

Biosynthesis Of Iron Oxide Nanoparticles Using *Martynia Annua* Aqueous Leaf Extract, Its Antioxidant Potential And Antibacterial Ability Against Selected Wound Pathogens

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Abstract

*The present study demonstrates a green and eco-friendly method for the biosynthesis of iron oxide nanoparticles (Fe₂O₃ NPs) utilizing the aqueous leaf extract of *Martynia annua*. The reduction of FeSO₄ to iron oxide was initially indicated by a visible color change from yellow to brownish-black. UV-Vis spectroscopy confirmed the synthesis with a characteristic absorption peak at 267 nm. Functional group analysis through FTIR revealed the presence of phytochemicals such as hydroxyl groups, aldehydes, unsaturated esters, primary and aromatic amines, and alkyl halides, which likely acted as reducing and stabilizing agents. XRD analysis displayed diffraction peaks at 2θ angles of 25.68°, 13.26°, and 8.24°, confirming the crystalline nature of the nanoparticles. SEM images showed that the nanoparticles were spherical, smooth, and well-crystallized, while EDAX analysis detected strong signals for iron and trace elements like calcium, sodium, and magnesium. DLS analysis revealed an average particle size of 181.7 nm, and zeta potential studies confirmed high negative surface charge, indicating good stability and polydispersity. The synthesized iron oxide nanoparticles exhibited dose-dependent antioxidant activity and showed effective antibacterial potential against selected wound pathogens. These nanoparticles, capped with bioactive compounds from *M. annua*, enhance their therapeutic and biocompatible properties. The study thus highlights a simple, sustainable, and effective approach for producing iron oxide nanoparticles with promising biomedical applications.*

Keywords: *Martynia annua*, Iron oxide nanoparticles, Green synthesis, Antioxidant activity

INTRODUCTION

Nanotechnology is an emerging field of modern research which deals with the synthesis of particle structure at nanoscale level. The nanomaterial having a small scale size of about billionth meter expresses unusual physical and chemical characteristics. These weird properties of the nanomaterial may due to an increase in surface area as the particles get smaller or they might subjected to quantum effects. Nanoparticles had wider applications such as antimicrobial, antioxidant, antiinflammatory, antiviral, cytotoxic, anticancer and anti HIV properties (Govindappa et al., 2018).

Metal and metal oxide nanoparticles exhibit different physiochemical properties and they are differ from their native bulk compounds in several aspects which include its surface, optical, thermal and electrical properties.

Nanoparticles synthesis is generally carried out in a wide array of methods. However, these methods are associated with the use of heavy equipment, huge amount of energy output, highly toxic and dangerous chemical compounds that generate biological hazards and are not ecologically safe. Biological synthesis of nanoparticles using plant or plant extracts, microorganism, enzymes are stated as ecofriendly, as they are cost

effective, nontoxic, simple, rapid and dependable (Venugopal et al., 2017).

Iron oxide nanoparticles, being an instrumental in medical world due to its several biomedical applications in cancer therapy, drug delivery, magnetic resonance imaging (MRI), wastewater treatment, magnetic beads in bacterial capturing and in designing of sensor to detect various biothread agents (Vicky et al., 2010). Iron oxide nanoparticles (IONPs) with added unique functions has shown promise role in a variety of fields which includes, cosmetics, bioremediation, diagnostics and materials engineering. Moreover applications of iron oxide nanoparticles in biomedicine are expanding at an exponential rate. It has shown to be effective in controlled release of medicines for MRI, therapy of solid tumours and tissue healing. The excellent characteristics of this nano material viz., its greater surface area, small band gap and stability can be ascribed to its wide variety of applications. Intense study on nanoparticles synthesis reveal that the green chemistry approach produce varied size and forms of iron oxide nanoparticles using bacteria, fungi and plant extracts (Yeary et al., 2005; Roh et al., 2006; and Senthil et al., 2012). The elimination of complex procedure in maintaining cell cultures makes the production of nanoparticles using plant extract is preferable than other biological processes.

The well-known tiny herbaceous annual plant *Martynia annua* found all over India, is a member of the Martyniaceae (or Pedaliaceae). This plant has biologically active component, the leaves and fruits possess antibacterial and antiepileptic properties which is used to cure epilepsy, inflammation and tuberculosis (Nagdu Dhruti, 2009). The edible leaves of the plant is used topically to treat thyroid glands in the neck, gargled sore throats and for domestic animal wounds (Gupta et al., 2015). The whole plant is used to treat fever, hair loss, sores, scabies, snake bites and back abscesses (Flora et al., 2013; Hiral Gadhave and Himanshu Pandya, 2017).

The chemical analysis of this plant reveals the presence of glycosides, tannins, polysaccharides, phenols, flavanoids and anthocyanins (Singhai and Lodhi, 2011). In view of above, the current study was designed to synthesise, characterize and assess the antibacterial, antioxidant activity of synthesized iron oxide nanoparticles using *Martynia annua* aqueous leaf extract.

MATERIALS AND METHODS

Synthesis and characterization of iron oxide nanoparticles

Synthesis of iron oxide nanoparticles was made by mixing 5mL of prepared plant extract with 5mL of FeSO₄ and incubated in a dark condition. The colour change of the combined reaction mixture was observed and recorded. Further confirmation of synthesized iron oxide nanoparticle was done using UV-Visible spectrometer (Shimadzu- 1800) operating in the wavelength range between 200-800 nm.

In addition, the biosynthesized iron oxide nanoparticles was characterized using different analytical techniques. The functional group present in the synthesized iron oxide nanoparticles was confirmed using FTIR spectroscopy. The average particle size, surface morphology and elemental composition of synthesized nanoparticles was studied using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) (SIGMA model, CASL ZEISS, German) operating at an accelerating voltage of 3.00 KV. The particle size distribution of nanoparticles was evaluated using dynamic light scattering measurements (DLS) and zeta potential analysis of nanoparticles was carried out using Malvern zetasizer neroseris compact scattering spectrometer (Malvern Instruments Ltd., Malvern, UK) for their surface charge.

Determination of antioxidant activity of synthesized iron oxide nanoparticles DPPH free radical scavenging activity

The free radical DPPH is used to evaluate the ability of compounds as freeradical scavengers and hydrogen suppliers. It is a rapid, simple and inexpensive method for testing antioxidant capabilities of biological compounds.

The free radical scavenging ability of synthesized iron oxide nanoparticles was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH (Nagdu Dhruti, 2009). The biosynthesized nanoparticles suspension of various concentrations (10-200µg/mL) was thoroughly mixed with 0.1 mM DPPH in 1 mL methanol solution. The mixture was allowed to stand 30 minutes in the dark and the absorbance was measured at 517 nm. An equal amount of DPPH and water was used as blank and ascorbic

acid was used as standard. Percentage inhibition of DPPH free radical was calculated based on the blank reading, which contain DPPH and distilled water without any extract using the following equation:

$$\text{DPPH Scavenging activity(\%)} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

Where A_{blank} was the absorbance of the blank and A_{sample} was the absorbance of the sample in the presence of the iron oxide nanoparticles. The antioxidant activity of the iron oxide nanoparticles was expressed as IC₅₀. The IC₅₀ value is the concentration (in µg/mL) of iron oxide nanoparticles that inhibits the 50% DPPH free radical formation.

Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) assay is common method that uses antioxidants as reductants in a redox linked colorimetric reaction, wherein Fe^{3+} is reduced to Fe^{2+} . Ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion reduction at low pH causes formation of a colored ferrous probe complex from a colorless ferric probe complex that determines the reducing power of a compound. The ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 mL TPTZ in 40 mM HCl and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in the proportion of 10:1:1 at 37°C. Freshly prepared 3.995 mL of working FRAP reagent was mixed with different concentrations (10-100 µg/mL) of the appropriately diluted iron oxide nanoparticles and mixed thoroughly. An intense blue color complex was formed when ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to ferrous form (Fe^{2+}) and the absorbance was recorded at 593 nm against reagent blank (3.995 mL FRAP reagent + 5 µL distilled water) after 30 min incubation at 37°C. Ferric reducing antioxidant power of iron oxide nanoparticles was expressed as ascorbic acid equivalents in µg per mg of iron oxide nanoparticles (Kumar et al., 2014).

Antibacterial activity of synthesized iron oxide nanoparticles Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) is the least concentration of the drug that inhibits the growth of microorganism which is measured by turbidity in the test samples. The minimum inhibitory concentration (MIC) of synthesized iron oxide nanoparticles was determined by following the standard protocol (Arasu et al., 2013). Primarily the synthesized iron oxide nanoparticles was sterilized using 0.2µm filter discs by mixing with double distilled water and then the sterilized solution was serially diluted to two-fold of known concentrations (6.25, 12.5, 25, 50, 100 µg/mL) using Muller-Hinton broth with appropriate control in 96-well micro titer plates. 5µL of the newly cultured bacterial suspensions of the test organism (*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* sp, *Bacillus subtilis* and *Enterococcus faecalis*) (1.5×10^6 CFU/mL) was transferred to 200 µL of sterilized MHB and the antibiotic amoxicillin was used as control. Following, proper mixing, the 96-well micro titer plates was covered with a sterile plate sealer and incubated at 37°C for 20 hours.

Statistical analysis

The data of all experimental parameter of the present study was analyzed using Minitab 16 statistical software which utilizes Statistical One Way Analysis of Variance (ANOVA) followed by Dunnett's test. The experimental groups would be compared with respective control groups and the values are expressed as mean ± Standard Error Mean (S.E.M). *P value < 0.05 was considered as significant.

RESULTS AND DISCUSSION

Synthesis, preliminary confirmation and characterization of iron oxide nanoparticles

Synthesized iron oxide nanoparticles was preliminarily confirmed based on the change in colour of reaction mixture from yellowish to brownish black once the reaction was finished (Fig. 1a). The acquired iron oxide nanoparticles was centrifuged and dried under ideal circumstances. The aqueous leaf extract of the study play dual role as reducing agent and stabilizing agent, which leads to the development of the nanoparticles. It also reduces and minimize the aggregation of nanoparticles as they are capping them with aldehyde and ketone groups. The colour change of the reaction mixture solution is due to the surface plasma resonance phenomenon on the surface of synthesized nanoparticles (Santhoshkumar et al., 2017). The formation and

stability of formed iron oxide nanoparticles was further confirmed by UV-visible spectrophotometer analysis. The sharp peak of UV- visible chromogram at the wavelength of 267 nm is corresponding to the surface plasma resonance of iron oxide nanoparticles (Fig. 1b). The UV visible chromatogram of the synthesized iron oxide nanoparticles was quite similar to the report of *Ocimum sanctum* leaf extract mediated green synthesized iron oxide nanoparticles (Balamurugan et al., 2014).

FTIR analysis of the synthesized iron oxide nanoparticle was carried out to find the possible biomolecules that way out for the reduction of Fe^{+} ions and capping of the reduced iron oxide nanoparticles. It figures out the presence of the phytochemical constituents like alkaloids, tannins, saponins, glycosides, flavanoids, anthocyanins, amino acid, steroids and phenols on the surface that protect the NPs from aggregation and thereby retain for long term stability. FTIR spectra of FeSO_4 shows the absorption peaks at $3444.07\text{--}3359.42\text{ cm}^{-1}$ was shifted to $3380.69\text{--}3359.77\text{ cm}^{-1}$ is assigned to OH stretch which confirms the presence of hydroxyl group. The peak value in the range of $1770.60\text{--}1683.74\text{ cm}^{-1}$ is associated with $\text{C}=\text{O}$ which denotes the presence of aldehydes and unsaturated esters. The peak range between $1558.38 - 1508.25\text{ cm}^{-1}$ is related to $\text{N}=\text{H}$, which confirm the presence of primary amines. The peak stretch of $1292.22\text{--}1340.43\text{ cm}^{-1}$ was shifted to 1384 cm^{-1} represents C-N linkage that ensures the presence of aromatic amines (Fig. 1c).

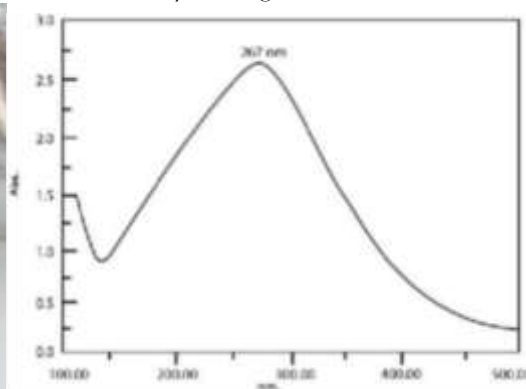
FTIR spectra of synthesized iron oxide nanoparticless shows absorption peaks in the range $3512.13\text{--}3284.55\text{ cm}^{-1}$ which is shifted to $3452.34\text{--}3205.47\text{ cm}^{-1}$, 1350.08 cm^{-1} that represents hydroxyl (OH) group. The peak value in the range of $3452.34\text{--}3205.47\text{ cm}^{-1}$ is related with N-H bond of primary amines. The other peak ranges of $3298.66 - 2935.46\text{ cm}^{-1}$ stretched to $3407.98\text{--}3265.26\text{ cm}^{-1}$ reveals the C-H and N-H bonds of alkyl halides and primary amines (Fig. 1d). The result of the present study is correlates with the previous study on from iron (III) precursor to magnetite and vice versa, which indicates the formation of iron oxide nanoparticles. The results of the previous study also reveals that the presence of phenolic compounds and flavanoids are the major factor it would responsible for the formation and stabilization of synthesized iron oxide nanoparticles (Wakeel et al., 2019).

The average size, quality and the crystalline nature of the synthesized iron oxide nanoparticles was determined using X-ray Diffraction (XRD) spectrum. Crystalline state of the compound determines saturation solubility, dissolution rate and stability behavior of the compounds. A powder X-ray diffraction method is the most reliable one to identify the physical nature of the particles. The XRD analysis of synthesized iron oxide nanoparticles formulation exhibit diffraction peaks in 2θ angle of 25.68° , 13.26° and 8.24° (Fig. 2a). It reveals that the increase in the degree of crystalline as increase the sharp distinctive peaks during the formation of iron oxide nanoparticle from *Martynia annua* fresh leaf extract using FeSO_4 . The results of the current study correlate with the study on green synthesis of iron oxide nanoparticles using *Lagenaria siceraria* and evaluation of its antimicrobial activity (Kanagasubbulakshmi and Kadirvelu, 2017).



(a)

Plant Extract $\text{FeSO}_4/\text{FeO NPs}$



(b)

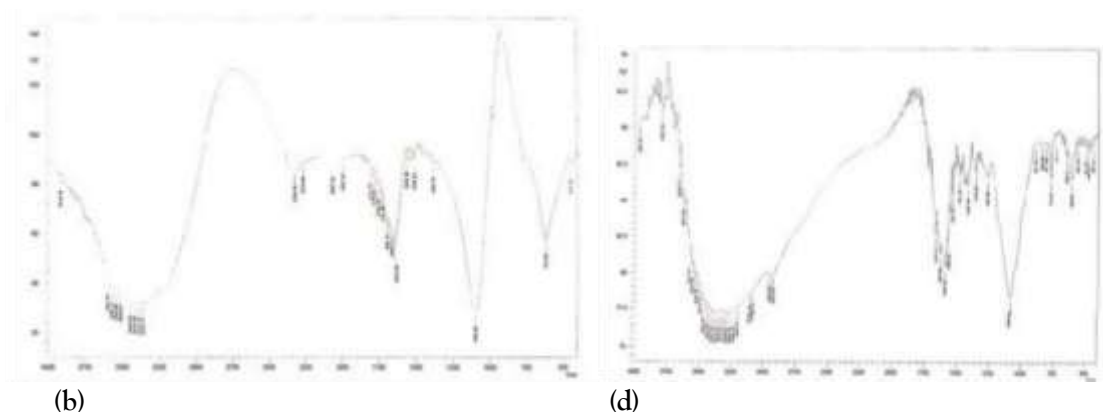


Fig. 1. Synthesis, confirmation and characterization of iron oxide nanoparticle using aqueous leaf extract of *Martynia annua* (a) Synthesis of iron oxide nanoparticle, (b) UV- visible spectrum of biosynthesized iron oxide nanoparticle, (c) FTIR spectrum of FeSO_4 , (d) FTIR spectrum of biosynthesized iron oxide nanoparticle.

The surface morphology of green synthesized iron oxide nanoparticles was analyzed using Scanning Electron Microscopy. The SEM image of the synthesized iron oxide nanoparticles reveals that they are small in size, well crystallized with smooth texture and spherical in shape (Fig. 2b). Latha and Gowri (2014) report the synthesis of iron oxide nanoparticles using the leaf extract of *Carica papaya* and they explained that the synthesized nanoparticles shows plate like structure with coarsened grains, uniformly distributed small spherical shaped particles.

Energy dispersive X- ray spectroscopy (EDX) is the quantitative technique used for elemental analysis of chemicals composition on the surface of the synthesized nanoparticles and to examine the purity of the metal oxide nano particles synthesized. The result of the present study reveal the presence of strong elemental signal of iron at 3 KeV which is typical for the absorption of metallic iron oxide nanocrystallites due to surface plasma resonance (Fig. c,d). The slight absorption peaks of Ca, Na and Mg in the spectrum might be due to the involvement of phytoconstituents as capping and stabilizing of iron ions through the respective related groups. The EDAX profile of the synthesized iron oxide nanoparticles reveal strong signals for iron ions and weaker signals for calcium, sodium and magnesium which are provenients from biomolecules. Energy Dispersive X-ray Spectroscopy (EDX) analysis of iron oxide nanoparticles showed the presence of elemental iron in the sample. The appearance of chloride and carbon in the EDAX spectrum is due to FeSO_4 precursors used in the synthesis and also attributed mainly to organic molecules present in the leaf extract (Kantha Arunachalam, 2013).

Dynamic light scattering (DLS) analysis is used to determine the particle size distribution profile of synthesized nanoparticles and capping agent enveloped the metallic particles along with the particle size of the metallic core. The particle size distribution has most important characteristic affecting the in vitro fate of nanoparticle. The particle size was measured by using Malvern particle size analyzer. The size distribution of analysis that the particle size of iron oxide nanoparticle is 181.7 nm (Fig. 3a). This result is in accordance with the study on one step green synthesis and characterization of synthesized iron oxide nanoparticles using aqueous leaf extract of *Teucrium polium* and their catalytic application in dye degradation with mean particle size of 284.5 nm (Mohammad Amin Jadidi Kouhbanani, 2019).

The zeta potential is the surface charge potential index as well as an important parameter to determine the stability of nanoparticles in the suspension (Erdogan et al., 2019). The Zeta potential value of biosynthesized iron oxide nanoparticles shows a peak at

-33.0 mV indicates that the surface of nanoparticles is negatively charged with good stability (Fig. 3b). It is evident that the iron oxide nanoparticless are polydispersed in nature. Due to its high negative zeta potential thus the electrostatic repulsive force between them results in the prevention of agglomeration of the nanoparticles and is also a key significant for long term stability in the solution. The result is similar with results of green synthesis of iron oxide nanoparticles using *Lagenaria siceraria* and evaluation of it antimicrobial activity where the zeta potential of FeSO_4 nanoparticles was -52 meV proves that the synthesised

NPs are highly stable due to the strong negative surface charge (Kanagasubbulakshmi and Kadirvelu, 2017). The other similar study on synthesis of iron oxide nanoparticles using the aqueous coprecipitation method and it was observed that the zeta potential of nanoparticles was constant around -39 mV (David Kovar et al., 2017).

DPPH free radical scavenging activity

DPPH, a stable free radical evaluates the ability of antioxidant compounds to scavenge free radicals. Recent studies reported that depends on configuration and conformation of chemical compounds, interaction of these potential antioxidant compound with DPPH would determine the free radical scavenging ability of the respective compounds (Oliveira et al., 2016). The free radical scavenging activity (IC₅₀) of ascorbic acid and synthesized iron oxide nanoparticles was found to be $22.69 \mu\text{g/mL}$ and $88.28 \mu\text{g/mL}$ respectively (Table 1; Fig. 4a). The biosynthesized iron oxide nanoparticles exhibit potent DPPH scavenging activity when compared with standard. It was observed that a significant decrease in the concentration of DPPH radical due to the scavenging ability of synthesized iron oxide nanoparticles. The study revealed that the synthesized iron oxide nanoparticles showed effective antioxidant activity due to capped phenolic compounds. Phenolic group facilitates electron donating ability during synthesis of the nanoparticle which import effective free radical scavenging activity.

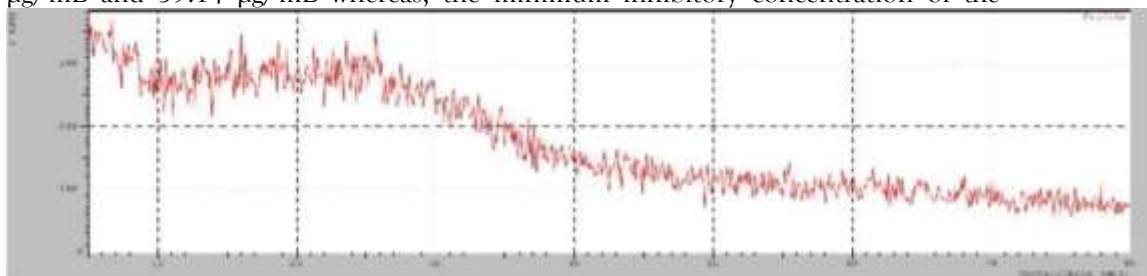
The existence of bioactive components in the extract and the surface of the functionalized iron oxide nanoparticles exhibit effective antioxidant and free radical scavenging activity (Alamelumangai et al., 2014). In addition, presence of polyphenolic content and phytochemicals like flavanoid, tannins, saponnins, glycosides in the extract are responsible for the free radical scavenging activity (Sastri et al., 2014). The green synthesized iron oxide nanoparticles can be a potent component in various biomedical applications due to its high biocompatibility and antioxidant activity. A similar result was reported in biogenic synthesis of magnetic iron oxide nanoparticles using inedible *Borassus flabellifer* seed coat (Sandhya and Kalaiselvam, 2020).

Ferric Reducing Antioxidant Power

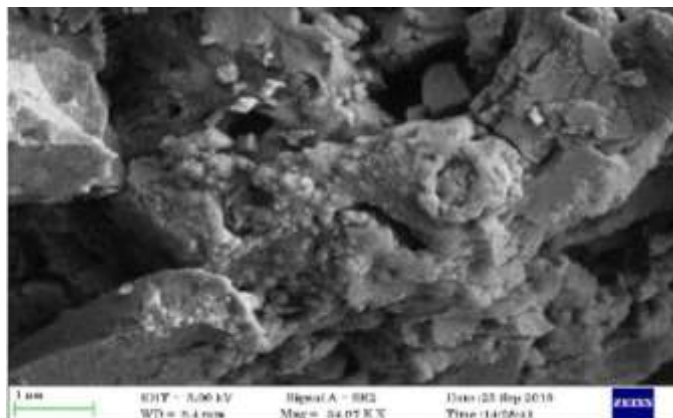
Ferric reducing antioxidant power is the reduction of complex 2, 4, 6 - tripyridyl- s-triazine into ferric chloride hexa hydrate. It utilizes ferricyanide and ferric ions as chromogenic oxidants and provides an immediate result in terms of vast range of antioxidants in a dose dependent level (Veeramani et al., 2022). The ferric reducing antioxidant power activity (IC₅₀) of ascorbic acid and synthesized iron oxide nanoparticles was observed as $4.35 \mu\text{g/mL}$ and $15.42 \mu\text{g/mL}$ respectively (Table 2; Fig. 4b). The iron oxide nanoparticles exhibited significant reducing ability higher than that of standard ascorbic acid. This might be due to the presence of high phenolic concentration. A similar result was reported in green synthesis and characterisation of iron nanoparticles from aqueous leaf extract of *Vitex leucoxylon* and its biomedical application (Mohammed et al., 2022). Another study on free radical scavenging activities of *Salvia brachyantha* and its protective effect against oxidative cardiac cell injury correlates with the results of the present study (Esmaeili and Sonboli, 2010).

Minimum Inhibitory Concentration

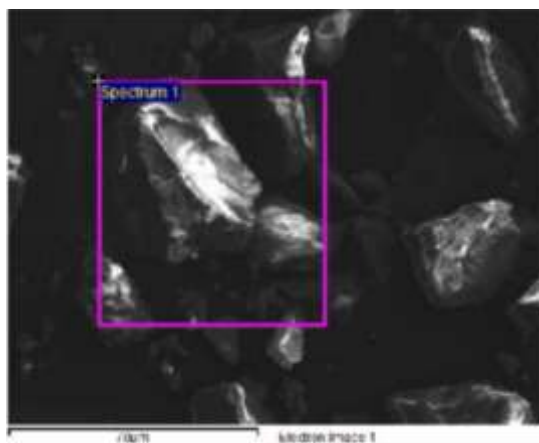
The antibacterial activity of biosynthesized iron oxide nanoparticles was assessed using minimum inhibitory concentration method against selected wound pathogenic organism. The minimum inhibitory concentration of commercial antibiotic, amoxicillin against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* sp, *Bacillus subtilis* and *Enterococcus faecalis* was observed as $5.61 \mu\text{g/mL}$, $4.28 \mu\text{g/mL}$, $1.14 \mu\text{g/mL}$, $3.26 \mu\text{g/mL}$ and $59.14 \mu\text{g/mL}$ whereas, the minimum inhibitory concentration of the



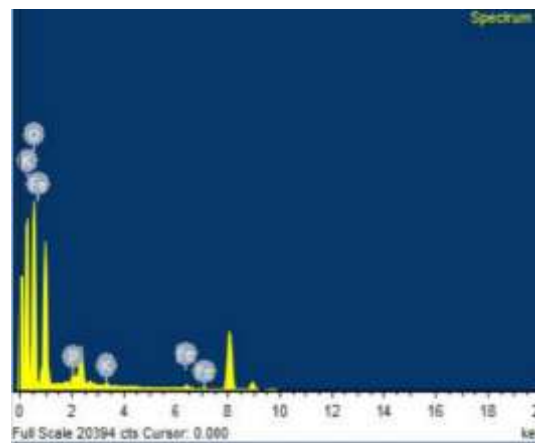
(a)



(b)

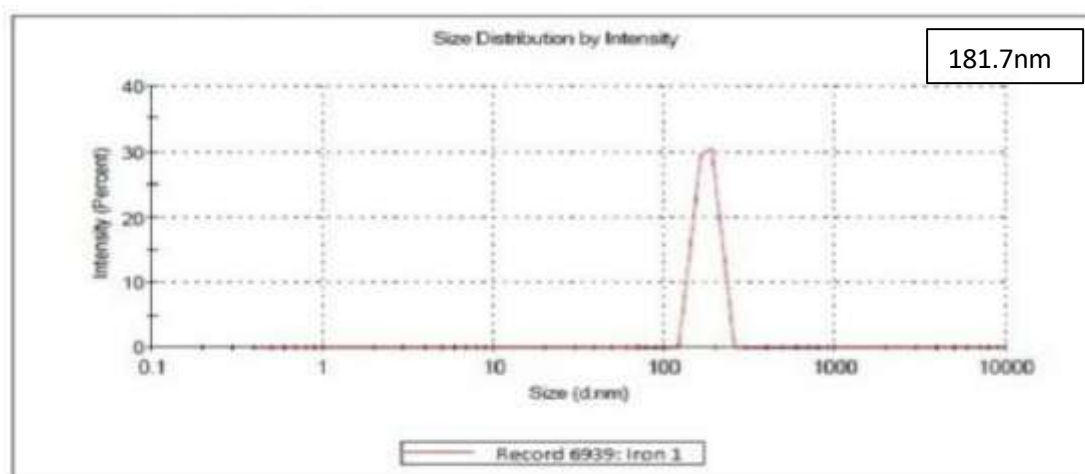


(c)



(d)

Fig. 2. Analytical characterization of iron oxide nanoparticles synthesized using aqueous leaf extract of *Martynia annua* (a) X-ray diffraction pattern of biosynthesized iron oxide nanoparticle, (b) Scanning electron microscopic image of biosynthesized nanoparticle, (c) EDAX image of iron oxide nanoparticle with bioorganic components, (d) EDAX pattern of biosynthesized iron oxide nanoparticles.



(a)

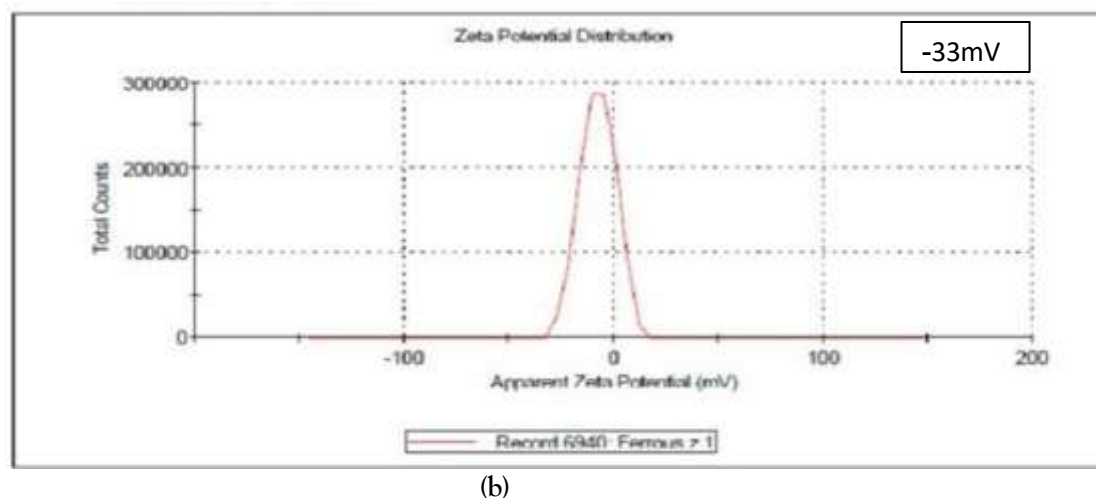


Fig.3. Dynamic light scattering analysis (DLS) of iron oxide nanoparticles synthesized using aqueous leaf extract of *Martynia annua* (a) Particle distribution of biosynthesized iron oxide nanoparticle, (b) Zeta potential spectrum of biosynthesized iron oxide nanoparticles.

Table 1. DPPH free radical scavenging activity of synthesized iron oxide nanoparticles using aqueous leaf extract of *Martynia annua*

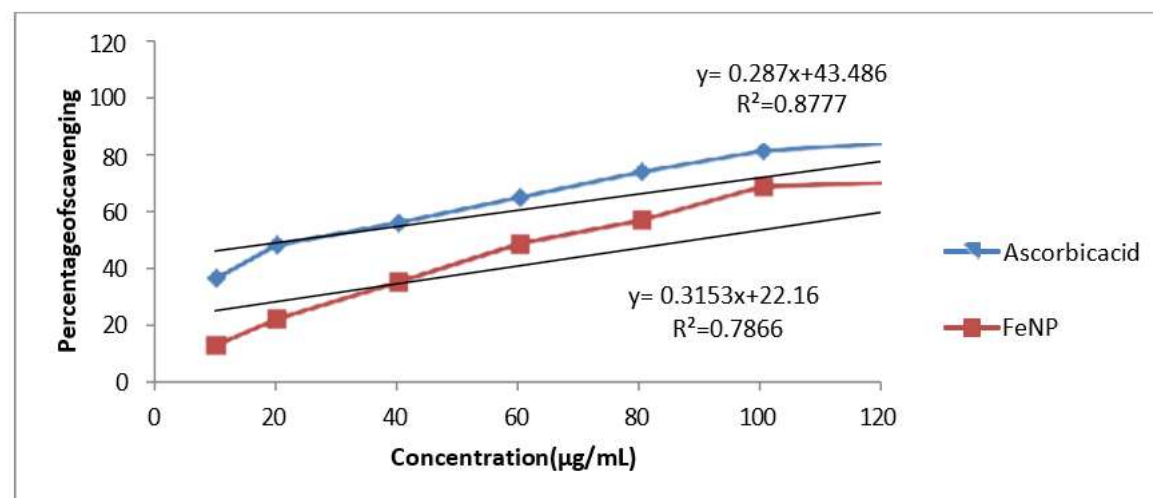
S. No	Concentration ($\mu\text{g/mL}$)	Free radical scavenging ability of Ascorbic acid (%)	Free radical scavenging ability of FeO NP (%)
1	10	36.16 ± 0.27	12.89 ± 0.42
2	20	47.73 ± 0.18	21.97 ± 0.63
3	40	55.43 ± 0.73	34.83 ± 0.28
4	60	64.27 ± 0.58	48.24 ± 0.76
5	80	73.19 ± 0.39	56.35 ± 0.14
6	100	80.45 ± 0.63	68.12 ± 0.45
7	200	93.57 ± 0.23	73.54 ± 0.59
IC50 ($\mu\text{g/mL}$)		22.69 ± 0.43	88.28 ± 0.46

Values are mean $\pm$ S.E.M (n =3)

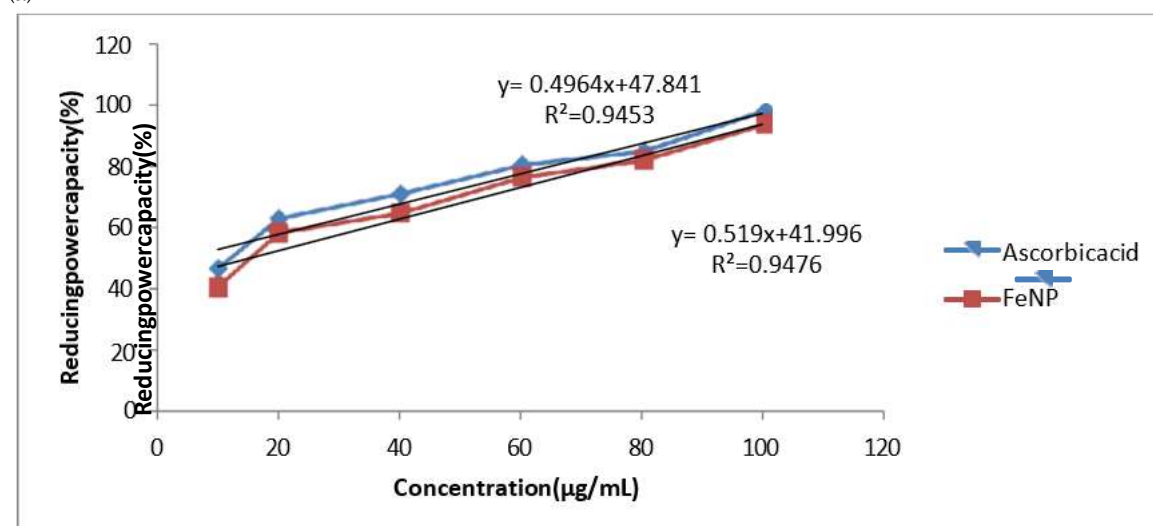
Table 2. Ferric Reducing Antioxidant Power (FRAP) activity of synthesized iron oxide nanoparticles using aqueous leaf extract of *Martynia annua*.

S. No	Concentration ($\mu\text{g/mL}$)	Ferric reducing power of Ascorbic acid (%)	Ferric reducing power of FeO NP (%)
1	10	46.49 ± 0.13	40.52 ± 0.43
2	20	62.52 ± 0.42	57.95 ± 0.25
3	40	70.49 ± 0.36	64.26 ± 0.63
4	60	79.84 ± 0.17	75.83 ± 0.54
5	80	84.16 ± 0.72	81.34 ± 0.25
6	100	97.43 ± 0.27	92.98 ± 0.72
IC50 ($\mu\text{g/mL}$)		4.35 ± 0.34	15.42 ± 0.47

Values are mean $\pm$ S.E.M (n =3)



(a)



(b)

Fig.4. Antioxidant potential of biosynthesized iron oxide nanoparticles using aqueous leaf extract of *Martynia annua* (a) DPPH free radical scavenging activity, (b) Ferric reducing antioxidant power activity.

Table 3. The Minimum Inhibitory Concentration (MIC) of synthesized iron oxide nanoparticles and amoxicillin antibiotic against tested wound pathogens

Concentration (µg/mL)	Percentage of Inhibition									
	Escherichia coli		Streptococcus sp		Staphylococcus aureus		Bacillus subtilis		Enterococcus faecalis	
	Std	FeO NP	Std	FeO NP	Std	FeO NP	Std	FeO NP	Std	FeO NP
6.25	42.84	22.83	44.89	19.88	44.5	23.7	42.79	29.47	10.11	7.48
12.5	54.88	43.47	50.89	35.85	56.23	38.1	56.99	48.29	24.33	12.51
25	62.25	51.78	62.24	38.85	65.89	38.25	63.74	62.18	37.41	24.31
50	71.37	58.12	74.22	42.15	78.97	64.96	71.58	65.52	52.78	39.14
100	85.26	69.39	80.55	58.69	92.42	75.85	86.69	85.26	67.94	52.67
MIC(µg/mL)	5.61	40.97	4.28	71.32	1.14	42.19	3.26	22.30	59.14	86.70

*Std - Amoxicillin

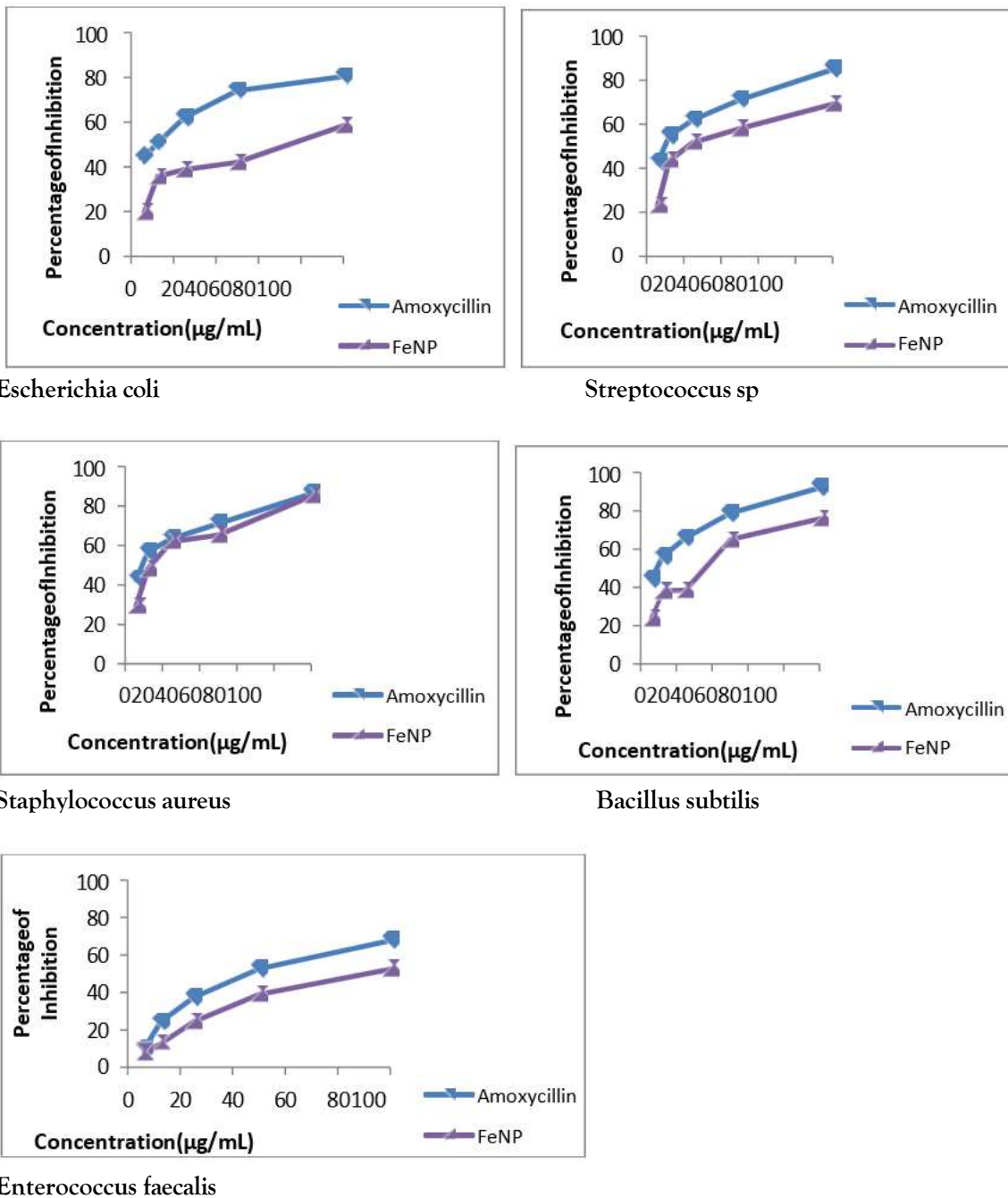


Fig. 5. Minimum Inhibitory Concentration effect of biosynthesized iron oxide nanoparticles using aqueous leaf extract of *Martynia annua* leaf extract against wound pathogens.

synthesized iron oxide nanoparticles against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus sp*, *Bacillus subtilis* and *Enterococcus faecalis* was recorded as 40.97 µg/ml, 42.19 µg/ml, 71.32 µg/ml, 22.30 µg/ml and 86.70 µg/ml (Table 3; Fig. 5). It was observed that the antibacterial activity was increased with decreasing particle size and concentration of nanoparticles was also play a vital role for antibacterial activity of the nanoparticle. A similar type of concentration dependent antibacterial behavior of iron oxide nanoparticles was observed in the synthesis of uniform ferrimagnetic magnetite nanocubes (Kim et al., 2009). Similarly, in another study on the use of supermagnetic nanoparticles for prosthetic film also reported concentration dependent bacterial inhibition (Taylor and Webster, 2009). It is also important to observe that iron oxide nanoparticles do not negatively influence all the cells of an organism and thus it can be explain that

appropriate external magnetic field, iron oxide nanoparticles may directed to kill bacteria species alone as needed throughout the body (Abbaszadegan et al., 2015).

CONCLUSION

The present study report a simple method for biosynthesis of iron oxide nanoparticles using aqueous leaf extract of *Martynia annua*. Preliminary confirmation on reduction FeSO_4 into iron oxide was observed based on colour change of reaction mixture from yellow to brownish black. Further, the synthesis was confirmed using UV visible spectroscopy in the spectrum range of 267 nm. FTIR analysis reveals the presence of various active biomolecules viz., hydroxyl aldehydes, unsaturated esters, primary amenes, aromatic amenes and alkyl halides. The XRD analysis of synthesized iron oxide nanoparticles formulation exhibit diffraction peaks in 2θ angle of 25.68° , 13.26° and

8.24° . It reveals that the increase in the degree of crystalline as increase the sharp distinctive peaks during the formation of iron oxide nanoparticle. The SEM image of the iron oxide nanoparticles reveals that they are small in size, well crystallized with smooth texture and spherical in shape. The EDAX measurement reveals the presence of strong signal for iron and weaker signals for Ca, Na and Mg. The DLS analysis report that, the particle size distribution is 181.7 nm. The zeta potential analysis reveals the synthesized iron oxide nanoparticles is polydispersed in nature with high negative surface charge. The antioxidant activity of iron oxide nanoparticle is based on dose dependent and particle size. The synthesized iron oxide nanoparticle also possess effective antibacterial activity against tested pathogenic organism. Iron oxide nanoparticle coated with the therapeutically vital bioentities from the extract which enhances the pharmacological value and biocompatibility. It concluded that the present study report effective route for green synthesis of iron oxide nanoparticle which enhanced the therapeutic properties.