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Biogenic green synthesis: environmentally friendly route for synthesis of iron nanoparticles utilizing *Ficus religiosa* leaves and assessment of their antimicrobial properties

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Abstract

A crucial aspect of nanotechnology is the creation of biologically driven methods for environmentally friendly synthesis of nanoparticles. In this research article, we show a green and biogenic approach to synthesizing metal nanoparticles by using plant extract. Iron nanoparticles have been synthesized by processing anhydrous ferric chloride with an extract from *F. religiosa* leaves. In this process, the extract from *F. religiosa* leaves serves as a reducing agent as well as a capping agent. Solutions have been prepared in the proper concentration and used with the proper procedure and time to make *Ficus religiosa*-iron nanoparticles (FRFeNPs). Stirring time, temperature, and rpm have been carefully maintained throughout the process as they have significantly influenced the properties of the nanoparticles. First confirmation comes from colour change during the reaction which indicate the production of FRFeNPs. Again, confirmation of iron nanoparticles formation during the reaction has been established by the UV-Vis Spectrograph showing peak at 450 nm. The synthesized FRFeNPs have been further characterized using different instruments, including a FTIR Spectroscopy, FESEM-EDX, XRD, and DLS. Antibacterial activity of iron nanoparticles has been assessed using the well-diffusion method. FRFeNPs tested against both gram +ve & -ve bacteria i.e. *Pseudomonas fluorescens*, *E. coli*, *S. aureus*, and *B. subtilis*, respectively. Significant activity of the FRFeNPs was demonstrated by antimicrobial activity.

Keywords: Nanoparticle, Green synthesis, FRFeNPs, Ferric chloride anhydrous, *Ficus religiosa*

1. Introduction

The Nanotechnology involves manipulating matter on the nuclear, atomic, and supermolecular scale[1,2]. The name "Nano" comes from the Latin word ‘nanus’ which also means dwarf[3,4]. The study and manipulation of matter at incredibly small sizes, mainly between 1 to 100nm is called

Nanotechnology. Nanotechnology includes the manufacture, strategy, classification, & implementation of equipment, structural features, as well as frameworks via handling both shape and size at the nanoscale. A nanometre is one thousand millionth of a meter, while nano size is defined as one thousand millionth of a given unit. Nanotechnology has a very wide area of applications like innovative methods for the production of new materials by consuming less energy[5], detection and diagnosis in medical fields[6–8], cosmetics[9], construction[10], environmental

remediation, paints, smart materials like self-cleaning glasses, nanoglues, automobile[8], food and agriculture[11].

Nanomaterials exhibit different properties compared to the bulk material because when nanomaterials were made from the bulk, their dimensions decrease at the atomic level. Generally, nanoparticles are categorized into two types: organic nanoparticles and inorganic nanoparticles. NPs made from carbon, such as fullerenes, are included in organic nanoparticles. NPs of semiconductors like ZnO and TiO_2 as well as metal nanoparticles (Al, Au, and Ag) come under inorganic NPs[12].

There are two distinct methodologies for synthesizing nanoparticles. The top-down or destructive method involves breaking down large material into little particles using various techniques like grinding, ball milling, laser & thermal ablation, and sputtering. The bottom-up or constructive process involves building up the material from atoms to complexes to nanoparticles for example production of nanoparticles through chemical, biological, or electrochemical methods[13].

Compared to chemical and physical methods of production, biosynthesis (Bacteria, Fungus, Plant, etc) of nanoparticles has recently been considered as cost-effective, safer, and more environmentally friendly. Currently, a vast range of physical, chemical, biogenic, and hybrid techniques can be used to develop a variety of NPs. The physical and chemical techniques for synthesizing nanoparticles involve toxic solvents, synthetic reducing agents and synthetic capping agents (like $NaBH_4$, ethylene glycol hydrazine and CTAB), a lot of energy, and non-biodegradable stabilising agents. Therefore, researchers have pioneered a number of synthetic pathways for nanoparticle production, which revealed a considerable advantage for nature and the environment via clean, non-toxic, and ecologically sustainable "green chemistry" techniques using bacteria, fungus, and plants[14,15].

A biogenic synthesis of metal nanoparticle is a bottom-up approach, which involves microorganisms (fungus, bacterium or algae)[16–18] and different plant based extracts such as extract of roots[19], leaves[20], peel[21], fruits[22], flower[23], seeds[24] and stems[25]. These green materials

contain polyphenols and proteins, which can act as reducing agent to synthesize metal nanoparticles[26]. Some examples show the capability of the green route over the conventional method for the manufacturing of metal nanoparticles, such as size of Fe_3O_4 nanoparticles produced via a green method using the leaves extract of *Hibiscus rosa-sinensis* comes out to be 2–80 nm. In this reaction leaves extract performs the role of both reducing and capping agents. Whereas the nanoparticles, which were made by a wet chemical method had a particle size of 87-400 nm. Nanoparticles manufactured through the green method were stable, unlike nanoparticles produced through the wet chemical method, which are not stable and start aggregation[27].

Padalia et al. utilized the flowers extract of *Tagetes erecta* to produce silver nanoparticles. The average particle size was 46.11 nm. Some of the nanoparticles have a spherical and hexagonal shape, while others have an irregular shape. Wang *et al.* prepared spheroidal shape Fe nanoparticles utilising a leaf extract of *eucalyptus.* The size of these nanoparticles was between 20 and 80 nm. As synthesized Fe nanoparticles showed great potential in the waste water treatment[28,29]. *Wei et al.* produced Fe nanoparticles utilising peel extract of *Citrus maxima*. The size of these nanoparticles was between 10 and 100 nm.[30]. Hoag *et al*. performed the manufacturing of iron nanoparticles utilizing plant material i.e. tea extract. TEM analysis signified the existance of spherical nanoparticles with sizes ranges from 5 to15 nm[31]. Venkateswarlu *et al.* produced magnetic (Fe_3O_4) nanoparticles by utilising low-cost bio reducing agent, plantain peel extract. TEM analysis showed that the nanoparticles had sizes ranging from 30 to 50 nm[32].

Nanoparticles showed very good antimicrobial behaviour. In today's world, microbial resistance towards tradition drugs is a big problem. So the need of new antimicrobial agents is increases. Plants extracts have always been used as antimicrobial agents. Nanoparticles typically have a size between 1-100 nm, which is highly suitable for their antimicrobial properties. On account of their exceptionally tiny size and huge surface area, NPs interact more extensively with the cell wall of bacteria[33,34].

Large perennial tree *Ficus religiosa* commonly known as peepal, which is glabrous when young and found out throughout India's plains but in Himalayas it can grow as tall as 170 metres. Generally, this tree is planted on the side of the street or road, particularly in the vicinity of temples [35]. It is a member of the *Ficus* genus and family *Moraceae*. It is one of the earliest trees mentioned in Indian literature. Herbal remedies are made from almost every component of this tree, including the fruits, leaves, seed and bark. It is a medicinal plant that has gained popularity as a reliable source of traditional medicine for the diagnosis of sevral conditions like

asthma, bacterial infections, diabetes, gonorrhea, hiccups, diarrhea, stomach issues, inflammatory and infectious diseases[36].

Ficus religiosa (Fig. 1) leaves are a good source of secondary metabolites that are utilised as larvicides and antimicrobials. They contain tannic acid, leucine, isoleucine, methionine, tryptophan, threonine, glycine, asparatic acid, serine, and arginine. The bark also contains bergaptol and bergapten. It is of bitter-sweet and acrid properties make it suitable for use as a laxative, purgative, aphrodisiac, and astringent[35,37,38].

Fig. 1 *Ficus religiosa* Leaf

2. Materials and Methods

2.1. Chemicals

All chemicals used in this research work were of analytical quality and employed without further refinement. $FeCl_3$ & ethanol were purchased from Loba Chemie Pvt. Ltd. India). Nutrient Agar purchased from Himedia Laboratories Pvt. Ltd. (India). *Ficus religiosa* leaves

(Fig. 1) were gathered from the campus of BMU, Rohtak. Solutions were made using deionised water.

2.2. FR leaves extract

Initially, leaves of *F. religiosa* were washed mutiple times with DI water to eliminate dirt and sandy particles. Dried those leaves in the sunlight to remove moisture content. The dried leaves were subsequently powdered by grinding. Weigh 10g of dried leaves and pour them in 100 ml of DI water in a 300 ml conical flask. The mixture was then subjected to reflux at 80 °C for a duration of 60 minutes. The aqueous leaves extract was filtered with the help of rotary vacuum pump using Whatman filter paper No. 1. In an Amber bottle filtrate was kept at 4°C for further use[39].

2.3. Biosynthesis of FR-FeNPs

150 ml of 0.1M $FeCl_3$ was taken in volumetric flask and stirred magnetically at 37°C for 15min. 75 ml of leaf extract was dispersed drop by drop into it. Solution was kept at 70-75°C and 1000 rpm until the colour change. Change in the colour from brown to Blackish colour indicate the reduction of Fe^{3+} and formation of FR-FeNPs. Confirmation of FR-FeNPs synthesis was achieved through UV-Vis spectroscopy. The solution was subsequently centrifuged for 20 minutes at 10000 rpm. Following the centrifugation process, the supernatant was discarded, and the pellets were rinsed with ethanol several times. Pellets were dried at 60°C upto 12 hrs and stored at 4°C for further characterisation[39].

2.4. Characterization

Characterization of newly synthesized FR-FeNPs were done using several characterization techniques like FESEM-EDX, FT-IR, XRD, DLS, UV-Vis. For the FTIR measurements, the dried FR-FeNPs samples were ground with KBr to create pellets. The FTIR spectrum was recorded within the range of 4000-400 cm^{-1} using a Spectrophotometer (Spectrum BX1, Perkin-Elmer). UV-Vis absorption spectra were captured using SHIMADZU UV-2450 spectrophotometer, across the range of 200-800 nm. Diffraction pattern were recorded on Malvern Panalytical Empyrean diffractometer having Cu-K Alpha wavelength is 1.540598 and Scan range is 4.99º to 90 º. Scan rate was 0.0001º. The particles size and Zeta potential of the newly produced iron nanoparticles were recorded with Litesizer 500 (Anton Paar, Austria). Surface morphology of FR-FeNPs were investigated by FESEM (Merlin Compact 6073, Carl Zeiss, Germany). EDX analysis performed on Oxford maxN.

2.5. Antibacterial Activity

The antimicrobial behaviour of synthesized FRFeNPs was assessed using the agar well diffusion method. The plates of nutrient media were prepared. The chosen bacteria's cultures were evenly spread on the agar plates in an amount of around 30µl. A sterile borer was used to create four wells on each plate, each measuring about 6 mm in diameter. Fe NPs, leaf extract, and $FeCl_3$ (20 µl) were added into the wells after the antibiotic disc (streptomycin) was placed on the plate. The inhibitory zones on the petri dish were observed after 24 hr of incubation at 37°C. We have checked antimicrobial behaviour toward both gram positive and Gram negative bacteria[40,41].

3. Results

3.1. UV-Visible Spectrophotometer

The synthesized NPs were evaluated by UV-visible spectroscopy and the result shown in Fig. 2a. It is clear that the graph shows the maximum absorption peak of 450 nm which is the confirmation of formation of iron nanoparticles[42]. The optical absorbance was studied using UV-Vis spectrophotometer with a resolution between 200-800 nm. It follows the Beer-Lamberts law, which states that "absorption is directly proportional to the path length and concentration to the solution".[43]

$$\boldsymbol{A = \varepsilon CL}$$

Here, A = absorption, ε = molar absorptivity constant, C = molar concentration of the sample, L = path length of the sample cell.

3.2. Fourier-Transform Infrared Spectroscopy

Infrared spectroscopic results reveals the presence of phenolic -OH (3470 & 3419 cm^{-1})[44]. 2938 cm^{-1} due to CH & CH_2 aliphatic hydrocarbon[44,45] while 1692 cm^{-1} indicates the carbonyl group[45]. Two bands at 1639 and 1618 cm^{-1} represent C=C aromatic ring stretching vibrations[39]. 1561, 1414, 1344, 1279 and 1020 cm^{-1} due to N-H stretching vibrations[46], CH_2 bending[47], C-N stretching vibration of aromatic amines[39], C-O stretching vibrations[48], and Carbon-Nitrogen (C-N) stretching vibration by aliphatic amines[39]. Bands at 628, 642, 524, and 475 cm^{-1} confirms the synthesis of FRFeNPs and represent different forms of iron nanoparticles[49] shown in (Fig. 2b).

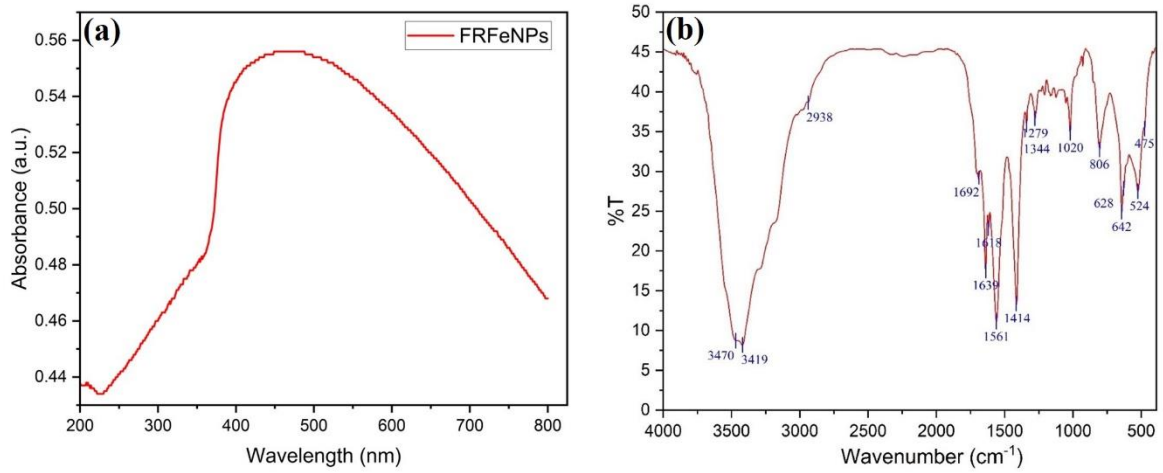

Fig. 2 (a) UV-Vis Spectral Analysis of FRFeNPs (b) FTIR Spectra of FRFeNPs

3.3. X-Ray diffraction (XRD)

The XRD analysis of the sample FRFeNPs was done over a range of 2θ from 4.994599997° to 90.00005004°. At the time of analysis XRD instrument has the following settings: Minimum step size 2 Theta: 0.0001, Minimum step size Omega: 0.0001, Cu-K-α1 wavelength: 1.540598, Cu-K-α2 wavelength: 1.544426, Generator voltage: 45, Tube current: 40, Scan step size: 0.0262606, Scan type: continuous, Time per step: 36.465. The major peaks in the pattern (Fig. 3a) have been identified, and their positions (2θ values) are 24.57, 33.46, 35.95, 41.25, 49.82, 54.41, 62.79 and 64.35°. These peaks represent the 012, 104, 110, 113, 024, 116, 214 and 300 planes. The Diffraction pattern of green synthesized Fe nanoparticles was matched with JCPDS card number 00-024-0072. XRD spectral analysis revels the presence of rhombohedral unit system[50,51].

3.4. Zeta potential

Zeta potential of the green synthesized Fe nanoparticles found to be -23mV (Fig. 3b), which indicates the presence of –ve charge on the synthesized nanoparticles. Negative charge indicates the repulsion between the synthesized nanoparticles and demonstrate their stability[52].

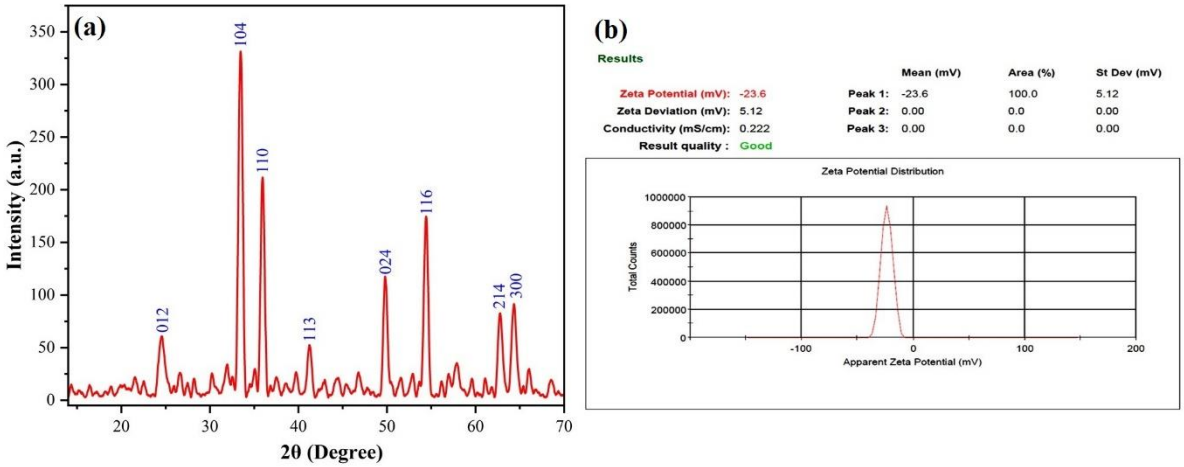

Fig. 3 (a) XRD analysis of FRFeNPs (b) Zeta Potential analysis of FRFeNPs

3.5. FESEM and EDX

Morphological investigation has much importance in understanding the shape and size of newly produced FRFeNPs. This analysis was done with FESEM. On the analysis of FESEM images, it is revealed that the nanoparticles having particle size in between 60 to 92 nm (Fig. 4a). FESEM images also reveal that the produced nanoparticles having spherical shape[44]. The presence of iron in a sample confirmed by EDX is shown in Fig. 4b[39].

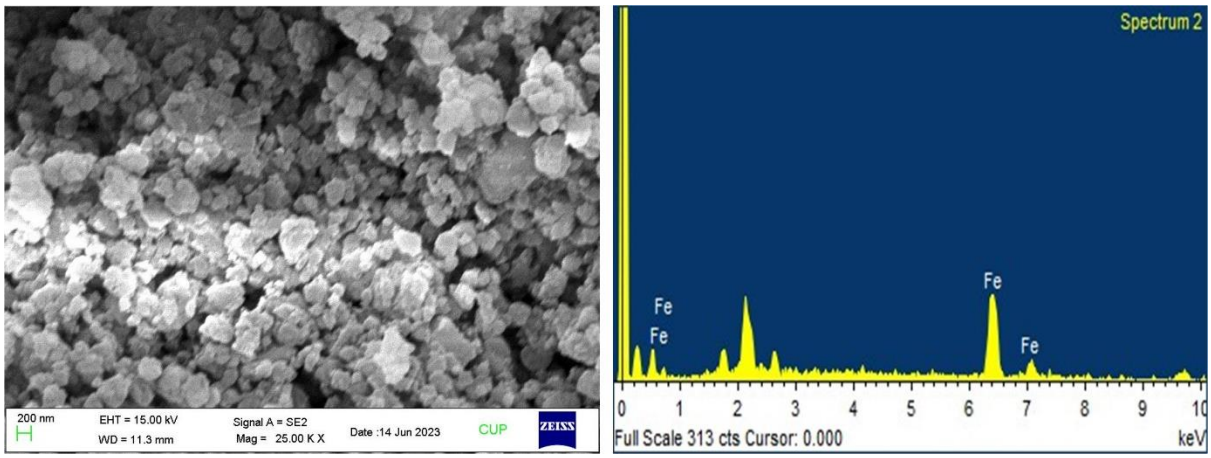

Fig. 4 (a) FESEM Image of FRFeNPs (b) EDX of FRFeNPs

3.6. Antibacterial Activity

Green synthesized FRFeNPs showed very good antimicrobial behaviour towards both type of bacteria (*Staphylococcus aureus*, *Bacillus subtilis, Pseudomonas fluorescens* and *Escherichia coli* i.e. gram positive & negative. The results shown in Table 1 reveal that synthesized FRFeNPs exhibited greater antimicrobial activity than both the plant extract and their

corresponding metal salt. Here, "A" represents control, "B" represents FRFeNPs, "C" represents metal salt ($FeCl_3$), and "D" represents plant extract. Zones of inhibition (ZOI) by control, FRFeNPs, metal salt, and plant extract are shown in Fig. 5(a-d). Graph shown in Fig. 5e describe the antimicrobial activity of FRFeNPs against both types of bacteria.

Table 1 Summary of ZOI

Sr. No.	Bacteria	Size in mm			
		A	B	C	D
1.	*Staphylococcus aureus* – (+ve)	30	28	26	20
2.	*Bacillus subtilis* – (+ve)	38	36	30	28
3.	*Pseudomonas fluorescens* – (-ve)	36	32	28	24
4.	*Escherichia coli* – (-ve)	36	30	26	24

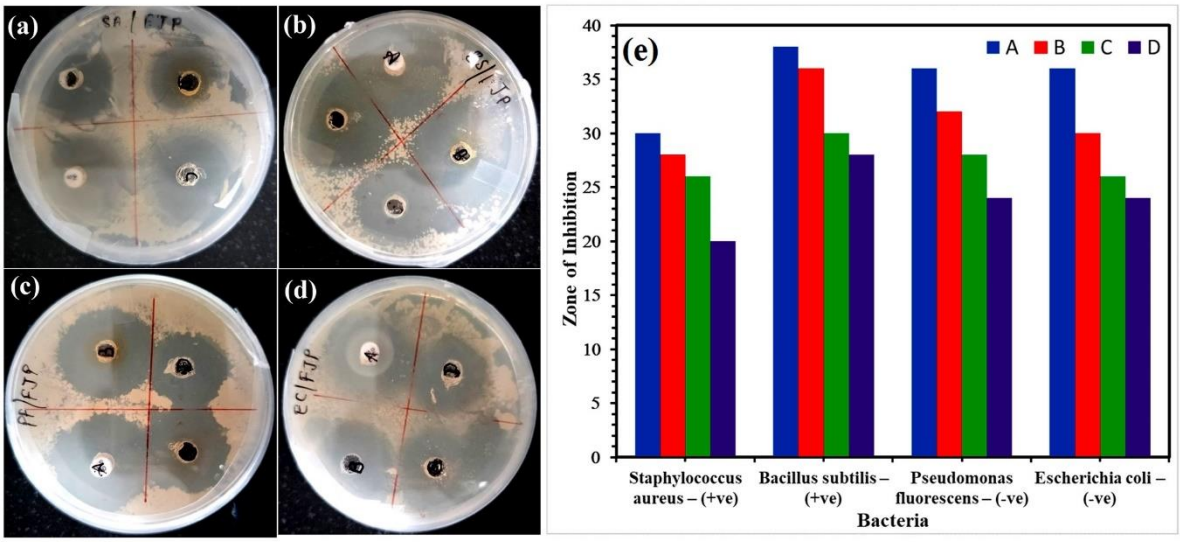

Fig. 5 Antibacterial activity against (a) *Staphylococcus aureus* (b) *Bacillus subtilis* (c) *Pseudomonas fluorescens* (d) *Escherichia coli* (e) Graph showing antibacterial activity of bacteria

3.7. Antibacterial mechanism of FRFeNPs

For nanoparticles to exert their antibacterial effects, they must come into contact with bacterial cells. After this nanoparticles start crossing the bacterial membrane and start disrupting shape and working of the cell. One of the most frequently suggested mechanism of metal oxide nanoparticles is oxidative stress (ROS). Pictorial representation in Fig. 6, Metal nanoparticles

perform antibacterial action by the production of reactive oxygen species (ROS). ROS are also termed as reactive species that contain oxygen. These species consists of superoxide radical, hydroxyl radical, H_2O_2, HOCl, Singlet Oxygen and Lipid Hydroperoxide (LOOH) etc. ROS is responsible for oxidative stress and causing the death of bacterial cell. The state known as oxidative stress is linked to damage by oxidation to biomolecules such as RNA, DNA, lipids, and proteins. While moderate oxidative stress can produce cell malfunction, fenceless oxidative stress often results in cell death. This phenomenon is responsible for antibacterial behaviour of the metal oxide nanoparticles. Unfortunately, till now the full mechanism behind the antibacterial action of FeNPs is not fully known[33,42,53].

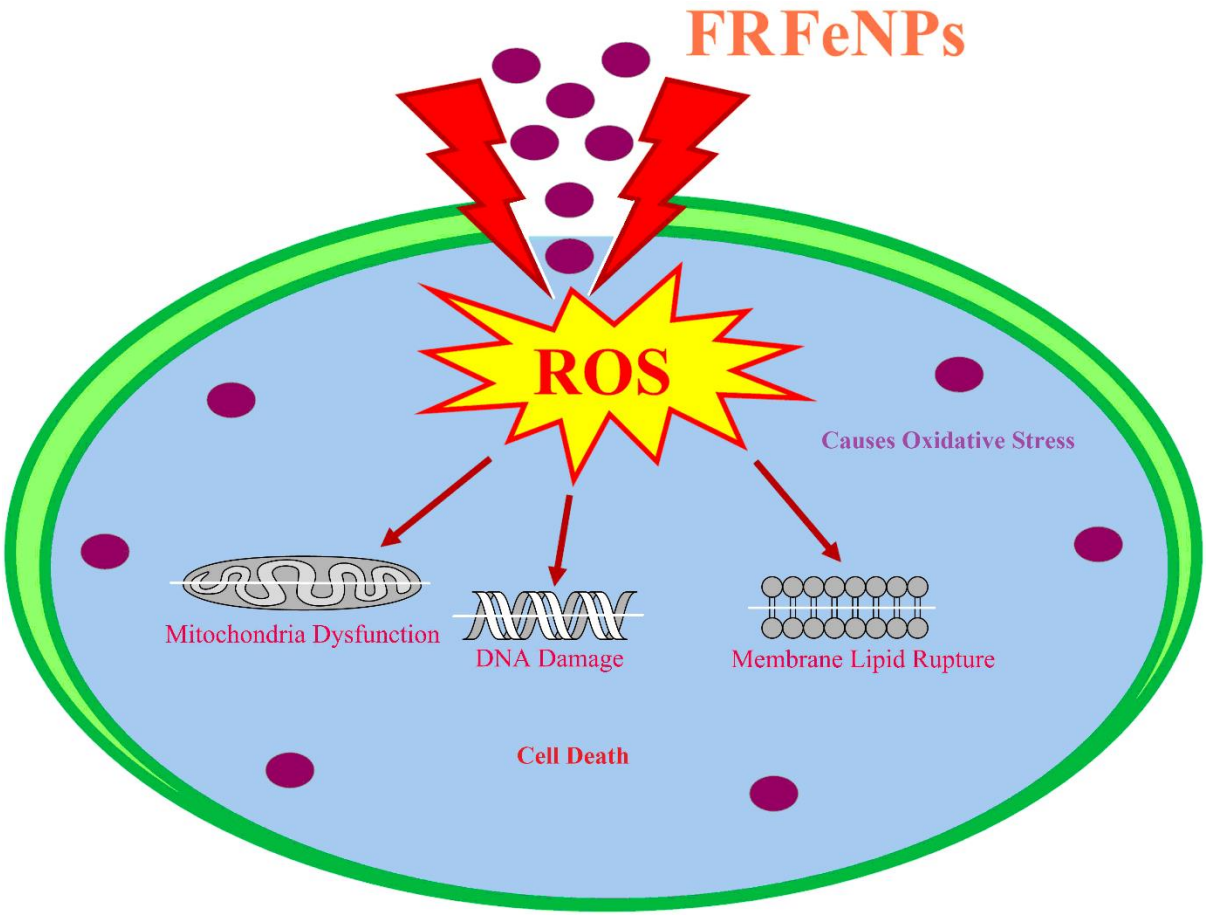

Fig. 6 A proposed antibacterial mechanism of FRFeNPs

4. Conclusion

The synthesis of iron nanoparticles via green route is the need of the hour. Iron nanoparticles were cheaply and efficiently prepared by a quick and green method. Ficus religiosa extract has several medicinal properties and plays a role as both a reducing and capping agent. Tremendous

about biogenic green method is the requirement of less energy and less time. *Ficus religiosa* leaves are easily and freely available across the Indian subcontinent. Overall, this synthesis method involves biodegradable and very cheap reducing and capping agent unlike the traditional manufacturing route for nanoparticles. Synthesized Ficus religiosa-based iron nanoparticles show better antimicrobial behaviour towards both gram +ve and gram -ve bacteria than both the leaves extract and metal salt.

5. Declaration of Competing Interest

The authors have stated that there are no financial or personal conflicts of interest pertaining to the article's content.

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