CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1092678

Available online at: http://www.iajps.com Research Article

ANTIBACTERIAL ACTIVITY OF GREEN BIOSYNTHESIS OF MAGNETIC IORN OXIDE NANOPARTICLE OF *MURRAYA EXOTICA* L. AQUEOUS EXTRACT AGAINST HUMAN PATHOGENS

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Abstract:

The green synthesis of magnetic iron oxide nanoparticles is a convenient, economical, rapid and eco-friendly method compared to physical and chemical synthesis methods. In the present study iron oxide nanoparticles synthesized by Murraya exotica L. leaves extract. The formation of iron oxide nanoparticles was confirmed by the colour change and further characterized by UV-Visible Spectroscopy, FT-IR analysis, DLS and XRD. The morphology and the size of nanoparticles were analyzed by SEM and HR - TEM analysis. The antibacterial efficacy of synthesized iron oxide nanoparticles exhibited considerable activity against the tested human pathogens. Our study shows that green synthesized iron oxide nanoparticles can be a good source for alternative therapy of bacterial diseases.

Key words: *Green synthesis, iron oxide, nanoparticles, Murraya exotica, antibacterial efficacy*

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Please cite this article in press as S. Amutha and S. Sridhar., ***Antibacterial Activity of Green Biosynthesis of Magnetic Iorn Oxide Nanoparticle of Murraya Exotica l. Aqueous Extract against Human Pathogens****, Indo Am. J. P. Sci, 2017; 4(12).*

INTRODUCTION:

In nanotechnology, magnetic iron oxide nanoparticles are microscopic particles and sized between 1 and 100 nanometers [1]. It has unique and most important property i.e larger surface area than superior particles which cause them to be more reactive to some other molecules. They are extensively synthesized by using physical and chemical methods. These synthesized methods are needed to use high energy, temperature, toxic chemicals and expensive. The primary goal of nanotechnology is to develop convenient, economical, rapid and eco-friendly green synthesis methods [2].

Magnetic iron oxide nanoparticles research is presently an area of passionate scientific interest due to a broad range of prospective applications. It is used as catalysis [3] high-density magnetic storage media [4] and chemical sensors [5]. It is an effective nano agent to remove a number of pollutants from water resources [6]. It has many important biomedical applications such as for targeted drug delivery in clinical trials [7], contrast agents in magnetic resonance imaging (MRI) [8], antibacterial activity [9] and anticancer activities [10].

Murraya exotica L. commonly known as Chinese box belongs to the family of *Rutaceae.* It is an evergreen shrub, habitually 2-3 m in height. It is traditionally used in India and China for treatment of diarrhea, dysentery, toothache and body pains from injury or trauma [11]. It was documented to exhibit antimicrobial [12] anti-inflammatory, antinociceptive [13], anti-oxidant [14] and larvicidal activities [15]. In addition, various bioactive compounds such as Colensenone and colensanone [16], cinnamic acid [17], coumarins [18] methoxylated flavonoids [19], alkaloids [20] and phytosterols [21] have been reported in *M. exotica* L leaves. Furthermore, a study by Lv *et al.* (2013) [22] revealed sesquiterpenes are the main constituents in essential oil of *M. exotica.* Considering the ethnomedicinal properties and reported activities of *M. exotica* L., in the present investigation was preferred for nanoparticles synthesis.

MATERIALS AND METHODS:

Materials: Collection and identification of plant

Fresh healthy leaves of *M. exotica* 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/2015/3147.

Fig 1: Habit of *Murraya exotica* L.

Scientific classification of *M. exotica* L.

Class	:	Magnoliopsida – Dicotyledons
Subclass	:	Rosidae
Order	:	Sapindales
Family	:	Rutaceae
Genus	:	*Murraya*
Species	:	*Murraya exotica* L. – Chinese box
Synonymous	:	*Chalcas exotica, Chalcas Paniculata, Murraya paniculata*

Synthesis of iron oxide nanoparticles using *M. exotica* extract

About 100 g of fresh healthy leaves of *M. exotica* were washed thoroughly with running tap water and double distilled water, cut into fine pieces and shade dried for 10 days under dark condition. After drying 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.

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 *M. exotica* extract was added to the mixture, immediately the light green colour of the *M. exotica* 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. From the first addition of sodium hydroxide the dark brown mixture changed to black suspended particles. 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

The surface Plasmon resonances (SPR) of synthesized iron oxide nanoparticles have been studied by UV-Vis double-beam bio-spectrophotometer Elico-Bl-198 using the software Spectral Treats Version 2.37.4 Rel-1 in the range of 300 to 700 nm. The diffraction pattern was recorded by Seifert Rayflex Software which provides control modules for the complete range of diffractometer accessories together with the corresponding analysis software XRD with Cu-Kα radiation. 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 µ. Morphological analysis of nanoparticles was done using Vega 3 Tescan SEM machine. The morphology of magnetic iron oxide nanoparticles was viewed under a Transmission electron microscope (HR-TEM, Jeol model 3010, at 200 Kv and 104.1µA).

Test Bacteria

The Bacterial cultures employed in this study are *Bacillus cereus, Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Micrococcus luteus, Proteus mirabilis, Proteus vulgaris, Pseudomonas fluorescens, Staphylococcus aureus* and *Vibrio fluvialis.*

Antibacterial analysis by disc diffusion method

The antibacterial activity of synthesized iron oxide nanoparticles were evaluated using disc diffusion method [23]. 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:

UV-Visible spectroscopy analysis

In the present investigation, the formation and stability of synthesized iron oxide nanoparticles was further confirmed by UV-Vis spectral analysis. *M. exotica* aqueous extract has the absorption peaks at 230-279 nm regions and a synthesized iron oxide nanoparticle has the absorption peak at 401 nm (Figure 2). It might be due to the excitation of surface plasmon vibrations in the iron oxide nanoparticles, which are very similar to the characteristics UV – visible spectrum of β Fe_2O_3 [24]. Balamurugan *et al*. (2014) [25] reported UV-Vis spectrum of iron oxide nanoparticles synthesized by *Eucalyptus globulus* leaf extract showed absorption peak around 402 nm.

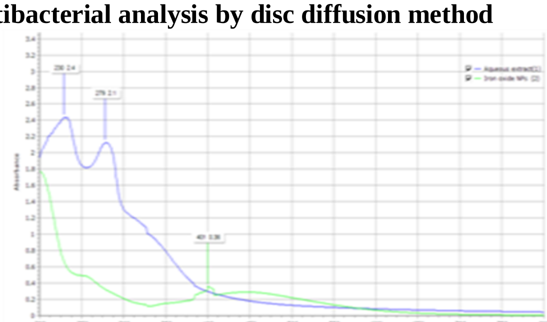

Figure 2. UV absorption spectrum of plant extract and synthesized iron oxide nanoparticles by *M. exotica* aqueous extract

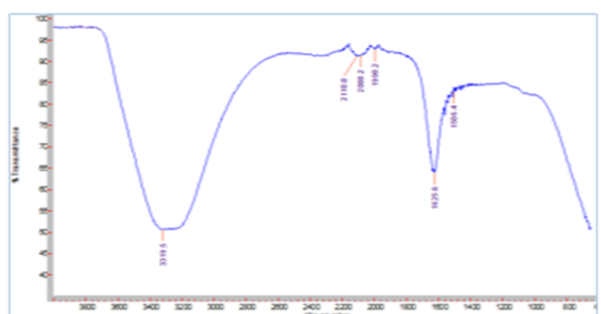

Figure 3. FT-IR spectrum of synthesized iron oxide nanoparticles by *M. exotica* aqueous extract

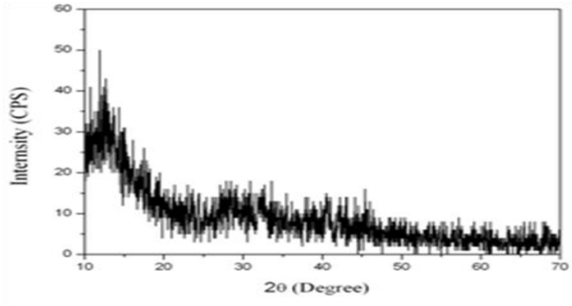

Figure 4. XRD patterns of synthesized iron oxide nanoparticles by *M. exotica* aqueous extract

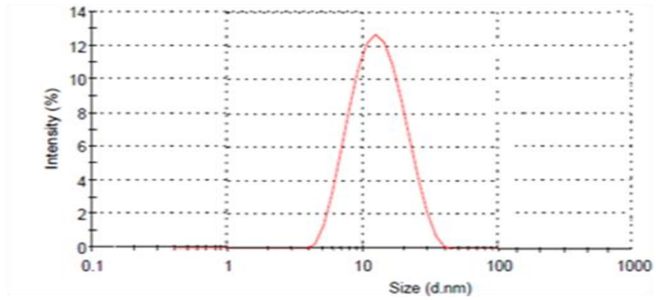

Figure 5. particle size analysis of synthesized iron oxide nanoparticles by *M. exotica* aqueous extract

FTIR analysis

Figure 3 shows the Fourier transform infrared (FTIR) spectra of magnetic nanoparticles. The strong absorption peaks at 3319, 2110, 2088, 1998, 1625 and 1506 $cm^{-1}$are assigned to O-H stretching, C≡N stretching vibrations, aliphatic C-H stretching, C-C multiple bond stretching, conjugated carbonyl (–C=O) group stretching vibration, O-H deformed vibtration and C-O stretching vibrations of synthesized iron oxide nanoparticles respectively. These functional groups are harmony with previous FT- IR spectrum of iron oxide nanoparticles synthesized by various extracts such as *Sargassum muticum* [26], *Passiflora tripartita* var. *mollissima* [27] and *Caricaya papaya* [28].

XRD analysis

The X-ray diffraction (XRD) patterns of Fe_3O_4 by *M. exotica* aqueous extract is shown in Figure 4. In figure 4, weak diffraction peaks with 2θ at 30.0°, 35.6°, 48.3°, 57.2° and 62.5° are observed, which indicate that the Fe_3O_4 particles have an amorphous structure.

DLS analysis

The particle size distributions of green synthesized iron oxide nanoparticles are shown in figure 5. The average size of iron oxide nanoparticles is found to be below100 nm. Similar work was done by Kumar *et al.* (2014) [27] who reported the average particle size of spherical iron oxide nanoparticles synthesized by *Passiflora tripartita* var. *mollissima* fruit is 22.3 ± 3 nm by DLS analysis.

SEM analysis

To determine the morphology and the average size of Fe_3O_4 particles, scanning electron microscopy (SEM) is used. The SEM image shows that magnetite nanoparticles have a mean diameter of about 50nm and a nearly spherical shape. The SEM image of iron oxide nanoparticles synthesized by *M. exotica* aqueous extract was shown in figure 6. and the size of the iron oxide nanoparticles ranges from 44.5 to 61.9 nm. This is comparable to the findings of Wang *et al.* (2014) [29] who reported the size of iron nanoparticles by using *Eucalyptus* leaves was diameter ranging from 20 to 80 nm. On the contrary Latha and Gowri (2014) [28] analysed the SEM image of iron oxide nanoparticles synthesized by *Carica papaya* leaf extracts demonstrated uniformly distributed spherical shaped particles. The increase in the size of nanoparticles confirms the presence of iron oxide nanoparticles with agglomerated in its structures.

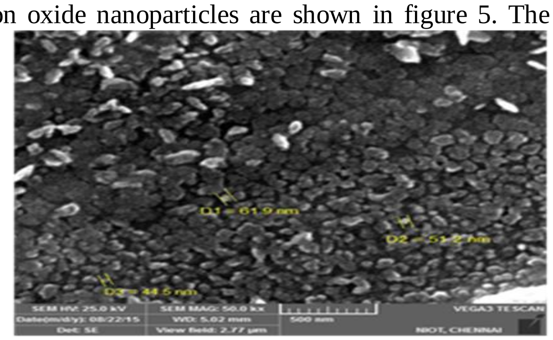

Figure 6. SEM image of synthesized iron oxide nanoparticles by *M. exotica* aqueous extract

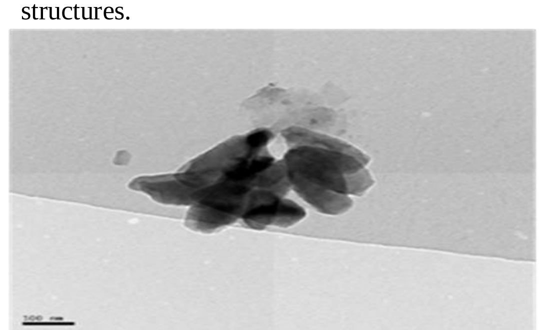

Figure 7. HR-TEM image of synthesized iron oxide nanoparticles by *M. exotica* aqueous extract

Table 1: Antibacterial activity of iron oxide nanoparticles synthesized by *M. exotica* aqueous extract

Name of the bacterial pathogens	Green synthesized magnetic iron oxide nanoparticles					Standard antibiotic
	10 µg/disc	15 µg/disc	20 µg/disc	25 µg/disc	30 µg/disc	Norfloxacin 20 µg/ disc
	Zone of inhibition (Diameter in mm)					
Bacillus cereus	10±1.0	12±1.0	15±1.7	16±1.0	18±2.0	20±0.0
Bacillus subtilis	12±2.0	14±1.7	15±1.0	16±1.0	18±2.0	22±0.0
Enterococcus faecalis	12±2.0	13±1.0	15±1.0	17±1.0	18±2.0	23±0.2
Escherichia coli	10±2.0	12±1.7	13±2.6	14±2.0	18±2.0	10±0.0
Klebsiella pneumoniae	8±1.0	9±1.0	10±2.0	12±2.0	14±1.0	16±0.1
Micrococcus luteus	10±1.7	12±1.0	13±0.9	15±0.9	16±0.5	13±0.2
Proteus mirabilis	9±1.1	10±1.0	8±0.7	11±0.6	13±1.0	14±0.0
Proteus vulgaris	12±0.9	13±1.4	15±0.9	17±1.4	18±1.4	16±0.0
Pseudomonas fluorescens	14±1.0	15±1.2	17±1.3	18±1.2	19±1.3	22±0.0
Staphylococcus aureus	7±0.8	8±1.0	10±1.2	11±1.0	12±1.0	15±0.0
Vibrio fluvialis	11±1.1	9±1.1	9±0.5	10±0.9	11±1.0	14±0.2

HR-TEM analysis

The morphology and structure of the iron oxide nanoparticles were further investigated by HR-Transmission Electron Microscopy. Figure 7 shows the TEM image of iron oxide nanoparticles synthesized by aqueous leaves extract of *M. exotica*. TEM image also revealed the successful synthesis of nanosized iron oxide particles, the average core diameter of 100 nm and the nanoparticles are agglomerated and cluster. The aggregation might be due to a magnetic property of Iron oxide nanoparticles. Iron oxide nanoparticles have a large surface to volume ratio and possess high surface energies. Accordingly, they tend to aggregate so as to minimize the surface energies [30].

Antibacterial activity

In the present investigation, the different concentrations of green synthesized iron oxide nanoparticles were exhibited variable degrees of antibacterial activity against the tested bacterial pathogens (Table 1).The activity of the magnetite iron oxide nanoparticles was concentration dependent; with the increase in concentration the activity was also increased. The inhibition activity of the iron oxide nanoparticles were compared with standard antibiotic Norfloxacin. The iron oxide nanoparticles showed minimum zone of inhibition (ranging 7-14mm) against the tested pathogens at 10 µg/disc concentration. The maximum zone of inhibition (ranging 17-19mm) was observed at 30 µg/disc concentration of iron oxide nanoparticles. These findings are in agreement with the earlier research on the antibacterial activity of iron oxide nanoparticles synthesized by *Lawsonia inermis* and *Gardenia jasminoides* leaves extract against *E. coli*, *P. mirabilis* and *S. aureus* [31]. Likewise, in another study by Groiss *et al*. (2017) [32] who reportediron oxide nanoparticles synthesized by leaf extract of *Cynometra ramiflora* exhibited effective inhibition against *E. coli* and *S. epidermidis.*

CONCLUSION:

For the first time, biosynthesis of magnetic iron oxide nanoparticles by using *M. exotica* L aqueous extract is reported. Measurement of UV, IR, XRD, DLS, SEM, and TEM analysis confirmed the structures. The antibacterial activity of iron oxide nanoparticles showed potent activity against human pathogens. On the basis of this research work, green synthesized iron oxide nanoparticles can be a good source for alternative therapy of bacterial diseases. The study can be extended for nanomedicine application and preclinical studies in relevant animal models.

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