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Green Synthesis of Iron Oxide Nanoparticles using *Lagenaria Siceraria* and Evaluation of its Antimicrobial Activity

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ABSTRACT

Magnetic iron oxide nanoparticles (MNPs) with appropriate surface chemistry exhibit many interesting properties that can be exploited in a variety of biomedical applications such as magnetic resonance imaging contrast enhancement, tissue repair, hyperthermia, drug delivery and in cell separation. In this study unexplored *Lagenaria siceraria* leaves extract was found to be capable in green synthesis of Iron oxide nanoparticles (Fe_3O_4-NPs) and their characteristics were studied by using UV-visible spectrophotometer, SEM, EDX, XRD, Zeta sizer, and FT-IR. Thus synthesised Fe_3O_4-NPs were naturally stabilised, cubic shaped and in the size range of 30 mm - 100 nm. The phytochemicals present in the leaf has a main role as reducing agent that assists to the eco friendly synthesis of Fe_3O_4-NPs with enhanced antioxidant property. Functional groups present on the NPs are mainly –OH and –COOH (FT-IR) makes it hydrophilic hence NPs does not need any further functional modification for applications. The antimicrobial property of synthesised Fe_3O_4-NPs was evaluated against Gram negative - *Escherchia coli,* Gram positive- *Staphylococcus aureus,* The Zone of inhibition was found to be 10 mm for *Escherchia coli,* and 8 mm for *Staphylococcus aureus.* Thus naturally stabilised Fe_3O_4-NPs with herbal property can be used in various biological applications.

Keywords: Fe_3O_4-NPs; Green synthesis; *Lagenaria siceraria*; Antimicrobial activity; Zeta potential

1. INTRODUCTION

The uses of environmentally benign materials like plant leaf extracts for the synthesis of iron nanoparticles offer numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol[1,2]. Chemical synthesis methods lead to presence of some toxic chemicals that may have adverse effect in the medical applications. Biological synthesis of nanoparticles by plant extracts is at present render exploitation as some researches worked on it[3,4].

Currently, a large number of physical, chemical, biological, and hybrid methods are available to synthesize different types of nanoparticles[5]. The nanoparticles formed by using each method shows specific properties. However, biosynthesis of metal nanoparticles by plants is currently under development. Green nanotechnology has attracted a lot of attention and includes a wide range of processes that reduce or eliminate toxic substances to restore the environment. The synthesis of metal nanoparticles using inactivated plant tissue[6], plant extracts[7], exudates[8], and other parts of living plants[9] is a modern alternative for their production. Green synthesis of nanoparticles makes use of environmental friendly, non-toxic and safe reagents[10].

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Biological systems such as plants microorganisms produce inorganic materials and most of these are present in nanoscale dimensions[11]. The cellular extracts from these biological organisms can be used to synthesize nanoparticles of different size and chemical compositions. Green synthesis of metal nanoparticles extracted from different parts (mostly leaf) of the plant is the most effective process of synthesis at a very affordable cost. During the synthesis bioreduction of metal ions takes place. According to the components present in the plant extract are responsible for the reduction of iron ions whereas water soluble heterocyclic components stabilize the nanoparticles formed. Appropriate precursors such as Ferric Chloride can be used for the reduction of plant extracts[12]. Green nanotechnology has attracted a lot of attention and includes a wide range of processes that reduce or eliminate toxic substances to restore the environment.

Lagenaria siceraria is a large, softly pubescent, annular, climbing or trailing herb growing throughout the India and worldwide. The entire plant is recognised to be beneficial in ethnic systems of medicine. The fruit is sweet, diuretic, antipyretic, antibilious, tonic for the liver, vulnerary, and antiperiodic. It can cure blood diseases in muscular pain and dry cough. Their phytochemicals include hydroxyl, carboxyl, and amino functional groups, which can serve both as effective metal-reducing agents and as capping agents to provide a

robust coating on the metal nanoparticles in a single step[13, 14]. The synthesis of metal nanoparticles using inactivated plant tissue, plant extracts, exudates, and other parts of living plants is a modern alternative for their production[15]. Green synthesis of nanoparticles makes sure of environmental friendly, non-toxic and safe reagents. Fe_3O_4-NPs have several biomedical applications, such as magnetic beads in bacterial capturing, designing of sensor to detect various bio thread agents and being an instrumental in medical world. Therefore we have reviewed the green chemistry type of Fe nanoparticles synthesis process[16]. In this study, we carried out synthesis of iron nanoparticles using dry powder of *Lagenaria siceraria* leaves. The synthesis of Fe nanoparticles is summarised because of their industrial and environmental importance. The pharmacological actions attributed to *Lagenaria siceraria* (LS) in Ayurvedic texts have been validated by scientific researches, and the result indicate potent antioxidant activity, diuretic activity, antihyperglycemic activity, anticancer activity, analgesic activity and antidepressant activity exhibited from major component of the plant[17-19]. In this work the characterisation and formation mechanisms of iron nanoparticles are discussed and its antimicrobial activity has been evaluated. The Fe_3O_4- NPs were prepared using ferric chloride as iron precursor and LS extract as reducing agent and stabilizer.

2. EXPERIMENTAL METHOD

2.1 Collection of Plant Material and Preparation of Extract

Lagenaria siceraria leaves were collected from, Courtralam, Thirunelveli District, Tamil Nadu, India. The plant was authenticated by Botanical Survey of India (No.BSI/SRC/5/23/2010-11/Tech-1585). The fresh leaves were used for all experimental procedures.

2.2 Chemicals

The chemical Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, 98%) and solvent used in the study were of highest purity and analytical grade purchased from Sigma Aldrich.

2.3 Extraction Preparation

The LS plant parts were washed and stored at - 4 °C. For the production of extract, ground, air-dried LS samples (seed, leaf, fruit) about 5 g were boiled with double distilled water (100 ml) in an Erlenmeyer flask (Fig. 1) while being continuously stirred for 15 min. The extract was cooled to room temperature after that filtered, and stored at – 4 °C for further use.

2.4 Preparation of Fe_3O_4-NPs

Iron oxide nanoparticles were synthesised by modified protocol from the previous studies[20-25]. Briefly, by adding 0.01 M $FeCl_3 \cdot 6H_2O$ solution to the LS extract in a 1:1 volume ratio. Fe_3O_4-NPs were immediately obtained with the reduction process. The mixture was stirred for 60 min and then allowed to stand at room temperature for another 30 min to obtain colloidal suspension. Mixture was centrifuged and washed several times with ethanol and then dried at 40 °C under vacuum to obtain the Fe_3O_4-NPs. LS leaves have the best reduction capability against ferric chloride when compared to other parts of the plants (seeds and fruit) that is observed by the external color change. From this observation leaves were selected for further procedures. After the confirmation test the Fe_3O_4-NPs were synthesised by using the above procedure for further characterisation.

2.5 Characterisation

Characterisation techniques help us to understand the specific properties of the substance or nanocrystals to be studied in an accurate rapid manner which is reliable to understand the measured values. The synthesised Fe_3O_4-NPs were subjected to various characterisation studies to understand the specific properties such as optical, structural, morphological, elemental composition, particle size, functional groups studies which could be made precisely using sophisticated techniques such as UV-VIS spectroscopy (SHIMADZU 3600 UV-Vis NIR model), Zeta sizer (Malvern make) XRD (PANalytical X'Pert Pro instrument with Cu Kα1 radiation of wavelength (l) of 1.5406 (°A)), SEM, EDS (FEI- QUANTA 200), FT – IR (SHIMADZU FTIR 8400S) instruments. These techniques were helpful to verify our method is well optimised and meeting the requirements.

2.6 Antimicrobial Activity Test

Antimicrobial property of synthesised Fe_3O_4-NPs was tested to explore the herbal functionality of NPs. Antimicrobial activity was determined by agar well diffusion method as reported elsewhere[12]. For antibacterial test the organisms used were,

Gram negative - *Escherchia coli*, Gram positive- *Staphylococcus aureus*

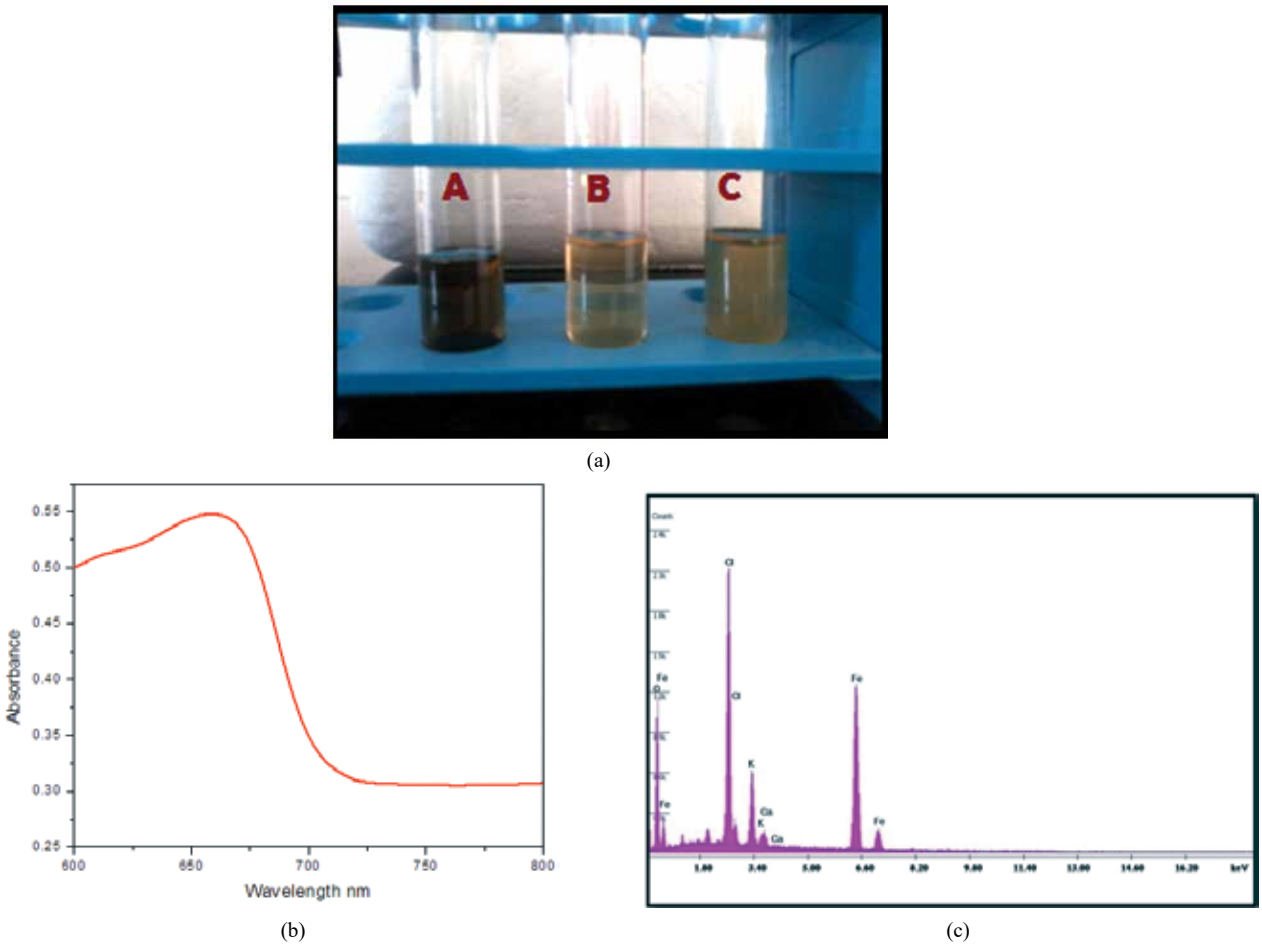
Reference drug – Ampicillin 20 mg/ml

3. RESULTS AND DISCUSSION

The phytochemicals include hydroxyl, carboxyl, and amino functional groups, which can serve both as effective metal-reducing agents and as capping agents to provide a robust coating on the metal nanoparticles in a single step and leads to the colour change Yellowish brown to brownish black[26]. This colour change gave the confirmation of the synthesis of Fe_3O_4-NPs (Fig.1(a)). This denotes the leaves of the LS plant have premier competence to synthesize of Fe_3O_4-NPs than other parts of the plant such as seed, fruit.

This preface test draw a parallel with the UV-visible absorption peak at 658 nm shows (Fig.1(b)) the plant debris as well as the protein bounded Fe_3O_4-NPs bout with the reviewed articles[11] the trivial difference shows the interference of chloride present in the plant extract naturally.

From EDX spectrum (Fig.1(c)), it shows the compounds such as Chloride, Calcium and Potassium are present in the reaction mixture other than Fe nanoparticles, these compounds interprets the complex of plant extract. It revealed that the extract from *Lagenaria siceraria* has recorded 48 per cent iron, 31 per cent oxygen in the total weight of Fe_3O_4-NPs. This is based on the bremsstrahlung X-ray intensity as a function of energy. And the Potassium changes the physical shape of the enzyme molecule, exposing the appropriate chemically active sites for reaction. Potassium also neutralises



(a)

(b)

(c)

Figure.1 (a) Confirmation test – visible interpretation of colour change (A) Leaves (B) Seeds and (C)Fruit, (b) Absorption spectrum of synthesised Fe_3O_4-NPs, and (c) EDX Spectrum of synthesised Fe_3O_4-NPs represents the elements present in the sample.

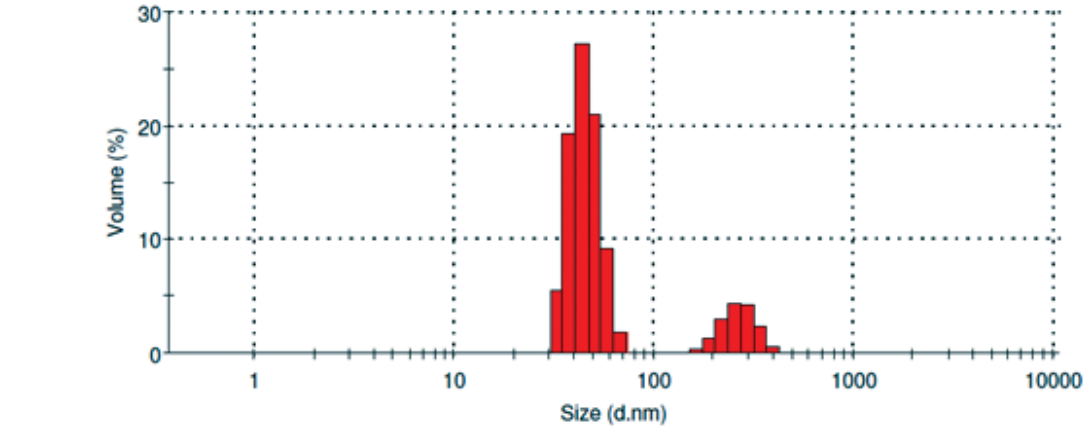
various organic anions and other compounds within the plant, helping to stabilize pH between 7 and 8 which is optimum for most enzyme reactions. The amount of K present in the cell determines how many of the enzymes can be activated and the rates at which chemical reactions can proceed.

Preliminary size confirmation was studied by Zeta sizer. Dynamic light scattering (DLS) principle shows the particle size distribution in the sample. Narrow size distribution in the region of 30 m - 100 nm shows the well size reduction by plant extract (Fig. 2(a)). Sample was further taken to SEM analysis for morphological analysis and size measurement as explained below.

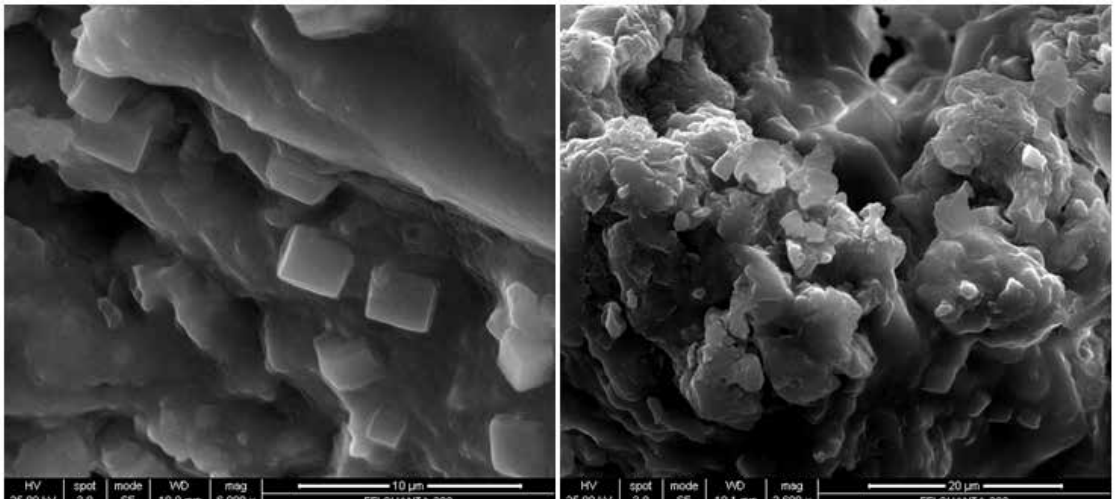
Formation of Fe_3O_4-NPs and its morphological dimensions were studied using the SEM. The study demonstrated that the average size of the NPs were in the range of 30 nm - 100 nm similar phenomenon was reported in the previous studies. That also exhibits the formation of cube shape of iron nanoparticles as shown in the Fig. 2(b). The cube shaped nanoparticles formation has induced by plant enzymes and chloride, potassium compounds present in the sample have an influence in the morphology of the nanoparticles. Due to the very narrow electron beam, SEM micrographs have a large depth of field yielding a characteristic three dimensional appearance useful for understanding the surface structure of a sample.

The X-ray diffraction patterns obtained for the Fe_3O_4-NPs synthesised using LS extract is shown in Fig.3. It is found that the exits strong diffraction peaks with 2θ values of 28.26°, 32.28° corresponding to the hkl value of 220,222, that denotes crystalline phase of Fe_3O_4-NPs matches with JCPDS card No. 39-1346 and JCPDS card No. 89-4319 for Fe_3O_4 - Nanoparticles, the grain size has calculated using Debye-Scherrer equation, which gives a relationship between peak broadening in XRD and particle size. Using the Scherrer equation the average crystallite sizes of the Fe_3O_4-NPs are found to be in the range of 14 nm - 18 nm. The results indicated that all the nanoparticles were in spinel structure with face-centered cubic phase.

FT-IR analysis gave the stretching vibrations at 3354 cm^{-1}, 1701.55 cm^{-1} and 624 cm^{-1} with in the region of 400-4000 cm^{-1} (Fig.4). These peaks represent the following bonding in the sample confirms the reducing agent role in the formation of Fe_3O_4-NPs. The peak at 3354 cm^{-1} corresponds to the -OH bond stretching denotes the aqueous phase as well as the reduction of the Ferric chloride, 1701 cm^{-1} corresponds to the C=O bond stretching denotes the phytochemicals present in the plant extract and amino acids which stabilise as well as act as a capping agents. Remaining unclear peaks represents small amount of organic acids which is responsible for the low pH of the sample which helps to the synthesis of the Fe_3O_4-NPs. The strong peak at 624 cm^{-1} corresponds to the inorganic stretching indicates the Fe_3O_4-NPs. The zeta potential of Fe_3O_4-NPs was



(a)



(b) (c)

Figure 2. (a) Particle size distribution of Fe_3O_4-NPs analysed by Zetasizer (b) and (c) SEM images of synthesised Fe_3O_4-NPs.

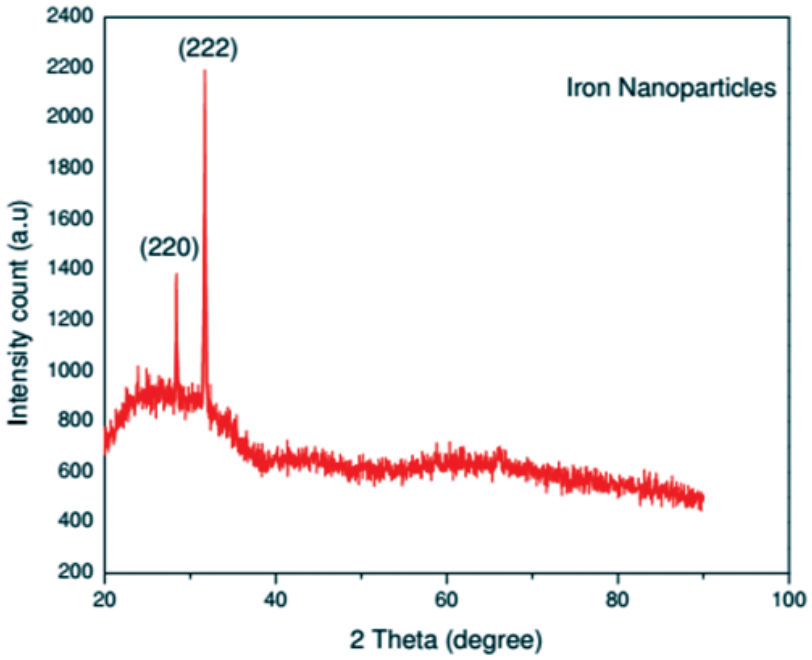


Figure 3. XRD spectrum of Fe_3O_4-NPs.

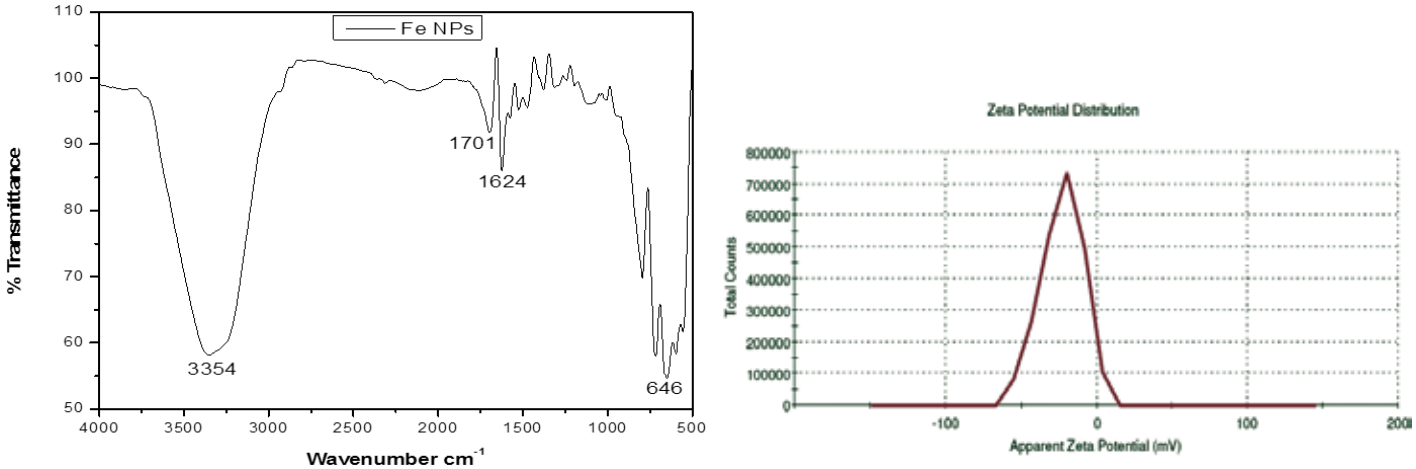


Figure 4. (a) FTIR spectrum of synthesised Fe_3O_4-NPs. (b) Zeta potential of naturally stabilised Fe_3O_4-NPs.

-52 meV proves the synthesised NPs are highly stable due to the strong negative surface charge.

he antimicrobial property of the synthesised Fe_3O_4-NPs revealed that the nanoparticles have the moderate antimicrobial activity when compare to reference drug the result shows higher antibacterial activity against *Eschesheria coli* (Fig. 5) whereas moderate activity was revealed against *Staphylococcus aureus,* the inhibitory activities in culture media of the Fe_3O_4-NPs reported in Table. 1, were comparable with standard antimicrobics Ampicillin. Synthesised NPs have equal antimicrobial potential when compare to original plant extract. Since the herbal property based functional groups of the plant covers surface of NPs it leads to the activation of antimicrobial properties. Less size of NPs further helps to the penetration of NPs inside the cell wall and leads to cell death. Moderate activity concludes that the more susceptible bacteria was *Eschesheria coli* and more resistant was *Staphylococcus aureus*. The Fe_3O_4-NPs was naturally stabilised and has more surface area that could be used to various applications. The factors responsible to the synthesis of Fe_3O_4-NPs should be separated and purified to avoid the plant impurities which influence the NPs characteristics.

Table 1. Antibacterial activity test of Fe_3O_4-NPs – Zone of inhibition

Name of the bacterial strain	Zone of inhibition in mm	
	Fe_3O_4-NPs (20 mg/ml)	Drug (20 mg/ml)
Staphylococcus aureus	8	14
Eschesheria coli	10	17

4. CONCLUSION

The phytochemicals present in the sample have acted as both capping and reducing agents that naturally stabilize the NPs as well. Though the yield of nanoparticle is less when compare to other chemical and physical methods this would be most preferable because of its non toxic way of green synthesis and natural functionalisation with herbal properties. The high zeta potential and functional groups can be used in various applications. Moderate antimicrobial activity against chosen pathogen proves the herbal properties of the plant. Very less size of the NPs address the plant extract as a reducing agent for an efficient synthesis of NPs. Future studies would include the validation of super paramagnetism which can be used in cancer therapy.

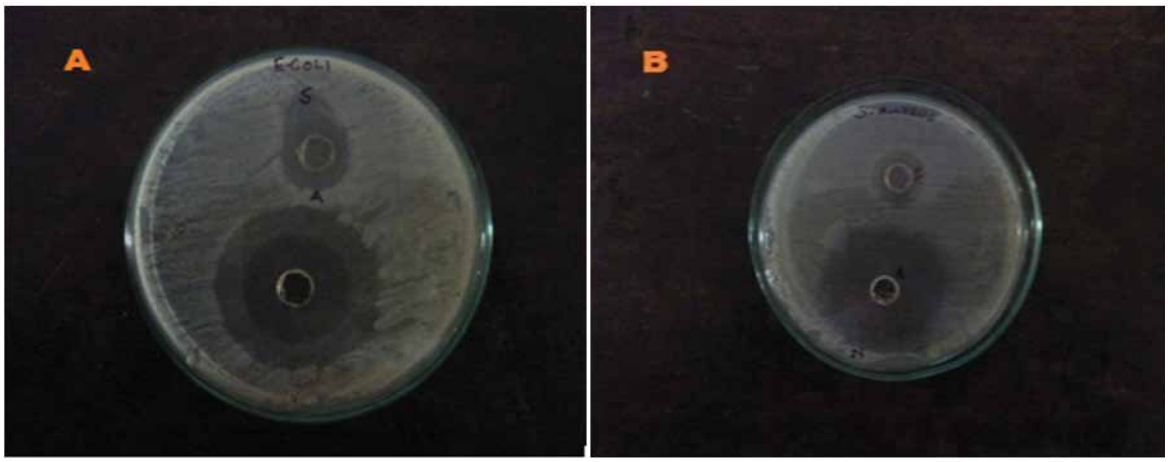


Figure 5. Antibacterial activity of Fe_3O_4-NPs and reference drug. (a) *E.Coli* . (b) *Staphylococcus aureus*,

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As the main author of this research paper, she has given her contribution in entire experiment part and has written the manuscript by addressing the research outcomes.

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He has been a supervisor for this work and has chosen an appropriate research problem which is to be addressed. His contribution also includes the guidance throughout the work and the correction of manuscript.