Mikania mikrantha leaf extract mediated biogenic synthesis of magnetic iron oxide nanoparticles: Characterization and its antimicrobial activity study

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Abstract

With an aim to introduce a new highly potent antimicrobial nanoparticles using an environment-friendly route, he present work reports the green synthesis of iron oxide nanoparticles (Fe_3O_4NPs) utilizing *Mikania mikrantha* leaf extract and its application as efficient antimicrobial agent. The green Fe_3O_4NPs have been described by X-beam diffraction (XRD), Ultraviolet-Visible (UV-Vis) spectroscopy, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Fourier Transform-Infra Red (FT-IR) investigation. The TEM image shows the rhomboidal Fe_3O_4NPs with average mean sizes 20.27 nm. The FT-IR investigation proved Fe_3O_4NPs have been balanced out through the associations of steroids, terpenoids, flavonoids, phenyl propanoids, phenolic acids and proteins present in the leaf extract. The synthesized Fe_3O_4NPs shows a very high antibacterial and antifungal property against 5 bacterial strains such as *Bacillus cereus, Acinetobacter johnsonii, Pseudomonas aeruginosa, Achromobacter spanius* and *Chromobacterium pseudoviolaceum* strain, and 4 fungal strains (*Aspergillus niger, Penicillium citirinum, Fusarium oxysporium,* and *Candida albicans*). The green synthesized iron oxide nanoparticles can interfere metabolic activities of microorganisms which determine its antimicrobial properties and could bring a promising application in the fields of medicine.

Keywords: Biosynthesis, Fe_3O_4 nanoparticles, Mikania mikrantha, Characterization, Antimicrobial activity.

1 Introduction

Nanoparticles synthesized from metal or metal-oxides are the trending study of plant engineering and nanotechnology [1],[2].The diverse and most imperative characteristics of the nanoparticles synthesized from metal/metal-oxide nanoparticles is that they discloses superior activity [3],[4].The bio-nanoparticles have lots of uses and application in the field of medical diagnostic [5], bio-engineering [6], drug-delivery [7],[8], antimicrobial [9],[10], larvisidal, catalyst for chemical reactions, and electronics-devices. Due to the global sensible use of nanoparticles (<100 nm) has ascribable for varieties in distinctive qualities. Nanoparticles biosynthesized are widely synthesized using chemical-synthesis method or physical-synthesis techniques, which uses one to prepare the particles with the common characteristics many ways like, heating, electrolytic study, sedimentation precipitation, wet chemical reaction, deposition, and sonicating the nanoparticles are being used for bio- synthesize of nanoparticles [11],[12],[13],[14]. Still these synthesis methods, are typically favourable and labour-intensive and are probably some projects using to the environmental and ecofriendly techniques using plants, fungi algae etc. were carried out. Green synthesis has advances over chemical and physical method because it is price operative, atmosphere friendly, vital structural plenty of engineering.

Metal nanoparticles are widely synthesized victimization physical and chemical processes, which allow one to accumulate particles with the popular characteristics, many ways like hydrothermal, conventional heating , anodization, deposition precipitation, wet chemical reaction, deposit, and sonication area unit being applied to synthesize the nanoparticles. However, these production methods are typically high-priced and effortful, hence green synthesis of nanoparticles has gain momentum these days [15],[16],[17]. Green synthesis has advances over chemical and physical method because it is value operative, atmosphere friendly, and easily [18],[19]. With the

recent speedy development and evolvement of technology, personalities have placed their religion in nanotechnology and believe that it will ameliorate their current living commonplace [20],[21]. As a consequence, the nanoparticle has drawn a large interest from researchers globally thanks to specific characteristics such as form, size, and distribution, that can be utilized in an exceedingly distinct field of applications [22],[23]. Synthesis of Fe_3O_4NPs has been dispensed because of its unique properties, like being super paramagnetic, biocompatible, perishable, and expected to be non-toxic to humans [24],[25]. These distinctive properties allow Fe_3O_4NPs to be wide employed in totally different areas of applications, like chemical process , magnetic storage media [26], biosensors [27], resonance imaging (MRI) [28],[29], and targeted drug delivery [30],[31],[32]. Numerous strategies of fabrication of Fe_3O_4NPs will be used, like sol gel technique, solid state synthesis and flame spray synthesis [33]. In distinction to the long chemical and physical strategies which involve sophisticated procedures, inexperienced technique is much easier and safer to use, and plant-mediated synthesis of nanoparticles remains a replacement theme and therefore the outcome is however to be studied.

With the recent speedy development and evolvement of technology, personalities have placed their religion in nanotechnology and believe that it will ameliorate their current living commonplace. As a consequence, the nanoparticles has drawn a large interest from researchers globally thanks to specific characteristics such as form, size, and distribution, that can be utilized in an exceedingly distinct field of applications [34],[35]. Synthesis of Fe_3O_4NPs has been dispensed as a result of its unique properties, like being super paramagnetic, biocompatible, perishable, and expected to be non-toxic to humans [36]. These distinctive properties allow Fe_3O_4NPs to be wide employed in totally different areas of applications, like chemical process, magnetic storage media, biosensors, resonance imaging (MRI), and targeted drug delivery [37]. Numerous strategies of fabrication of Fe_3O_4NPs will be used, like sol gel technique, solid state synthesis and flame spray synthesis. In distinction to the long chemical and physical strategies which involve sophisticated procedures, inexperienced technique is much easier and safer to use, and plant-mediated synthesis of nanoparticles remains a replacement theme and therefore the outcome is however to be studied. There are a few of successful studies in synthesizing Fe_3O_4NPs by plant extract. as an example, fruit extract of *Psidium guajava* [38], leaf extract of genus *Perilla frutescens* [36],[29], *Tridax procumbens* [39] and *Caricaya papaya* [28], peel extract of plantain [40],[41], and additionally seed extract of grape proanthocyanidin [42]. Here, in continuation to our interest in the green synthesis of nanoparticles such as silver nanoparticles using *Mikania mikrantha* leaf extract for its antimicrobial activities [43], we reported the use of *Mikania mikrantha* leaf extract as a reducing and stabilizing agent for the green synthesis of iron oxide nanoparticles. The Fe_3O_4NPs obtained were fully characterized by Fourier transform infrared (FT-IR), transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDS) and X-ray diffraction (XRD), Scanning electron microscope (SEM) and Vibrating sample magnetometer (VSM). The as-synthesized Fe_3O_4NPs were studied to observe its efficacy as antimicrobial agents.

2 Experimental

A brief description of all the used reagents, chemicals, experimental procedure, and various analysis data obtained by different spectroscopic methods are covered under this section.

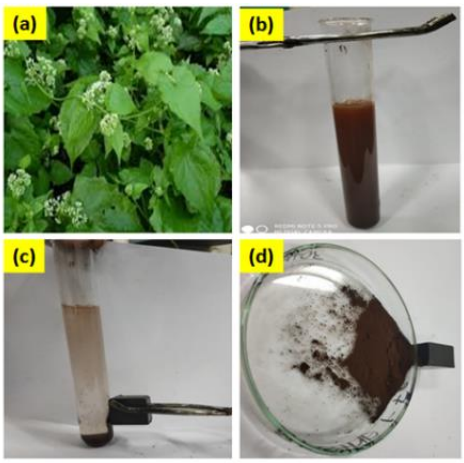

FIGURE 1: a) *Mikania mikrantha* plant with leaves, b) Reaction mixture, c) Magnetic property of the precipitate Fe_3O_4 nanoparticles in the reaction mixture, d) washed and dried magnetic Fe_3O_4 nanoparticles

2.1 Materials

Ferrous sulphate ($FeSO_4.7H_2O$), Ferric chloride ($FeCl_3$), were purchased from Sigma-Aldrich. Double distilled deionized water was used for carrying out the whole experiment. *Mikania mikrantha* leaves for the experiment were collected from NIT Silchar campus (Silchar, Assam, India).

2.2 Preparation of Mikania mikrantha leaf extract

Fresh leaves of *Mikania mikrantha* plants were collected, washed thoroughly 3-4 times with double distilled water to remove external impurities, dust particles and finally chopped to fine pieces. 40 g of the finely chopped *Mikania mikrantha* leaf were boiled in 100 mL of double distilled water at 90 °C for 20 min. The solution of the *Mikania mikrantha* leaf extract was then cooled to room temperature and filtered using filter paper of Whatman No. 41 grade and was stored at -4 °C for future experimental purpose.

1.2.3 Synthesis of magnetic iron oxide nanoparticles

5 mmol of $FeSO_4.7H_2O$ and 10 mmol of $FeCl_3$ (Fe^{2+}:Fe^{3+}=1:2 molar ratio) were dissolved in 100 mL of distilled water at 80 °C. 25 mL *M. mikrantha* leaf extract was then added drop by drop to the reaction mixture with continuous stirring. The reaction solution slowly changes its color from colorless to dark brown. The reaction mixture was then allowed to settle to form the precipitate. The formed Fe_3O_4NPs was easily separated by using an external magnet. The Fe_3O_4NPs precipitate moved towards the magnet and sticks to the wall of the test-tube hence justifying its magnetic property (Fig. 1).

1.2.4 Screening of Antimicrobial Property of synthesized Fe_3O_4NPs.

Test microorganisms:

In vitro antimicrobial screening includes 5 bacterial and 4 fungal strains. The bacterial strains includes *Bacillus cereus* strain SN_SA, *Acinetobacter johnsonii* strain SB_SK, *Pseudomonas aeruginosa* strain SN1, *Achromobacter spanius* strain GCCSB1 and *Chromobacterium pseudoviolaceum* strain GCC-SO4, having NCBI-GenBank accession number MH482928, MH482927, KF031122, MK000623 and MH109305 respectively. The laboratory fungal isolates include *Aspergillus niger*, *Penicillium citirinum, Fusarium oxysporium, Candida albicans*. Bacterial strains were grown and maintained on nutrient agar medium, while fungi were maintained on potato dextrose agar (PDA) medium.

3 Results and Discussion

In this chapter, we have discussed the biosynthesis of an inexpensive, Fe_3O_4NPs using *Mikania mikrantha*. Furthermore, the synthesized Fe_3O_4NPs was characterized by various analytical and spectroscopic techniques.

3.1 FT-IR analysis

The FT-IR spectroscopic studies were carried out to investigate interaction between the surface of Fe_3O_4NPs and the organic functional groups of the biomolecules present in the *M. mikrantha* plant leaf extract. The FT-IR spectrum of aqueous extract of *M. mikrantha* was shown in Fig. 2. The absorption bands at 3302 cm^{-1} and 3317 cm^{-1} are due to are because of the extending vibrations of O-H bunch in alcohols and phenolic mixes and extending vibrations of N-H in auxiliary amides. The groups at 1641 and 1636 cm^{-1} are normal for amide I and amide II groups, individually. Appearance of two small new peaks at 585 and 626 cm^{-1} is the characteristic absorbance peak of Fe_3O_4 confirming the formation of Fe_3O_4NPs from plant source [44].

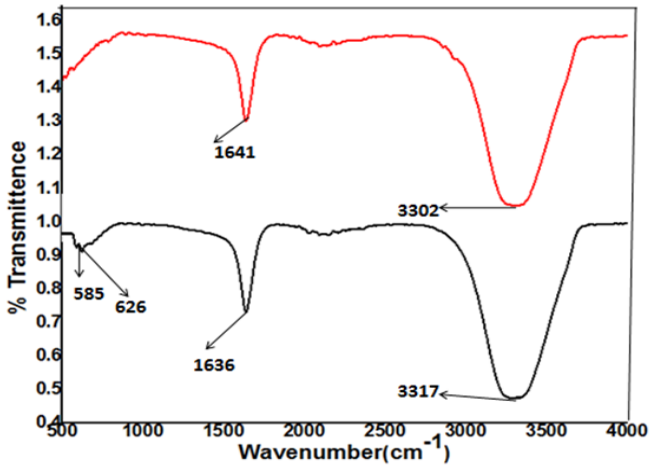

FIGURE 2: FT-IR of *Mikania mikrantha* Fe_3O_4 nanoparticles

3.2 XRD analysis

The phase composition and nature of the biosynthesized Fe_3O_4NPs from *Mikania mikrantha* leaf extract was analyzed by XRD analysis (Fig. 3) with intense Bragg's angle ranging from 100 to 800 and was found that the XRD peaks values are 30.27, 35.60, 36.75, 43.41, 53.88, 57.38 and 62.90 corresponding to (220), (311), (222), (400), (422), (511) and (440) proving the crystalline form of the nanoparticle with (JCPDS: 75-1610).The average crystallite size is 20.27 nm which is calculated by Debye-Scherer's formula.

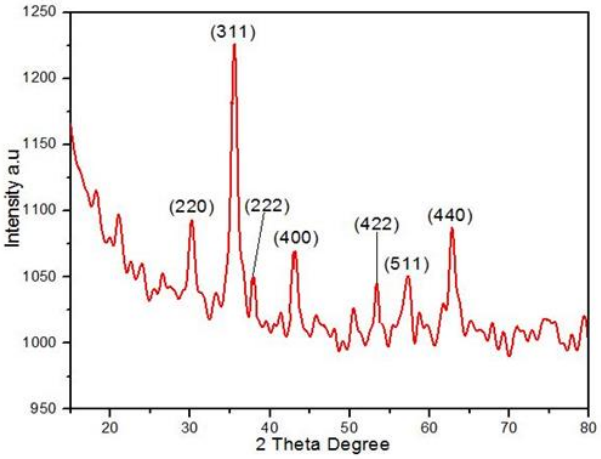

FIGURE 3: XRD pattern of *Mikania mikrantha* Fe_3O_4 nanoparticles

3.3 TEM, SAED analysis

The TEM analysis elucidates morphology and size of the synthesized resultant Fe_3O_4NPs. In Figure 4, the TEM images at various magnifications (a-d) and selected area electron diffraction (SAED) patterns (e) and nanoparticles size distribution graph are shown [45]. From the TEM images of synthesized Fe_3O_4NPs we have seen poly-dispersed and the majority of them being rhomboidal in shape with particle size ranged from 15 to 20 nm and a mean of 20.27 nm. SAED pattern confirms the crystalline nature of the NPs. The nanoparticles distribution curve shows that majority of them are in the range of 20 nm. The Fe_3O_4NPs were seen surrounded by thin layer, which is predicted to be the capping agents, organic compounds from the *Mikania mikrantha* leaf extract.

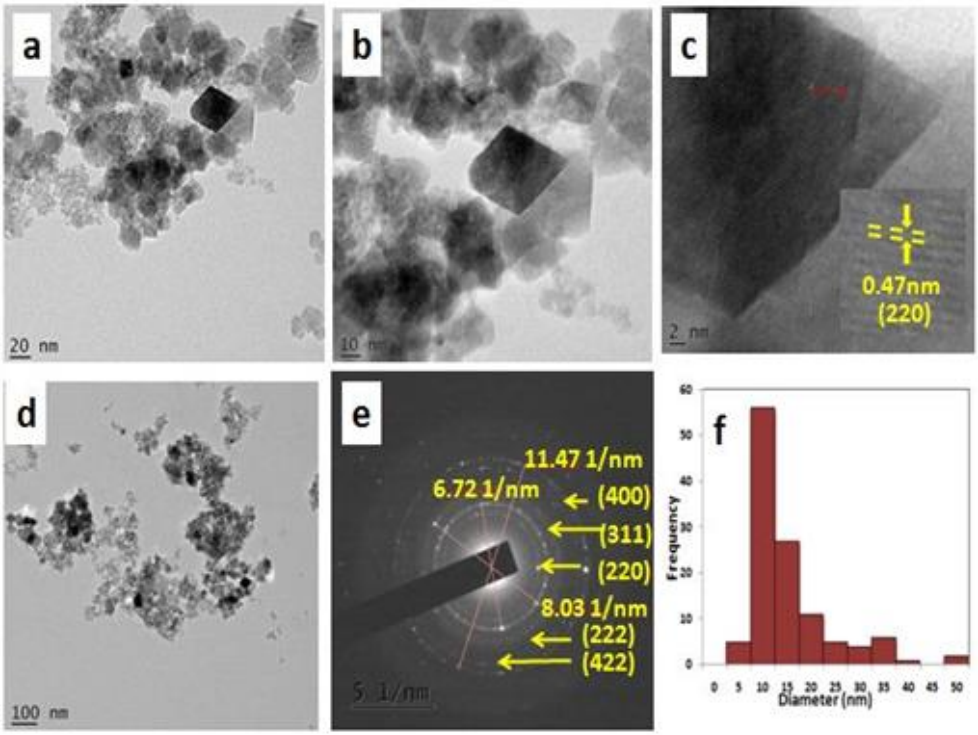

FIGURE 4: TEM images (a-d), SAED pattern (e) and size distribution graph of Fe_3O_4 nanoparticles

3.4 SEM and EDX analysis

SEM image confirmed the rhomboidal shape, well dispersed nature of the nanoparticles with average 15-20 nm sized nanoparticles and a mean size of 20.27. To have more insight of the chemical composition and further information of the metallic nature of the synthesized nanoparticles we have studied the EDX [46]. In this study generally the interaction takes place between X-rays and the compound to be investigated results in reflection of the peak of metal or compounds present in the sample. The obtained peak amplitude shows the presence of particular elements in the compound. Iron generally depicts amplitude peak at 0.7-7 keV. From our EDS analysis we can see the presence compounds showing peaks at 0.4, 6.5 and 7.0 respectively. Elemental compositions percentage of the synthesized Fe_3O_4NPs from *Mikania mikrantha* leaf extract were also calculated (Figure 5).

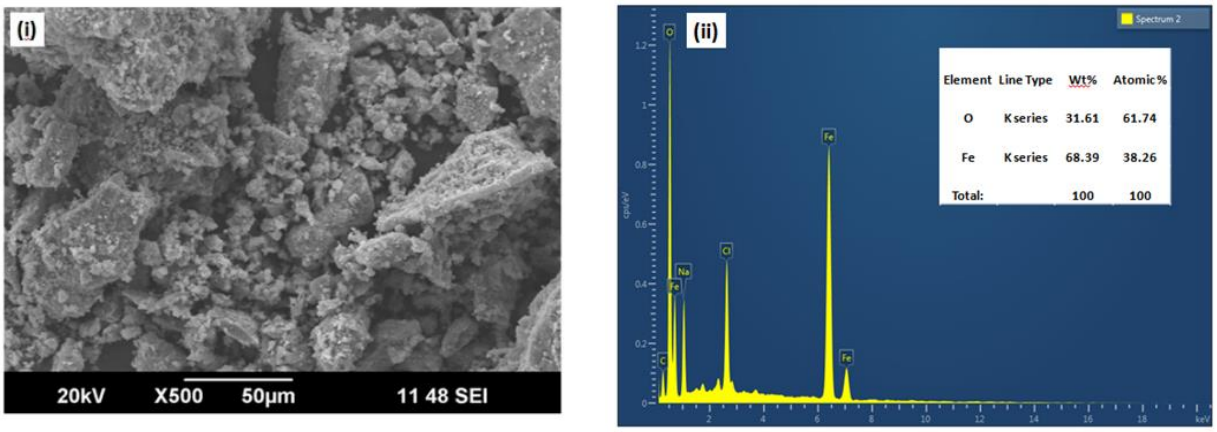

FIGURE 5 (i) SEM (ii) EDS pattern of Mikania mikrantha Fe_3O_4 nanoparticles

3.5 VSM analysis

The vibrating sample magnetometer (VSM) is carried out for checking the magnetic properties of biosynthesized Fe_3O_4 nanoparticles from *M. mikrantha* leaf extracts. The super paramagnetic property and magnetic nature of Fe_3O_4 nanoparticles magnetization measurement is also recorded with VSM (Fig.6). The magnetization curve displays zero remanence and co-ercivity, with a specific high magnetization saturation at 60.50 emu/g respectively which stipulates the super paramagnetic properties of the biosynthesized Fe_3O_4 nanoparticles, which closely matched with the reported literature [47]. Due to its super paramagnetism, the biosynthesized Fe_3O_4 nanoparticles solution responds to the magnets when bought closer an applied magnetic field despite of having any fixed magnetization and disperse back very fast as soon as the magnet is being removed [48]. Hence the biosynthesized Fe_3O_4 nanoparticles from *Mikania mikrantha* leaf extract may have varied applications in, biological, antimicrobial and biomedical studies because of its high super paramagnetic property and easy recoverability using an external magnet.

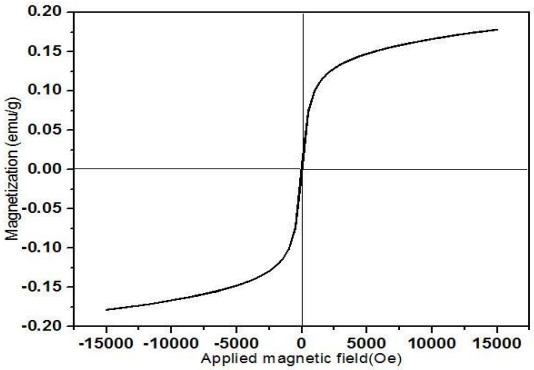

FIGURE 6: VSM pattern of Mikania mikrantha mediated Fe_3O_4NPs

3.6 Antimicrobial activity

Antimicrobial activity was performed by agar well diffusion method, with slight modifications. Freshly prepared molten Muller Hinton Agar (MHA) media for bacterial culture and Potato Dextrose Agar (PDA) media for fungal culture were poured to uniform depth of 5 mm and allowed to cool at room temperature [49],[43],[50]. After solidification, wells were made in MHA media by 6 mm sterilized cork borer. 1-2 drops of media poured in the bottom with the help of sterile micropipette. 1-2 ×107 cfu/mL of test microorganisms were spread onto the surface of MHA media using a glass spreader. 50 µl of Fe_3O_4NPs of three different concentrations (1.0 mM, 0.5 mM and 0.25 mM) were then filled in three wells. DMSO was also used as negative control. The plates were then incubated for 24 h at 37 ºC for bacterial culture and 48 h at 25 °C for fungal culture. The antibacterial activity of Fe_3O_4NPs was evaluated by measuring the zone of inhibition (ZOI) in millimeter with a very high antibacterial activity as shown in Figure 7 and Table 1 and was found that *Bacillus cereus* strain SN_SA shows the highest ZOI of 13 mm at 1.0 mM, 10 mm at 0.5 mM and 8 mm at 0.25 mM, followed by *Acinetobacter johnsonii* strain SB_SK 13mm at 1.0 mM, 10 mm at 0.5 mM and 6 mm at 0.25 mM, *Chromobacterium pseudoviolaceum* strain GCC-SO4showing 13 mm at 1.0 mM, 6mm at 0.5 mM, and 5 mm at 0.25 mM, *Pseudomonas aeruginosa* strain SN1 showing 10 mm at 1.0 mM, 5 mm at 0.5 mM, and 0 mm at 0.25 mM and *Achromobacter spanius* strain GCCSB1 showing the least among the five bacterial strains with 9 mm at 1.0 mM, 7 mm at 0.5 mM, and 6 mm at 0.25 mM.

Table 1. Antibacterial activity of measurement of ZOI at three concentrations of Fe_3O_4 nanoparticles against five pathogenic bacteria a) *Bacillus cereus* b) *Acinetobacter johnsonii strain* SB_SK c) *Pseudomonas aeruginosa* d) *Achromobacter spanius* strain GCCSB1 e *Chromobacterium pseudoviolaceum* strain GCC-SO4) (i) 0.25 mM (ii) 0.5mM (iii) 1mM

Test microorganisms Bacterial isolates	Test sample 1.0 mM	Test sample 0.5 mM	Test sample 0.25 mM	Control 0
1. *Bacillus cereus* strain SN_SA	13	10	8	0

2. *Acinetobacter johnsonii* strain SB_SK	13	10	6	0
3. *Pseudomonas aeruginosa* strain SN1	10	5	0	0
4. *Achromobacter spanius* strain GCCSB1	9	7	6	0
5.*Chromobacterium pseudoviolaceum* strain GCC-SO4	13	6	5	0

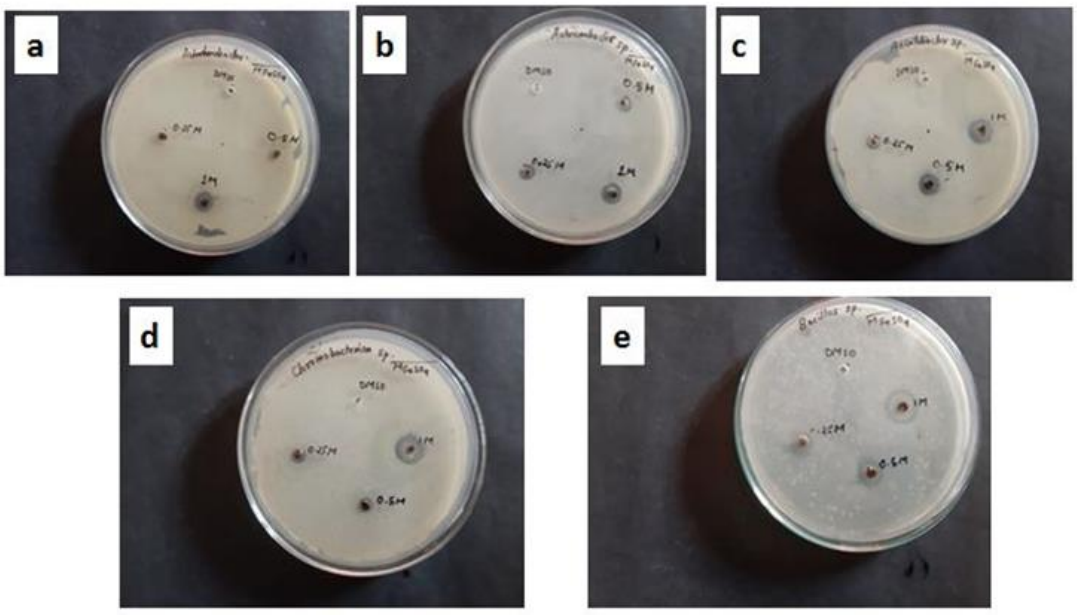

FIGURE 7: Antibacterial activity, ZOI caused by biosynthesized *Mikania mikrantha* Fe_3O_4 nanoparticles against five pathogenic bacteria. a) *Bacillus cereus* b) *Acinetobacter johnsonii* strain SB_SK c) *Pseudomonas aeruginosa* d) *Achromobacter spanius* strain GCCSB1e *Chromobacterium pseudoviolaceum* strain GCC-SO4)

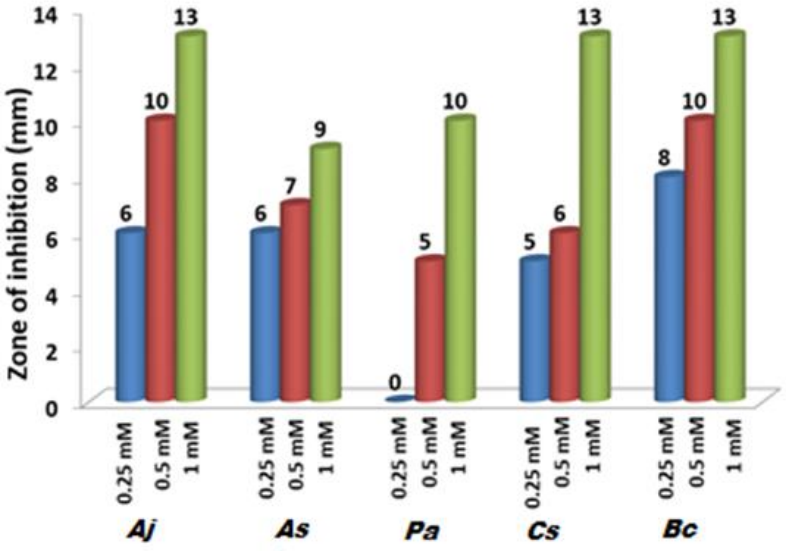

FIGURE 8: Bar chart of Antibacterial activity, ZOI caused by biosynthesized *Mikania mikrantha* Fe_3O_4 nanoparticles against five pathogenic bacteria a) *Bacillus cereus* b) *Acinetobacter johnsonii* strain SB_SK c) *Pseudomonas aeruginosa* d) *Achromobacter spanius* strain GCCSB1 e) *Chromobacterium pseudoviolaceum* strain GCC-SO4) at concentration (i) 0.25 mM (ii) 0.5mM (iii) 1mM

The antifungal activity of Fe_3O_4NPs was also evaluated by measuring the ZOI in millimeter with also very high antifungal activity as shown in Figure 9 and Table 2 and was found that among the four fugal strain studied for antifungal activity the *Candida albicans* showing the highest ZOI 20 mm at 1.0 mM, 19 mm at 0.5 mM, and 14mm at 0.25 mM, followed by *Penicillium citirinum*, showing 14 mm at 1.0 mM, 13 mm at 0.5 mM, and 10mm at 0.25 mM, *Aspergillus niger* showing 10 mm at 1.0 mM, 8 mm at 0.5 mM, and 6 mm at 0.25 mM, and *Fusarium oxysporium* showing the least antifungal activity 10mm at 1.0 mM, 7mm at 0.5 mM, and 0 mm at 0.25 mM respectively.

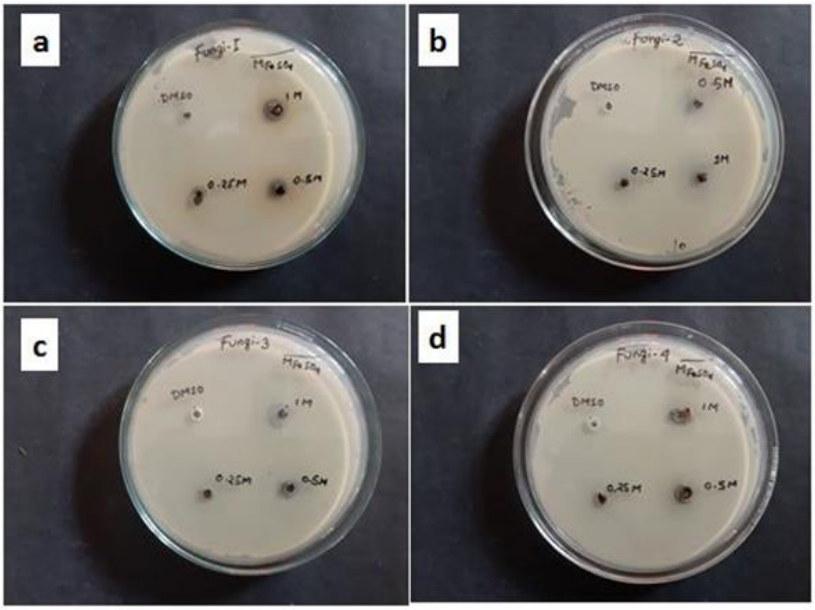

FIGURE 9: Antifungal activity, ZOI caused by biosynthesized *Mikania mikrantha* Fe_3O_4NPs against four pathogenic fungi a) *Aspergillus niger* b) *Penicillium citirinum* c) *Fusarium oxysporium* d) *Candida albicans* (i) 0.25 mM (ii) 0.5 mM (iii) 1 mM

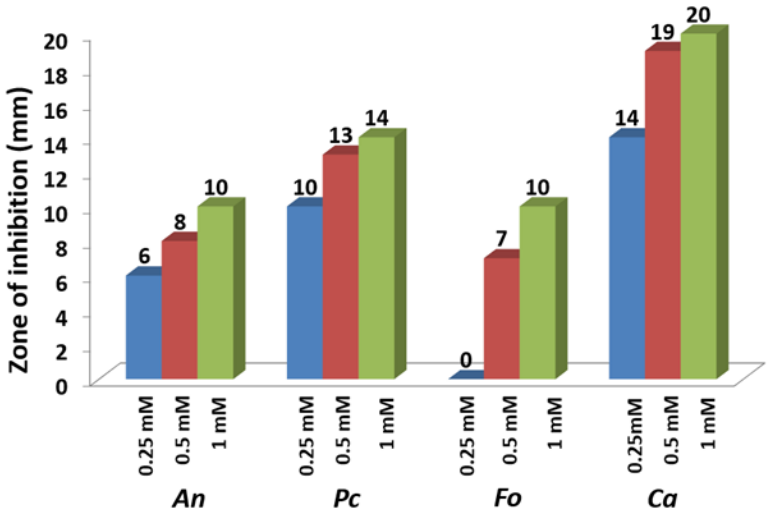

FIGURE 10: Bar chart of antifungal activity ZOI caused by biosynthesized *Mikania mikrantha* Fe_3O_4 nanoparticles against four pathogenic fungi a) *Aspergillus niger* b) *Penicillium citirinum* c) *Fusarium oxysporium* d) *Candida albicans* at concentration (i) 0.25 mM (ii) 0.5 mM (iii) 1.0 mM

Table 2 Antifungal activity of measurement of ZOI at three concentrations of Fe_3O_4 nanoparticles against four pathogenic fungi a) *Aspergillus niger* b) *Penicillium citirinum* c) *Fusarium oxysporium* d) *Candida albicans* (i) 0.25mM (ii) 0.5mM (iii) 1mM

Test microorganisms Fungal isolates	Test sample 1.0 mM	Test sample 0.5 mM	Test sample 0.25 mM	Control 0
1. *Aspergillus niger*	10	8	6	0
2. *Penicillium citrium*	14	13	10	0
3. *Fusarium oxysporium*	10	7	0	0
4. *Candida albicans*	20	19	14	0

4 Conclusions

In summary, magnetic iron oxide nanoparticles were successfully synthesized using *Mikania mikrantha* leaf extract by heating at a temperature of 90 °C. The impacts of strengthening temperature on molecule size and auxiliary properties of nanoparticles were monitored utilizing FT-IR, XRD, SEM, TEM and VSM analysis. The FT-IR indicated a wide retention band identified with Fe_3O_4 vibration band. The XRD examination affirmed that the forerunner was Fe_3O_4 and the combined Fe_3O_4 nanoparticles have rhomboidal structure. The XRD and TEM investigation demonstrated that the normal molecule size of Fe_3O_4 particles increments with expanding strengthening temperatures. Furthermore, the SEM results demonstrated the arrangement of well dispersed rhomboidal formed nanoparticles. From the TEM examination, the normal molecule sizes of the Fe_3O_4NPs were 15-20 nm respectively with a mean size of 20.27 nm. Due to the increasing number of microbes resistant to antibiotic drugs available in the market, a new molecule with high inhibiting efficacy towards microbes is in great demand. Hence, several NPs have been investigated in this line. Interestingly our newly synthesized Fe_3O_4NPs shows a very high antibacterial and antifungal property against 5 bacterial strains and 4 fungal strains with maximum activity against *Bacillus cereus* strain SN_SA and *Candida albicans* strains hence proving its efficacy in biological application as antimicrobial agents. Owing to its high antibacterial and antifungal property, our newly introduced Fe_3O_4NPs will be a good addition to the existing antimicrobial drugs available in the market.

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