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Research article

Green synthesis of magnetic iron oxide nanoparticle using leaves of *Glycosmis mauritiana* and their antibacterial activity against human pathogens

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Abstract

The potential effect of *Glycosmis mauritiana* leaf extract for the formation of iron oxide nanoparticles and its application on antibacterial activity was discussed. The efficiency of *G. mauritiana* leaves are used as a bio-material for the first time as reducing agent. Synthesized iron oxide nanoparticles were characterized by UV–Vis spectrometry, DLS, XRD, FT-IR, SEM and TEM analysis. The results revealed that iron oxide nanoparticles has the absorption peak at 404 nm, spherical shaped and average size of particle is found to be below 100 nm. The green synthesized iron oxide nanoparticles showed good antibacterial activity against the tested pathogens. The present study highlights the potential application of iron oxide nanoparticles can be explored for biomedical industries.

Introduction

Iron oxides are chemical compounds composed of iron and oxygen. The synthesis of iron oxide nanoparticles using plant materials offer several benefits of eco-friendliness and compatibility for various applications as they do not use toxic chemicals for the synthesis protocol [1]. Iron oxides nanoparticles have been reported for various biomedical applications such as drug delivery [2], magnetic resonance imaging (MRI) [3], detection, diagnosis, and treatment of illnesses, such as neurological disease [4], cancer [5] and cardiovascular disease [6] due to their small size, biocompatibility, high magnetism and low toxicity [7].

Glycosmis is a clearly defined genus of the family Rutaceae comprising about 40 species [8].Various species of this genus used as a traditional medicine for the healing of various diseases [9]. *Glycosmis mauritiana* (Lam.) commonly known as Ash sheora is important species of *Rutaceae*. It is a shrub and distributed in south and Southeast Asia counties like India, Sri Lanka, Myanmar, Thailand, Malaysia and Indonesia. This plant has been reported for various biological activities such as antimicrobial and antioxidant activities [10] and [11]. Phytochemical analysis of leaves of *G. mauritiana* revealed the presence of various classes of phyto-constituents such as alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, saponins, steroids and tannins [10]. Furthermore, alkaloids, two acrid one alkaloids and a flavones glycoside were isolated from the aerial parts of *G. mauritiana* reported by Intekhab *et al.* (2011) [12]. Consequently, in the present research work is designed to synthesis of iron oxide nanoparticles using *G. mauritiana* extract.

Experimental

Collection and identification of plant

Fresh healthy leaves of *G. mauritiana* were collected from Thiruvanamalai local park (Figure 1) and were authentically identified by Prof. P. Jayaraman, Institute of Herbal Science, Plant Anatomy Research Centre, West Tambaram, Chennai, India as Rutaceae with voucher specimen number PARC/2015/3146.

Figure 1. Aerial view of *G. mauritiana.*

Scientific classification of *G. mauritiana* (Lam.)

Class	:	Magnoliopsida – Dicotyledons
Subclass	:	Rosidae
Order	:	Sapindales
Family	:	Rutaceae
Genus	:	*Glycosmis*
Species	:	*mauritiana*
Synonymous	:	*Limonia pentaphylla* Auct; *Glycosmis pentaphylla* Auct. *Limonia mauritiana*

Preparation of *G. mauritiana* aqueous leaves extract

About 100 g of fresh healthy leaves of *G. mauritiana* were shade dried and the leaves were powdered using kitchen blender. The powdered leaves were soaked in the 200 ml of double distilled water for overnight in a fridge for 4°C and then the rinsed mixtures were boiled for 10 minutes. The extracts were cooled to room temperature and then filtered through Whatman filter paper (No.42).

Synthesis of iron oxide nanoparticles using *G. mauritiana* extract

Iron oxide nanoparticles were synthesized by taking $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ (1:2 molar ratios) and were dissolved in 100 ml of double distilled water in a 250 ml beaker and heated at 80°C with mild stirring using magnetic stirrer under atmospheric pressure. After 10 minutes, 20 ml of the aqueous solutions of *G. mauritiana* extract was added to the mixture, immediately the light green colour of the *G. mauritiana* extract of the mixture changed to dark brownish colour. After 10 minutes, 20 ml aqueous solution of sodium hydroxide was added to the mixtures with the rate of 3 ml per minutes for allowing the iron oxide precipitations uniformly. The mixture was allowed to cool down to room temperature and the iron oxide nanoparticles were obtained by decantation to form magnetite. The magnetites formed were washed 3 times with double distilled water and 3 times with ethanol and air dried at room temperature.

Characterization of iron oxide nanoparticles

The absorbance spectra of sample were measured in wavelength within the range from 300-700 nm using a UV-Vis double-beam bio-spectrophotometer Elico-Bl-198. Particle size of magnetic iron oxide nanoparticles was measured by laser diffractometry using a Nano Size Particle Analyzer in the range between 0.6 nm to 6.0 µm. Structure and crystalline size of nanoparticles were determined by XRD using SHIMADZU (Model XRD¬6000). The functional group of nanoparticles were recorded by FT-IR spectroscope (Shimadzu, IR Affinity 1, Japan), with a scan range from 4000 to 500 cm^{-1} with a resolution of 4 cm^{-1}. Morphological analysis of magnetic iron oxide nanoparticles was done using Vega 3 Tescan SEM machine and HR-TEM Jeol model 3010 instrument operated at 200 Kv and a beam current of 104. 1µA.

Screening of antibacterial activity

Bacterial strains

The clinical isolates of *Bacillus cereus*, *B. subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia, Micrococcus luteus*, *Proteus mirabilis*, *P. vulgaris, Pseudomonas fluorescence*, *Staphylococcus aureus* and *Vibrio fluvialis* were obtained from Department of Bacteriology, King Institute of Preventive Medicine, Guindy, Chennai.

Antibacterial analysis by disc diffusion method

The antibacterial activity of synthesized iron oxide nanoparticles were evaluated using disc diffusion method [13]. A set of sterile discs (6 mm, Hi-media) were impregnated with different concentrations of iron nanoparticles *i.e.* 10 µg/ disc (10µg/µl), 15 µg/ disc (15µg/µl), 20 µg/ disc (20µg/µl), 25 µg/ disc (25µg/µl) 30 µg/ disc (30µg/µl) respectively. Subsequently, culture plates were prepared by pouring 20 mL of Mueller-Hinton agar (Hi-media) medium and bacterial suspension swabbed on the medium plates using sterile cotton swab and the plates were kept aside for few minutes. The discs were gently pressed and incubated in inverted position for 24 hours at 37°C.The discs with Norfloxacin (20 µg/ disc) were placed on the MHA plates maintained as positive control. After the incubation period, the susceptibility of the test organisms was determined by measuring the diameter of the zone of inhibition using Himedia zone scale and the obtained results were tabulated for evaluation.

Results and discussion

Green synthesis of iron oxide nanoparticles using *G. mauritiana* extract

In the present study, the iron oxide nanoparticles were prepared by green synthesis method using *G. mauritiana* extract. Likewise various plants extract such as *Moringa oleifera* [14], *Lagenaria siceraria* [15], *Mangifera indica, Murraya Koenigii, Azadiracta indica, Magnolia champaca* [16], *Lawsonia inermis* and *Gardenia jasminoides* [17] and *Hordeum vulgare* and *Rumex acetosa* [18] have been reported for synthesis of iron oxide nanoparticles. When *G. mauritiana* extract and aqueous $FeCl_3$ and $FeCl_2$ solution were mixed, the color of the reaction mixture instantaneously turned dark brown from light green. Black precipitates appeared in beaker were observed. The purified iron oxide nanoparticles and their magnetic property were confirmed towards aggregation the magnetic bead (Figure 2).

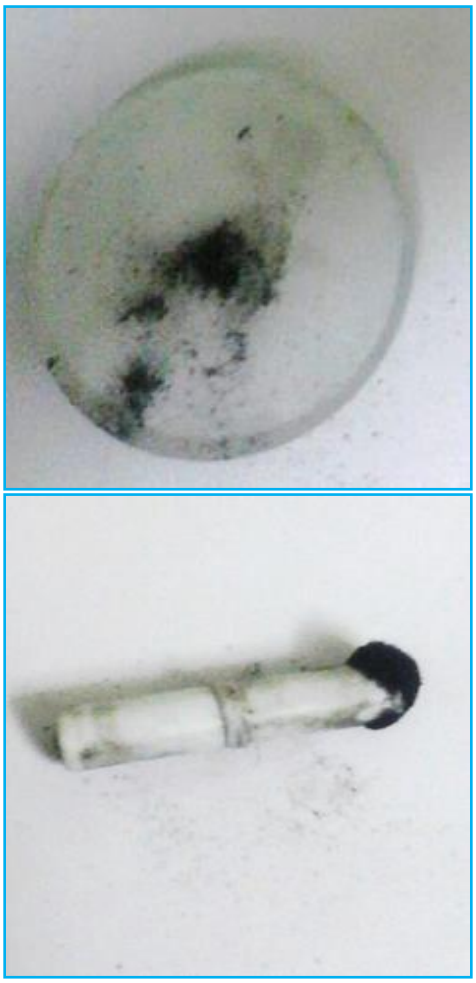

Figure 2. Purified magnetic iron oxide nanoparticles and its aggregation towards the magnetic bead.

UV-Visible spectroscopy analysis

The formation of iron nanoparticles was further explained by measuring their absorbance with UV-Vis spectrophotometer over the range from 200 to 800 nm. For *G. mauritiana* extract, there was two absorption peaks at 202 and 279 nm and synthesized iron oxide nanoparticles has the absorption peak at 404nm (Figure 3). These results are harmony with of findings of Turakhia *et al.* [19] who reported green synthesis of iron nanoparticles from *Spinacia oleracea* showed the sharp peak at 404 nm in UV–Vis spectrum. Absorption peak was observed between 400-450 nm regions due to the excitation of surface Plasmon vibrations in the iron oxide nanoparticles solution, which is identical to the characteristics UV-visible spectrum of Iron oxide nanoparticles [20].

DLS analysis

The particle size distribution of green synthesized iron oxide nanoparticles is shown in Figure 4. The average size of iron oxide nanoparticles is found to be below 100 nm. Similar work was done by Kanagasubbulakshmi and Kadirvelu [15] who reported the average particle size of cube shape iron oxide nanoparticles is 100 nm synthesized by *Lagenaria siceraria*.

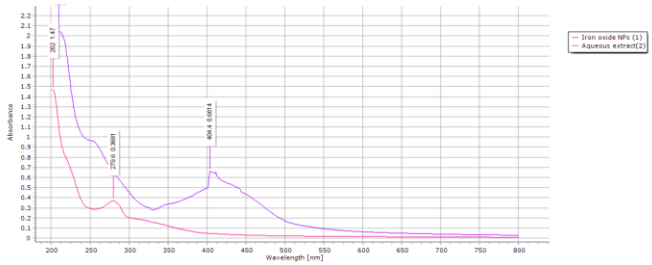

Figure 3. UV-visible spectrum image of synthesized iron oxide nanoparticles and *G. mauritiana* aqueous extract.

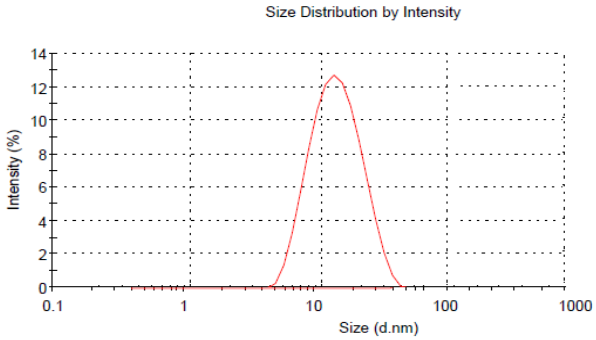

Figure 4. PSA of synthesized iron oxide nanoparticles by *G. mauritiana* aqueous extract.

XRD analysis

The powder XRD pattern of the prepared iron oxide nanoparticles using *G. mauritiana* aqueous extract is shown in Figure 5. The major strong characteristic peaks of iron oxide particles are obtained at 2θ = 24.14, 33.14, 35.61, 40.84, 49.45, 54.06, 62.42 and 64.00 which are corresponding to amorphous structure (220), (311), (400), (442), (511), (440) of iron oxide. All the reflection peaks could be indexed to rhombohedral structure, for iron oxide (JCPDS NO. 89-8104). These findings are analogous with the crystalline nature of iron oxide nanoparticles [21].

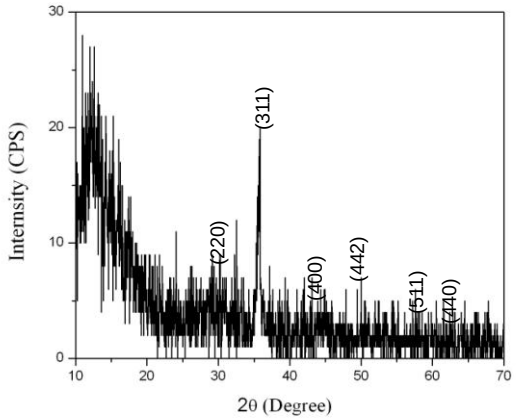

Figure 5. XRD patterns of iron oxide nanoparticles synthesized by *G. mauritiana* aqueous extract.

FT-IR analysis

FT-IR analysis of synthesized iron oxide nanoparticles gave the stretching vibrations at 3334.7 cm^{-1}, 2115.9 cm^{-1} and 1628.7 cm^{-1} with in the region of 400-4000 cm^{-1} (Figure 6). The peak at 3334.7 cm^{-1} corresponds to the –OH stretching frequency, 2115.9 cm^{-1} corresponds to the C≡N stretching vibrations, 1632 cm^{-1} for conjugated carbonyl (–C=O) group stretching vibrations. The identified functional groups are found in previous FT – IR analysis of iron oxide nanoparticles synthesized by Tie Guanyin tea extract [22], and aqueous extracts of *Sageretia thea* [23].

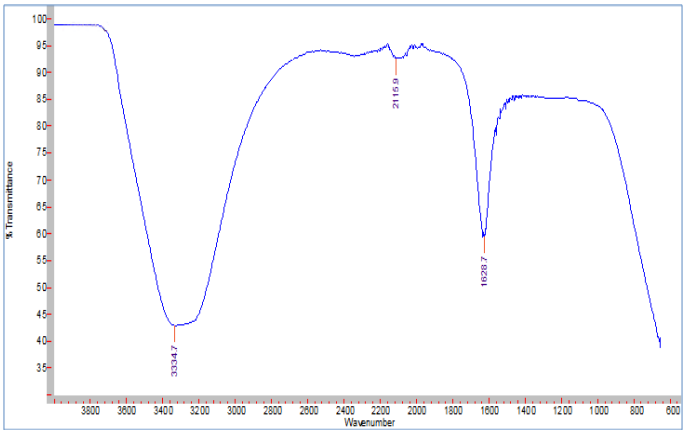

Figure 6. FT-IR spectra of synthesized iron oxide nanoparticles by *G. mauritiana* extract.

SEM analysis

The morphological dimensions of synthesized iron oxide nanoparticles were studied using the SEM. The study demonstrated that the size of the nanoparticles was in the range of 58-79 nm, similar phenomenons were reported in the previous studies [24]. And also exhibits the formation of spherical shape of iron nanoparticles as shown in the Figure 7. In an another study by Kuang *et al.* [25] used three different tea extracts, namely, green tea , oolong tea and black tea to synthesis iron nanoparticles and the SEM image revealed the irregular spherical iron nanoparticles indicating the chain-like structure.

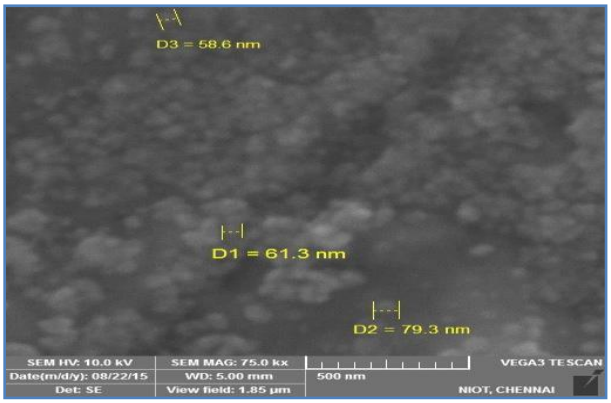

Figure 7. SEM image of the biosynthesized iron oxide nanoparticles.

HR-TEM analysis

Morphologies of the nanoparticles synthesized during bioreduction were confirmed by employing HR-TEM analysis (Figure 8). Iron oxide nanoparticles exhibited spherical nanostructures with the average core diameter of 20 nm and the particles were seen to be agglomerated. Similarly, Makarov *et al.* [18] reported electron-dense spherical iron oxide particles with a diameter of up to 30 nm were formed in the *Hordeum vulgare* extracts and electron dense amorphous particles up to 40 nm in diameter synthesized from *Rumex acetosa* by TEM analysis.

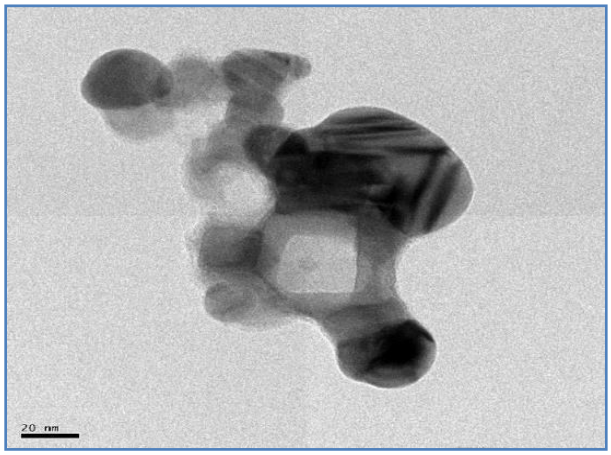

Figure 8. HR - TEM image of the synthesized iron oxide nanoparticles by *G. mauritiana* extract.

Screening of antibacterial activity of synthesized iron oxide nanoparticles by *G. mauritiana* aqueous extract

The results of antibacterial activity of *G. mauritiana* mediated iron oxide nanoparticles were presented in Table 1. The results showed the zone of inhibition of bacterial growth on agar plates as a function of the different concentrations of iron oxide nanoparticles. The growth of bacterial pathogens was inhibited gradually with increase in concentration of iron oxide nanoparticles. The inhibition activity of the iron oxide nanoparticles were compared with standard antibiotic Norfloxacin.The iron oxide nanoparticles exhibited minimum antibacterial activity against the tested pathogens at 10 µg/disc concentration. The maximum antibacterial activity was observed at 30 µg/disc concentration of iron oxide nanoparticles. Results of antibacterial activity were comparable with the findings of Kanagasubbulakshmi and Kadirvelu [15] who found that iron oxide nanoparticles synthesized by *Lagenaria siceraria* possesses significant antibacterial activity. In addition, Suganya *et al.* [26] reported considerable antibacterial activity of iron oxide nanoparticles from leaf extract of *Passiflora foetida* against *K. pneumonia, P. aeruginosa, S. aureus* and *E. coli.*

Table 1. Antibacterial activity of iron oxide nanoparticles synthesized by *G. mauritiana* aqueous extract.

Name of the Bacterial pathogens	Green synthesized magnetic iron oxide nanoparticles					Standard antibiotic
	10 µg	15 µg	20 µg	25 µg	30 µg	Norfloxacin 20 µg/ disc
	Zone of inhibition (Diameter in mm)					
B. cereus	7±1.0	8±2.0	9±1.7	10±2.0	11±1.0	13±1.0
B. subtilis	13±2.0	16±1.7	17±1.0	18±2.0	19±2.6	20±1.7
E. faecalis	12±2.0	13±1.7	15±1.0	16±1.0	18±2.0	20±2.0
E. coli	12±1.7	15±1.6	16±2.0	18±1.0	19±1.0	20±2.0
K .pneumoia	7±0.9	8±1.0	9±0.8	10±1.0	12±1.0	14±1.2
M. luteus	10±1.0	12±1.2	13±1.0	15±1.5	16±2.0	13±2.0
P. mirabilis	8±1.0	9±1.2	8±1.2	10±1.7	11±1.0	12±1.7
P. vulgaris	12±1.4	14±1.5	16±2.0	18±2.0	19±1.7	18±1.5
P. fluorescence	13±1.0	15±1.5	16±1.5	17±2.0	18±1.7	20±2.0
S. aureus	8±0.9	12±1.0	13±1.0	15±1.5	16±1.2	18±1.7
V.fluvialis	9±1.0	8±1.0	8±1.2	9±1.2	10±1.5	12±1.7

Conclusion

This is the first report on rapid and efficient synthesis of iron oxide nanoparticles using traditional medicinal plant *G. mauritiana*. The particles were found to be almost spherical shapes and stable. The particles exhibited effective antibacterial activity against the tested pathogens. The present research work revealed the green synthesized iron oxide nanoparticles can be potential source for antibacterial therapy.

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