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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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, antinoiceptive [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 ethnomedical 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.
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 biospectrophotometer Elico-Bi-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 Fe₂O₃ [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.
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 vibration 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\(_3\)O\(_4\) 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\(_3\)O\(_4\) 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 below 100 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\(_3\)O\(_4\) 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.
The activity of the magnetite, osculating 17-25µg/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 reported iron
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.

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

<table>
<thead>
<tr>
<th>Name of the bacterial pathogens</th>
<th>Green synthesized magnetic iron oxide nanoparticles</th>
<th>Standard antibiotic</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>10 µg/disc</td>
<td>15 µg/disc</td>
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<tr>
<td>Bacillus cereus</td>
<td>10±1.0</td>
<td>12±1.0</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
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<td>14±1.7</td>
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<td>9±1.0</td>
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<tr>
<td>Micrococcus luteus</td>
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<td>12±1.0</td>
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<tr>
<td>Vibrio fluvialis</td>
<td>11±1.1</td>
<td>9±1.1</td>
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</tbody>
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