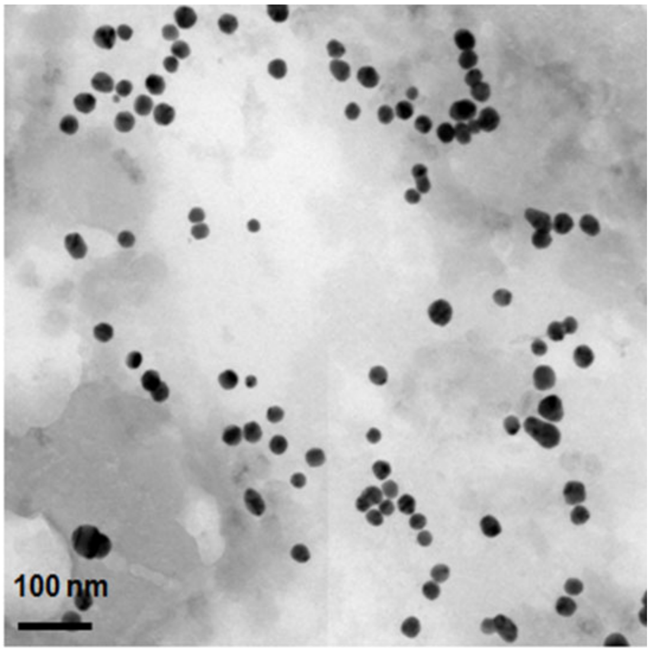

Fig. 2. TEM image of biosynthesized iron nanoparticles

FTIR studies: The FTIR spectra of biosynthesized iron nanoparticles using *G. sylvestre* leaves extract resulted in the stretching vibrations of 3335.6 cm^{-1}, 2116.8 cm^{-1} and 1612.5 cm^{-1} with a range of 4000-400 cm^{-1} (Fig. 3). The peaks at 3335.6 cm^{-1} correspond to the OH stretching vibrations, at 2116.8 cm^{-1} correspond to the stretching vibrations of C≡N and at 1514.5 cm^{-1} correspond to the stretching vibrations of the conjugated carbonyl (–C=O) group. The identified functional groups were found similar to the reported FTIR analysis of iron nanoparticles (FeNPs) biosynthesized by *Musa ornata* flower sheath extract [11], apple peel extracts [33] and *Urtica dioica* leaf extract [34].

Antibacterial activity: Fig. 4a shows the region of the reticence of bogenic FeNPs and control samples against *B. subtilis* (Gram-positive). It was found that the sample of iron nanoparticles produced 27.5 mm inhibition zone and zone of inhibition (ZOI) by control antibacterial drug was found to be 26.0 mm. Fig. 4b shows the zone of the reticence of Fe nano-

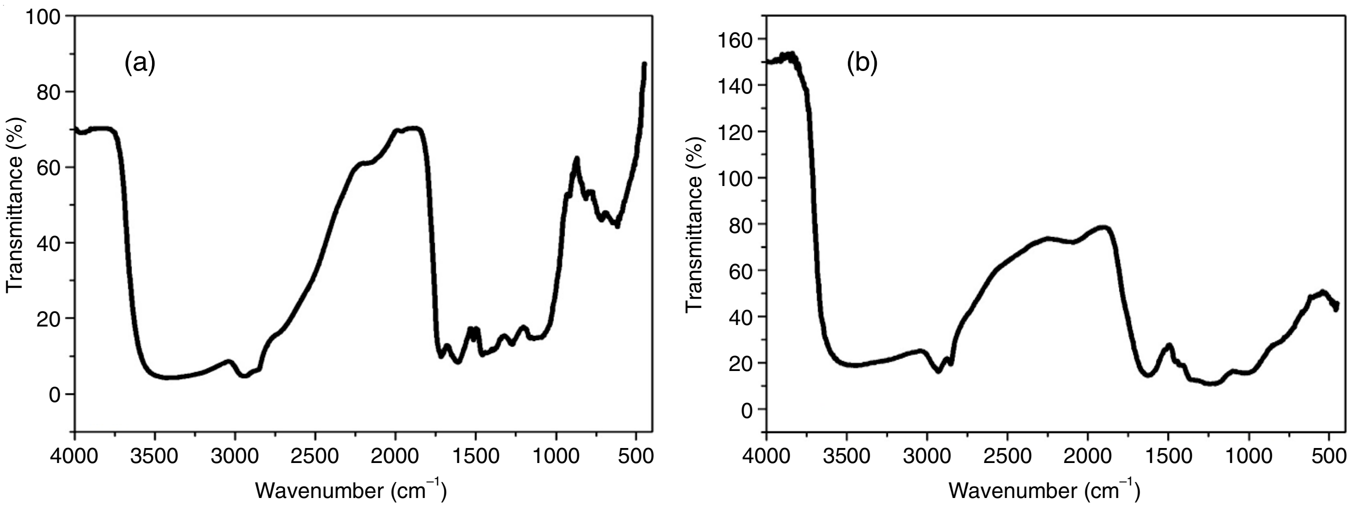

Fig. 3. FTIR spectra of *G. sylvestre* leaves extract (a) and biosynthesized iron nanoparticles using *G. sylvestre* leaves extract (b)

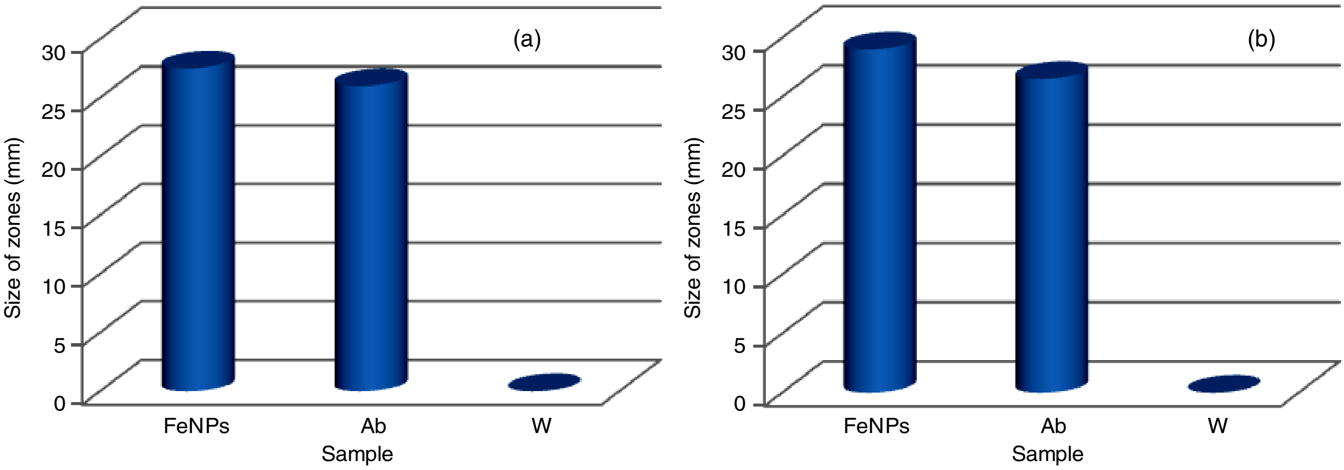

Fig. 4. Graph showing relative antibacterial activity of iron nanoparticles synthesized from *G. sylvestre* leaves extract (against *B. subtilis*) (a) and (against *E. coli*) (b) compared with standard antibiotic [where Fe-NPs, Ab and W are iron nanoparticles (from *G. sylvestre*), antibiotic drug (control) and water (control), respectively]

particles and control samples against *E. coli* (Gram-negative) were measured. It is found that FeNPs produced 29 mm inhibition zone and zone of inhibition (ZOI) by control antibacterial drug was found to be 26.5 mm.

Thus, it is evident that the biosynthesized FeNPs displayed high antibacterial activity against *E. coli* (Gram-negative) bacteria as compared to *B. subtilis* (Gram-positive) bacteria. Table-1 indicates a maximum zone of inhibition for *E. coli*.

TABLE-1
ZONE OF INHIBITION FOR BOTH *Bacillus subtilis* (GRAM-POSITIVE) AND *Escherichia coli* (GRAM-NEGATIVE) BACTERIA IN mm

Test micro-organism	Diameter of zone of inhibition (mm)		
	Distilled water (W)	Antibiotic (Ab)	FeNPs
Escherichia coli (MTCC 040)	0	26.5	29.0
Bacillus subtilis (MTCC 441)	0	26.0	27.5

Conclusion

In this research, iron nanoparticles were biosynthesized using biological method by using *Gymnema sylvestre* leaves extract with high stability. Using UV-visible absorption spectroscopy, particle size analyzer and transmission electron microscopy studies also recognized the formation of iron nanoparticles. The biosynthesized iron nanoparticles exhibited the strong absorbance peaks at 436 and 267 nm. Moreover, the average particle size of iron nanoparticle was found to be 405.8 nm. The antibacterial activity of iron nanoparticles (FeNPs) biosynthesized by these methods was evaluated by Agar well diffusion. The iron nanoparticles exhibited more activity towards *E. coli* (Gram-negative) bacteria than *B. subtilis* (Gram-positive) bacteria.

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