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Research Article

# Mirabilis jalapa Flower Extract as Therapeutic Agent and Cellular Delivery by Nanoparticles

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#### **Abstract**

Bio friendly green modest syntheses of nanoparticles are the present research in the extremity of nanotechnology. This study has been undertaken to explore the determinants of iron nanoparticles from 1 mM FeSO $_4$  solution through profuse concentration of aqueous flower extract of *Mirabilis jalapa* reducing besides immobilizing agent. The attribute of iron nanoparticles was studied by using UV-VIS spectroscopy SEM and XRD. The XRD spectrum of the iron nanoparticles established the presence of elemental copper signal. Green synthesized iron nanoparticle manifests the zone of inhibition against isolated human pathogenic (Streptococcus species, Bacillus species, Staphylococcus species, Klebsiella species and E. coli) bacteria. The analytical chassis contains the flower pigment betalain the natural food dye resources can efficiently use in the production of iron nanoparticle and it could be utilized in various fields in therapeutics and nanotechnology.

Keywords: Nanoparticles, Mirabilis jalapa, UV-VIS spectroscopy, SEM- XRD.

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#### **INTRODUCTION:**

Mirabilis jalapa (four O' Clock plant) belongs to the family Nyctaginaceae. Mirabilis jalapa bloom all the summer long. They have antifungal, antimicrobial, antiviral, antispasmodic and antibacterial properties<sup>1</sup> Mirabilis jalapa flower contains betalain pigments are classified in to red (crimson) betacyanin's and yellow betaxanthins2. Antibacterial properties of iron are documented since 1000 B.C., when iron vessels were used to preserve water3. Environmentally friendly antimicrobial nano paint can be developed4. Iron has been known to possess strong antimicrobial properties both in its metallic and nanoparticle forms hence; it has found variety of application in different fields<sup>5</sup>. The FeSO<sub>4</sub> attached Fe nanoparticles can be used for the treatment of water and easily removed using magnetic field to avoid contamination of the environment<sup>6</sup>. The iron nanoparticles are reported to show better wound healing capacity, better cosmetic appearance and scar less healing when tested using an animal model<sup>7</sup>. Although few iron-containing compounds are approved by the FDA for direct food contact, copper incorporated food packaging is quite widespread in Japan7. Silica gel micro-spheres mixed with silica thiosulfate are used for long lasting antibacterial activity8 and treatment of burns and various infections9. Iron has been known to possess strong antimicrobial properties both in its metallic and nanoparticle forms hence; it has found variety of application in different fields. The FeSO<sub>4</sub> attached Cu nanoparticles can be used for the treatment of water and easily removed using magnetic field to avoid contamination

of the environment<sup>10</sup>. Iron nanoparticles can be used for water filtration<sup>11</sup>. Use of plant sources offers several advantages such as cost-effectiveness, eco-friendliness and the elimination of high pressure, energy, temperature, and toxic chemicals necessary in the traditional synthesis methods<sup>12</sup>. The most important application of iron and iron nanoparticles is in medical industry such as topical ointments to prevent infection against burns and open wound. Iron ions (Fe+) and its compounds are highly toxic to microorganisms exhibiting strong biocidal effects on many species of bacteria but have a low toxicity towards animal cells13. It has also been proposed that the antibacterial mechanism of iron nanoparticles is related to the formation of free radicals and subsequent free radical-induced membrane damage<sup>14</sup>. The iron nanoparticle containing poly vinyl nano-fibers also show efficient antibacterial property as wound dressing<sup>15</sup>. Iron zeolite is used in food preservation, disinfection and decontamination products<sup>16</sup>. It was also observed that when treated with Fe+, E. coli, a gram-negative bacterium, sustained more structural damages than the gram-positive Staphylococcus aureus 17. Iron impregnated medical devices like surgical masks and implantable devices show significant antimicrobial efficacy<sup>18</sup> conducted a study to observe the effects of copper ions on gram positive (Staphylococcus aureus) and gram-negative bacteria (Escherichia coli). Use of plant sources offers several advantages such as effectiveness, eco-friendliness and the elimination of high pressure, energy, temperature, and toxic chemicals necessary in the traditional synthesis methods<sup>19</sup>. Cell membrane detachment from the cell wall, cell wall damage, and electron dense granules outside and, in some instances, inside the cell. It was proposed that condensation of DNA occurred as a protective measure in order to protect the genetic information of the cell<sup>20</sup>. Iron zeolite is used in food preservation, disinfection and decontamination of products<sup>21</sup>. Iron sulfadiazine depicts better healing of burn wounds due to its slow and steady reaction with serum and other body fluids<sup>22</sup>. Toxicity from copper is observed in the form of argyria, only when there is a large open wound and large amount of copper ions are used for dressing. There are no regular reports of iron allergy<sup>23</sup>. Studies shows that copper nanoparticles anchor to and penetrate the cell wall of Gram-negative bacteria<sup>24</sup>. cell membrane detachment from the cell wall, cell wall damage, and electron dense granules outside and, in some instances, inside the cell. It was proposed that condensation of DNA occurred as a protective measure in order to protect the genetic information of the cell<sup>25</sup>. Iron sulfadazine depicts better healing of burn wounds due to its slow and steady reaction with serum and other body fluids<sup>26</sup>. The main objectives of this study were to (1) Synthesize the Iron nanoparticles using aqueous flower extract of Mirabilis jalapa, (2) characterization of Iron nanoparticles by using UV-Vis spectroscopy, SEM-XRD (3) analyse antimicrobial properties against human pathogenic bacteria.

#### **MATERIALS AND METHODS:**

#### i. Sample collection:

Mirabilis jalapa flower extract were collected from Thodupuzha, Idukki district of Kerala state, India. The fresh flowers were collected and washed with distilled water. The plant materials were thoroughly washed with distilled water and fresh weight were determined. The samples are then oven dried at 50°C for 24 h. The dried samples were powdered using a waring blender and stored in air-tight bottles until further analysis.

# ii. Extraction Method:

Mirabilis jalapa flower extract was prepared with 10 g of fresh flower taken in a beaker. It was thoroughly washed with tap water and then with distilled water for at least 2 times and cut into small pieces. The chopped flowers were boiled in 50ml of distilled water for 5 minutes. The flower pigment extract was then cooled and filtered. The filtered flower pigment samples collected and stored in air tight bottles at 4°C until further analysis.

# iii. Synthesis of Iron Nanoparticles:

Stock solution was prepared by dissolving 1mM Iron sulphate (FeSO<sub>4</sub>; Merck, Chennai, India) and volume made up to 250 ml with distilled water. 5ml of Mirabilis jalapa flower extract was added to 100 ml of 1mM FeSO<sub>4</sub> solution and allowed to react at room temperature.

#### iv. Test Microorganisms:

The common human pathogenic organisms used (Staphylococcus, Bacillus, Streptococcus, Salmonella, Klebsiella and Escherichia coli). The test organisms were obtained from microbial stock cultures, District hospital Thodupuzha, Idukki, Kerala.

#### v. Characterization of Iron Nanoparticles:

*UV -Vis Spectroscopy* the periodic scans of the optical absorbance between 380 and 500nm with a UV- Vis spectrophotometer (Model 118, Systronics, Mumbai, India) at a resolution of 1 nm were performed to investigate the reduction rate of iron ions by *Mirabilis flower* pigment extract. The reaction mixture was diluted 20 times and used

for UV-Vis spectrophotometry. Deionized water was used to adjust the baseline.

#### vi. Antibacterial Assay:

Nutrient agar and Muller Hinton agar plates were made according to standard microbiological protocol. Filter paper discs of approximately 6 mm diameter were soaked with  $10\mu l$ ,  $20\mu l$ ,  $30\mu l$ ,  $40\mu l$ ,  $50\mu l$  respectively of the flower pigment extract, FeSO<sub>4</sub> and copper nanoparticle separately and allowed to dry at room temperature for 15 minutes. Muller Hinton Agar plates were prepared and the test microorganisms were inoculated by the spread plate method. Prepared discs were placed in the previously prepared agar plates. Each plate of every test organisms contained discs impregnated with Fe nanoparticle, flower extract, copper sulphate solution and an antibiotic disc. The discs were pressed down to ensure complete contact with the agar surface and distributed evenly so that they were not closer than 24 mm from each other, to Center. The agar plates were then incubated at 37°C. After 16 to 18 hours of incubation, each plate was examined. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of inhibition were measured, including the diameter of the disc where the antibiotic was used as control 27.



Figure 1: a) Mirabilis jalapa plant with flower b) Mirabilis jalapa fresh flower extract c) Mixture of Mirabilis jalapa flower extract and iron sulphate over 2 hours incubation.

### **RESULTS AND DISCUSSIONS:**

# i. Synthesis of Iron Nanoparticles:

After the addition of *Mirabilis jalapa* flower extract to FeSO<sub>4</sub> solution a visible colour change from transparent to dark green was observed which indicates the formation of copper nanoparticle. This occurred due to the reduction of copper ions present in the solution due to terpenoids present in *Mirabilis jalapa* flower extract. After 90 minutes there was no change in the intensity of colour developed, which indicates the completion of reduction reaction. The reduced iron particles are in the range of nano size.

#### ii. Characterization of Iron Nanoparticles:

*UV Spectrometry* the UV absorption spectrum of iron nanoparticles from *Mirabilis jalapa* flower extract of different concentrations was obtained as given in Figure 2.

# iii. Anti-Bacterial Assay:

For *Mirabilis jalapa* flower the zone of inhibition was found to be 13-26 mm for *Klebsiella species*, 14-28 mm for *Bacillus species*, 11-25mm for *E. coli*, 12-25 mm for *Staphylococcus species*, 14-25 mm for *Salmonella species*, and 12-27 mm for *Streptococcus species*. The study done by<sup>28</sup> the zone of inhibition found was 13-26 mm for *Klebsiella species*. Silver ions and iron salts are used as antimicrobial agents<sup>29</sup>. However, the high concentrations of iron salts restrict the use of them in present day medicine. Use of metal

nanoparticles decreases the concentration of iron and other metal salts<sup>30</sup>. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio which allows them to interact closely with microbial membranes and is not merely due to release of metal ions in solution or in culture plates<sup>31</sup>. The mode of action of both iron nanoparticles and iron ions were reported to be similar, although the nanoparticles were reported to be effective at significantly lower concentration than that of the ions<sup>32</sup>. According to<sup>33</sup>, the attachment of both iron ions and nanoparticles to the cell membrane caused acclimatization of envelope protein precursors causing dissipation of the protein motive force.

#### iv. SEM-XRD Analysis:

SEM-XRD analysis proved the effective formation of iron nanoparticles in *Mirabilis jalapa* flower pigment extract. SEM-XRD analysis was carried out in instrument JSM 6390 with acceleration voltage 20 kV. SEM reveals information about the sample including external morphology, chemical composition and crystalline structure and orientation of materials making up the sample<sup>35</sup>. SEM provides detailed high-resolution images of the sample by rastering a focused electron beam across the surface and detecting secondary or back scattered electron signal. The XRD imaging of the iron nanoparticles was performed to confirm the presence of elemental metal signal and provides quantitative compositional information<sup>36</sup>.

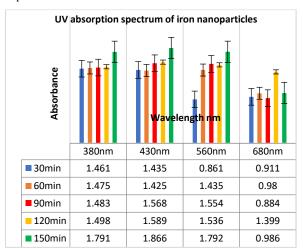
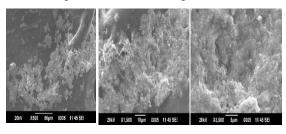


Figure 2: Mirabilis jalapa flower extract UV absorption spectrum of iron nanoparticles



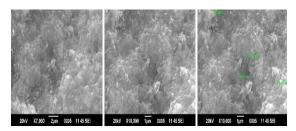


Figure 3: Mirabilis jalapa flower extract iron nanoparticles - SEM microgram of iron nanoparticles.

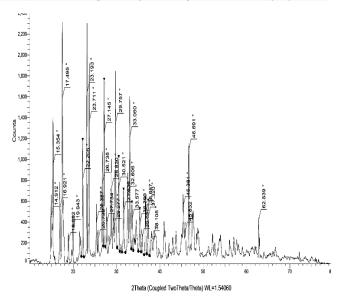


Figure 4: *Mirabilis jalapa* flower extract iron nanoparticles - X-Ray Diffractogram Fe (Coupled Two Theta/Theta)

Table 1: Zone of inhibition against various bacteria (Escherichia coli, Salmonella typhi, Staphylococcus species, Klebsiella species, Bacillus species,) using iron nanoparticles produced by Mirabilis jalapa flower pigment 1/100 dilution.

Microorganisms	control	Sample	Measure zone of inhibition in mm				
			10 μl	20 μl	30 μl	40 μl	50 μl
Escherichia coli	11	13	16	19	20	23	25
Salmonella typhi	13	11	15	17	19	24	27
Staphylococcus aureus	12	15	17	19	21	23	25
Klebsiella species	13	14	16	17	19	24	26
Bacillus species	14	12	16	18	23	26	28
Streptococcus species	12	12	14	16	18	23	25

# **CONCLUSION:**

The Mirabilis jalapa flower extract was draw up form fresh by boiling it 5 minutes. The prevail extract was of reddish in colour. Freshly prepared flower extract was appended to iron sulphate solution and the reaction takes place at room temperature which resulted in the synthesis of iron nanoparticles. The synthesized nanoparticles were characterized by UV-VIS spectrometry, SEM and XDX measurements. The UV-VIS spectra of iron nanoparticles formed in the reaction media has absorbance peak at 380 nm. It has been indicated that Mirabilis jalapa extract is competent of fabricating iron nanoparticles that shows good firmness in solution. The synthesized iron nanoparticles were distinguished by UV-VIS spectrum, SEM and XDX measurements. This green synthesis method is substitute to chemical method, since it is contemptible, non-polluting and green-friendly. The results showed that Mirabilis jalapa flower plays a salient role in the reduction and stabilization of iron-to-iron nanoparticles. Further, these synthesized iron nanoparticles from *Mirabilis jalapa* shows antibacterial activity on human pathogenic bacteria.

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### **Conflicts of Interest: Nil**

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