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Green Synthesis of Iron Oxide Nanoparticle Using *Funaria hygrometrica* Extract, and the Study of Its Antimicrobial Activities

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ABSTRACT

Over time, microbial resistance to antimicrobial drugs has gradually raised and is therefore a considerable threat to public health. *To tackle the menace of multi-resistant microbial activities which pose challenges to* scientist, Iron oxide nanoparticles (Fe_2O_3 NPs) *due to their importance in medicinal chemistry been known to exhibit antimicrobial activities against some microbes* was synthesized using *Funaria hygrometrica* to investigate its antimicrobial activities against some selected microbes *and then compared with two standard drugs* ciprofloxacin and Terbinafine. *Funaria hygrometrica*, a commonly found moss species, was employed as a natural source of reducing, capping, and stabilizing agents for the synthesis of Fe_2O_3 nanoparticles by co-precipitation method. The synthesized Fe_2O_3 nanoparticles were characterized using techniques such as UV-spectrophotometer with a peak appearing at 335.00 ± 0.877 nm to indicate the band gap for synthesized iron from the *Funaria hygrometrica*, X-ray diffraction (XRD), with spectrum characteristics diffraction peaks at two-theta angles of 24.1°, 33.1°, 35.61°, 39.3°, 40.8°, 43.5°, 49.4°, 54.0°, 57.6°, 62.4°, 63.96°, and 71.91°, which conformed with haematite (Fe_2O_3), Scanning Electron micrograph/Energy Dispersive Xray (SEM/EDX) Spectroscopy which revealed its morphology of heterogenous dark surface with the elemental composition of 76.86% Fe and 23.14% O. Also, nanoparticles' diameter and structure were determined from Transmission Electron Micrograph (TEM) of Fe_2O_3 nanoparticle to be 63.142 ± 16.633 nm, hexagonal crystal system, and roughly spherical. The synthesized Fe_2O_3 nanoparticles were screened against some selected microbes to determine its Zone of Inhibition (ZOI), Minimum Inhibitory Concentration (MIC) at 100 mg/L doses to inhibit the growth of *Escherichia coli* bacteria and *Aspergillus flavus, Trichophyton mentagrophyte* fungi and the Minimum Bactericidal and Fungicidal concentration (MBC/MFC) which completely inactivating *Salmonella typhi* at a dose of 100 mg/L.

1.Introduction

To address microbial resistance, there has been an increase interest in creating new antimicrobial drugs from numerous sources in recent years. These new medicines are in danger of losing their effectiveness due to the rise in microbial resistance since history is repeating itself nowadays [1]. Due to treatment failures caused by bacteria becoming resistant to multiple drugs, an effect caused by highly mutative capacity, microbial biofilms, planktonic cells, and rapid morphological changes, which leads to microbial resistance significant and is now a public health concern on a global level [2]. According to the World Health Organization, infectious disease agents such as bacteria, viruses, fungi, and

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parasites are thought to be responsible for roughly 17 million deaths globally each year. (WHO) [3]. Antimicrobial activity screening has therefore received more focus to assess antibiotic susceptibility for epidemiology, drug development, and therapeutic outcome prediction. The potential of nanoparticles in the treatment of various microbial infections has been shown in several studies. It is now impossible to ignore the therapeutic applications of nanoparticles in viral and microbial research, whether as broad-spectrum inhibitors or delivery agents. They were an essential substance as delivery agents in many ways due to their large surface area to volume ratio. Nanoparticles (NPs), with a diameter ranging from 1-100 nm (nm), are of paramount relevance in modern science and have been synthesized from a variety of materials with special properties channeled to meet particular therapeutic requirements. [4]. Because of the potential applications of nanoparticles in many different fields, such as medicine [5], drug delivery [6], electronics [7], optics [8], agriculture [9], wastewater treatment [10], as sensor support [11], among others, there has been a significant increase in the amount of research on them in recent years.

The antimicrobial effectiveness of the metallic nanoparticles depends upon two important parameters: (a) the material employed for the synthesis of the nanoparticles and (b) their particle size [12]. Metallic nanoparticles can prevent or overcome the development of biofilms and multidrug resistance due to a variety of reasons. They function through a variety of methods; nanoparticles can combat drug resistance, therefore, for microorganisms to circumvent the mechanisms of nanoparticles, several gene mutations must occur simultaneously in each cell. However, it is unusual that numerous biological gene mutations will occur simultaneously in the same cell. [13]. Additionally, nanoparticles of different metallic oxides and other metallic compounds have demonstrated strong antibacterial efficacy. A variety of pathogenic microorganisms, including MDR isolates, are susceptible to the metal oxide [14]. Although the exact mechanism is still unclear, [14] reports that reactive oxygen species (ROS) are released and interact with the cell wall. Reactive oxygen species (ROS) generation can cause oxidative stress on microbial cells, which can lead to the cell's destruction and antimicrobial activity. According to[15], iron oxide nanoparticles also cause oxidative stress, which damages the proteins and DNA of fungus cells, in some cases, fungal hyphae are deformed by nanoparticles, which can also prevent the hyphal transition that frequently results in fungicidal activity [16]. Therefore, the most promising approach for abating or avoiding microbial drug resistance is the utilization of nanoparticles.

Nanotechnology through green synthesis has a wide set of techniques for lowering and eliminating toxic and hazardous compounds that are damaging to the environment. The utilization of plant extracts, has been explored as an effective stabilizing and reducing agent through polyphenols, organic acids, proteins, and phytochemicals as the principal reducing agents. Green' material/nanoparticle synthesis based on biocomponent-derived materials/nanoparticles is applied extensively both in the field of environmental remediation and in other important areas like pharmaceutical, food, and cosmetic industries. The biosynthesis of metals and their oxide materials/nanoparticles using marine algae and marine plants is an area that remains largely unexplored [17]. Research on the already-published literature showed that *Funaria hygrometrica* has not been the subject of extensive purifying or phytochemical research. Studies in existing literature revealed that phytochemical studies confirm the presence of alkaloids, flavonoids, glycosides, and terpenoids as important phytoconstituents. Hence, this study aimed to synthesize iron oxide nanoparticles from methanol extract of local *Funaria hygrometrica* as it contains valuable secondary compounds that are very significant as the principal reducing and capping agents which is then used in the study of its antimicrobial activities.

Fig. 1. Funaria hygrometrica

2. materials and Methods

2.1 Materials

All the solvents used were of general-purpose (analar) grade. Deionized water, Iron (iii) nitrate $Fe(NO_3)_3$, Ammonia Solution (28%), Hydrochloric acid, sodium hydroxide pellets, and Chromium sulphate pellets. UV–visible spectrometry (Agilent, Cary-Win) with 300–800 nm wavelength range was used to measure the absorbance band gap in the UV-Vis spectrum at various intervals in aqueous solutions and contrast them with pure distilled water for Fe_2O_3 nanoparticles synthesized from *Funaria hygrometrica* extract's band gap. The X-ray diffraction (XRD: EMPYREAN) analysis was carried out using an Xpert-pro X-ray diffractometer with Cu KαI radiation with λ=1.54056Å. The scans were made at a temperature of 25^0C within the 2Θ range at a scan rate of $8.00^o min^{-}1$ to determine Fe_2O_3 nanoparticles crystal-synthesized from *Funaria hygrometrica* extract. Scanning Electron Microscopy/Energy Dispersive Xray (JOEL JSM-7600F) *SEM-EDX* analysis also provides high-definition images of the sample surface together with information on chemical composition and the distribution of elements in Fe_2O_3 nanoparticles synthesized from *Funaria hygrometrica* extract.

2.2 Procedure for Synthesis of Fe_2O_3 nanoparticles

Fresh *Funaria hygrometrical* was collected in Zaria City, Kaduna State, washed repeatedly with deionized water, shade dried to preserve the phytochemicals, ground to powder using mortar and pestle, and stored in an airtight container. The crude methanol extract was obtained by maceration of a 50 g sample of *Funaria hygrometrica* in methanol for 48 hours. The extract was filtered using a muslin cloth and concentrated using a rotary evaporator. Fe_2O_3 nanoparticles from *Funaria hygrometrical* extract were synthesized by the co-precipitation method. The mixture of Ferric nitrate $[Fe(NO_3)_3]$ solution of 0.5M in a 500-ml beaker on a stirrer heater at a temperature of 70 °C and *Funaria hygrometrical* extract (volume of 100 ml of a distilled extract of 10 g of *Funaria hygrometrical* extract) in the ratio of 2:1 [18], with a small amount of 28% NH_3OH was added dropwise to aid precipitation while being gently stirred for 1hr on a magnetic stirrer. The solution was then centrifuged at 3000 rpm for 15 min to collect Fe_2O_3 nanoparticle precipitate and then filtered. Deionized water was added to the Fe_2O_3 nanoparticle residue collected and then stirred. It was centrifuged and filtered again to wash off the impurities. It was finally dried overnight at 90^0C for 24 hours and calcined at 250°C to further remove volatile impurities. At this point, dark brownish Fe_2O_3 nanoparticles was formed [18] for application in the study of its antimicrobial activities.

2.3 Antibacterial Evaluation

The antimicrobial activities of synthesized Iron oxide nanoparticles were determined using some pathogenic microbes. The microbes were obtained from the Department of Medical Microbiology A.B.U. Teaching Hospital Zaria. The following organisms were tested against the synthesized Fe_2O_3 nanoparticle from *F. hygrometrica* extract: Bacteria: *Escherichia coli, Salmonella typhi.* Fungi : *Candida albicans, Aspergillus niger, Aspergillus flavus, Trichophyton mentagrophyte.*

2.3.1 Culture Media Preparation

Mueller-Hinton agar (Oxoid England) served as the microorganisms' growing medium while the synthesized Fe_2O_3 nanoparticle from *F. hygrometrica* extract was screened using the diffusion method. According to the manufacturer's instructions, the medium was sterilized and prepared. Standard inoculums of the test microorganisms in the amount of 0.1 mL was seeded into the sterilized medium. The inoculum was evenly distributed across the surface of the medium using a sterile swab. The synthesized Fe_2O_3 nanoparticle from *F. hygrometrica* extract (1 mg) was diluted to a solution concentration of 100 mg/mL in DMSO (10 mL). A standard cork borer (6 mm in diameter) was used to make a well in the middle of each inoculation media. After that, 0.1 mL of the synthesized Fe_2O_3 nanoparticle from *F. hygrometrica* extract solution was then transferred to the well on the inoculated medium. After 24 hr incubation period at 37 ^{o}C, the plates were checked for inhibitory zones. The measurement of the zone was done with a transparent ruler, and the result was given in millimeters [19]. Ciprofloxacin and Terbinafine, two common commercial medications, were utilized as controls for activity assessments at a concentration of 10 µg/mL.

2.3.2 Minimum Inhibitory Concentration (MIC) Determination.

Using a broth dilution approach, the MIC was determined [19]. Test tubes containing 10 mL of Mueller-Hinton broth (Oxoid, England) were made, filled with the broth, sterilized at 121 °C for 15 minutes, and then allowed to cool. After adjusting the suspension's turbidity to the 0.5 McFarland standard, the suspension's microbe was injected, and it was then incubated at 37 ^{o}C for 6 hours. The suspension was diluted with a regular saline solution to a concentration of around 1.5 × 108 CFU/mL after incubation. A two-

fold serial dilution of the synthesized Fe_2O_3 nanoparticle from *F. hygrometrica* extract was carried out in the sterilized broth to produce concentrations of 100, 50, 25, 12.5, and 6.25 g/mL, respectively. The minimum inhibitory concentration was determined by inoculating 0.1 ml of the test microbes into the sterile broth using various concentrations of the compounds, monitoring the turbidity (growth) of the test broth for 24 hours at 37 ^oC, and reporting the lowest concentration of the sterile compound that showed no turbidity [20].

2.3.3 Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) Determination.

The MBC/MFC was conducted to ascertain whether the microorganisms tested had been annihilated or whether just the compounds had prevented their growth. The MIC was serially diluted, subcultured, and incubated for 24 hours at 37 ^oC before the plates were checked for colony growth. The plate with the lowest Fe_2O_3 nanoparticle synthesized from *F. hygrometrica* extract, the concentration was the MBC/MFC devoid of a colony [20].

3.Results and Discussions

3.1 Synthesized Iron Oxide Characterization.

3.1.1 UV-Vis Spectrophotometric Technique

By measuring the absorbance measurements in the UV-Vis spectrum at various intervals in aqueous solutions and contrasting them with pure distilled water, this method is a technique for detecting the conversion of iron (III) to iron oxide nanoparticles by measuring their band gaps. The spectrophotometer (Agilent, Cary-Win) with a range of 200 to 800 nm was used for this analysis. This technique was used for detecting the conversion of iron (III) to iron oxide nanoparticles by measuring their band gaps. The UV-Vis spectrum for methanol extract of *F. hygrometrica*, Iron (III) Nitrate salt, and of Fe_2O_3 nanoparticle synthesized from *F. hygrometrica* extract are shown in Figs. 2-4. The band gap peaks for the methanol extract of *F. hygrometrica* were observed at 420 nm and 662 nm in Fig 2, for iron (III) nitrate solution at 295 nm in 3, which are distinct from Fe_2O_3 nanoparticle's band gap peak at 335 nm. This validated the synthesis of a new chemical molecule as seen in where the sharp peaks undergo significant changes to a new one in the range of 295-335 nm for the formation of iron oxide nanoparticles. The absorption peak at 335 nm may be a result of light scattering by Fe_2O_3 NP due to particle size, crystal structure as well and the excitation of surface plasmon resonance.

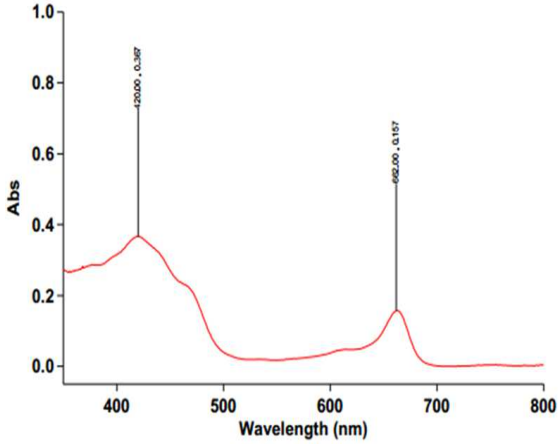

Fig. 2. UV-Vis spectrum for methanol extract of *F. hygrometrica*.

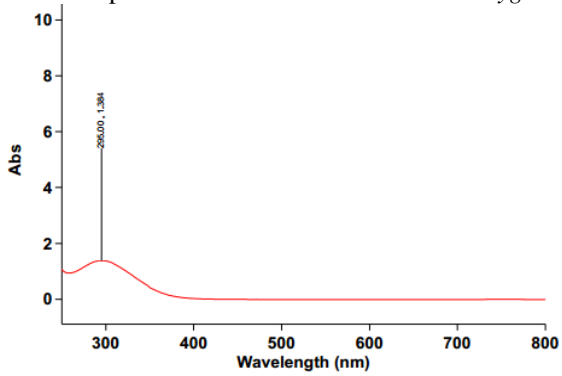

Fig. 3. UV-Vis spectrum for Iron (III) Nitrate salt.

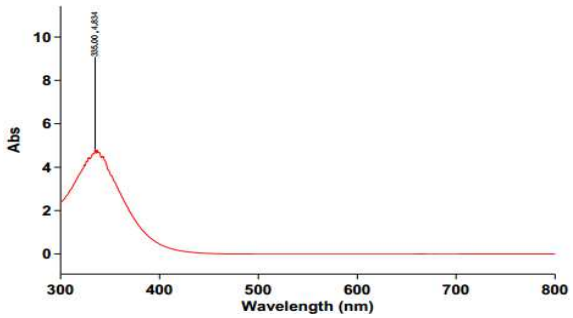

Fig. 4. UV-Vis of Fe_2O_3 nanoparticle synthesized from *F. hygrometrica* extract.

3.1.2 X-ray diffraction (XRD: EMPYREAN)

The X-ray diffraction analysis was carried out using an Xpert-pro X-ray diffractometer with Cu KαI radiation with λ=1.54056Å. The scans were made at a temperature of 25^0C within the 2Θ range at a scan rate of 8.00°min^{-1}. The XRD data of the iron oxide nanoparticle shows a strong agreement with the standard Fe_2O_3 (Reference code: 96-101-1241), having the lattice parameter (a=b=5.0381 Å, c=13.7556 Å). Furthermore, the analysis (as given in Fig. 5) shows well-defined reflection characteristics at diffraction peaks at two-theta angles of 24.1°, 33.1°, 35.61°, 39.3°, 40.8°, 43.5°, 49.4°, 54.0°, 57.6°, 62.4°, 63.96°, and 71.91°, which can be indexed to the (0,1,2), (1,0,4), (1,1,0), (0,0,6), (1,1,3), (2,0,2), (0,2,4), (1,1,6), (0,1,8), (2,1,4), (0,3,0), and (1,0,10) planes of Fe_2O_3 in a hexagonal crystal system respectively. This was consistent with other findings about Fe_2O_3 nanoparticle crystals. [21].

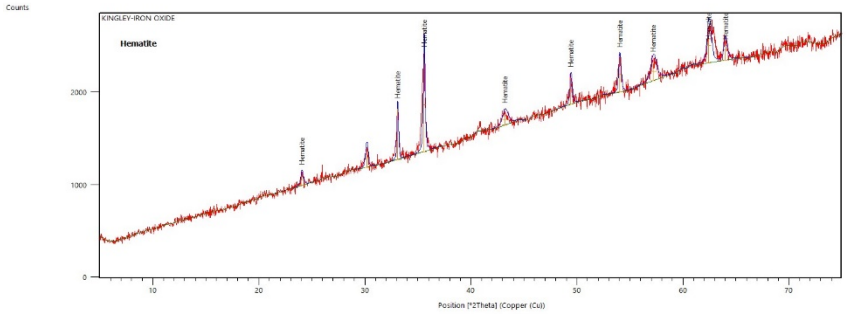

Fig. 5. XRD pattern of Fe_2O_3 nanoparticle synthesized from *F. hygrometrica* extract.

3.1.3 Scanning Electron Microscopy/Energy Dispersive Xray (JOEL JSM-7600F)

SEM/EDX

SEM-EDX analysis provides high-definition images of the sample surface together with information on chemical composition and the distribution of elements. The microscope has an integrated EDX system that was used to record the sample's SEM image. The qualitative and quantitative status of the elements was provided by EDX analysis. The EDX spectrum of Fe_2O_3 nanoparticle in Fig6 shows strong emission signals of Oxygen and Iron elements which confirms that the Fe_2O_3 nanoparticle prepared was essentially at peaks 76.86 % (Fe) and 23.14 % (O) which is in line with literature [22]. While the surface morphology of the produced Fe_2O_3 nanoparticle is depicted in the SEM micrograph in Figure 7. Clusters of irregularly shaped dark grains of various sizes are seen in the SEM micrograph of the synthesized Fe_2O_3 nanoparticle in Figure 7.

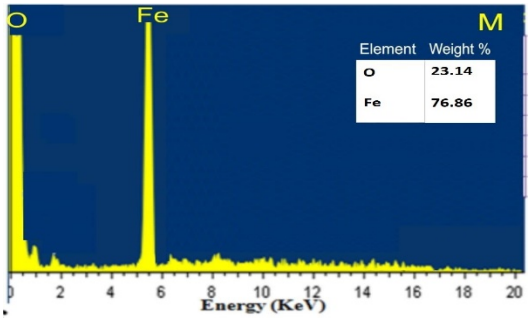

Fig. 6. EDX spectrum plot of Fe_2O_3 nanoparticle synthesized from *F. hygrometrica* extract.

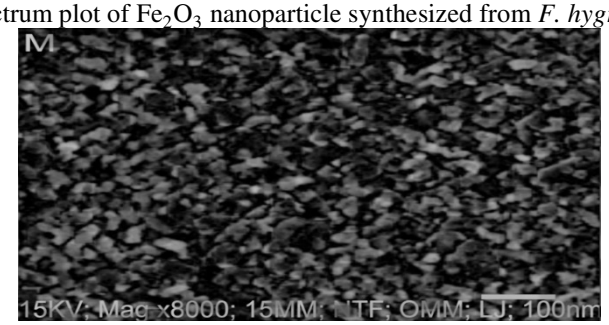

Fig. 7. Scanning Electron Micrograph (SEM) of the Fe_2O_3 nanoparticle synthesized from *F. hygrometrica* extract.

A picture of porous Fe_2O_3 nanoparticle captured by TEM is shown in Figure 8 and is relatively spherical. Using the Image J software and treating the particles like spheres, the particle diameter distribution of nanoparticles was evaluated, and from there the particle diameter distribution was determined to have an average diameter of 63.142 ± 16.633 nm.

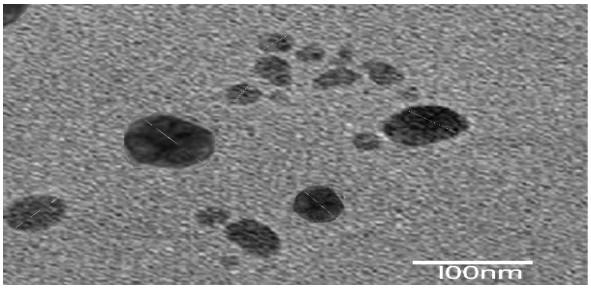

Fig. 8. Transmission Electron Micrograph (TEM) of the Fe_2O_3 nanoparticle synthesized from *F. hygrometrica* extract.

3.2 Antibacterial Activities

3.2.1 Zone of Inhibition (mm)

The antimicrobial activity of Fe_2O_3 nanoparticles were assayed in vitro by agar well diffusion method against two bacteria: *Escherichia coli, Salmonella typhi* and four fungi species: *Candida albicans, Aspergillus niger, Aspergillus flavus, Trichophyton mentagrophyte*.

Table 1. Zone of Inhibition (mm) at varying concentrations (mg/cm^3) of the Fe_2O_3 nanoparticle synthesized from *F. hygrometrica* extract against the Microorganism

TEST ORGANISM	Fe_2O_3 nanoparticle (100 mg/cm^3)	Fe_2O_3 nanoparticle (50 mg/cm^3)	Fe_2O_3 nanoparticle (25 mg/cm^3)	Ciprofloxacin	Terbinafine
Escherichia coli	13	-	-	35	-
Salmonella typhi	15	12	-	30	-

Candida albicans	-	-	-	-	19
Aspergillus niger	12	-	-	-	17
Aspergillus flavus	12	-	-	-	18
Trichophyton mentagrophyte	12	-	-	-	14

Drug Concentration
Ciprofloxacin =10 µg/cm^3
Terbinafine = 10 µg/cm^3.
The significant antimicrobial activity of the Fe_2O_3 nanoparticle was compared to the standard antimicrobial drugs, Ciprofloxacin (10 µg/cm^3) and Terbinafine (10 µg/ml).

Table 1 summarizes the microbial growth (zone) inhibition by Fe_2O_3 nanoparticles of *F. hygrometrica*. The Fe_2O_3 nanoparticle investigated showed maximum antimicrobial activity against *Semolina typhi* and *Escherichia coli* based on growth (zone) inhibition at 15 mm and 13 mm respectively.

Fig. 9: Zone of Inhibition exhibited by Fe_2O_3 nanoparticle

3.2.2 Minimum Inhibition Concentration (MIC)
Minimum Inhibition Concentration (MIC) at varying concentrations (mg/cm^3) of the Fe_2O_3 nanoparticle synthesized from *F. hygrometrica* extract against the Test Microbes
.

Table 2. Minimum Inhibition Concentration (MIC) at varying concentrations (mg/cm^3) of the Fe_2O_3 nanoparticle synthesized from *F. hygrometrica*

TEST ORGANISM	100 mg/cm^3	50 mg/cm^3	25 mg/cm^3	12.5 mg/cm^3	6.25 mg/cm^3
Escherichia coli	Ox	+	+	+	+
Salmonella typhi	-	Ox	+	+	+
Candida albicans	+	+	+	+	+

Aspergillus niger	+	+	+	+	+
Aspergillus flavus	Ox	+	+	+	+
Trichophyton mentagrophyte	Ox	+	+	+	+

KEY
_ No turbidity (growth)
Ox MIC
+ Turbidity (growth)

Error! Reference source not found. shows the Minimum Inhibition Concentration (MIC) of the Fe_2O_3 nanoparticle mg/ml required to inhibit the growth of microbes. The most effective growth inhibition was observed on *Semolina typhi* at 50 mg/cm^3 followed after by 100mg/cm^3 for *E. coli*, *A. flavus*, and *T. mentagrophyte* (Table 2).

3.2.3 Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

Table 3. Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of the Fe_2O_3 nanoparticles synthesized from *F. hygrometrica* extract against the Microbes.

TEST ORGANISM	100 mg/cm^3	50 mg/cm^3	25 mg/cm^3	12.5 mg/cm^3
Escherichia coli	+	+	+	+
Salmonella typhi	Ox	+	+	+
Candida albicans	+	+	+	+
Aspergillus niger	+	+	+	+
Aspergillus flavus	+	+	+	+
Trichophyton mentagrophyte	+	+	+	+

KEY
Ox MBC/MFC,
+ colonies growth

Table 3 investigated the Minimum Bactericidal/Fungicidal Concentration of Fe_2O_3 nanoparticle required to inactivate a microbe. The concentration of Fe_2O_3 nanoparticle required to inactivate microbe was observed only for *Semolina typhi* at 100mg/cm^3, therefore, synthesized Fe_2O_3 nanoparticle from *F. hygrometrica* can serve or can be used as an antibacterial against *Semolina typhi* in the treatment of wastewater and drug development.

4. Conclusion

The presence of secondary metabolites in *Funaria hygrometrica* served as reducing, capping, and stabilizing agents during the synthesis of Fe_2O_3 nanoparticles. The nanoparticles' structure and diameter were determined to be roughly spherical and 63.142 ± 16.633 nm (using Image J software on TEM) respectively, and the synthesized nanoparticles was crystalline nature as verified by X-ray diffraction analysis (XRD). The XRD data of the iron oxide nanoparticle shows a strong agreement with the standard Fe_2O_3. That is, at diameter 63.142 ± 16.633 nm, Fe_2O_3 nanoparticles exhibited various degrees of activity against the selected microbes as indicated by the zone of inhibition [23]. For each test microorganism, minimum inhibitory concentration (MIC) values were determined, which ranged from 50 – 100 mg/cm^3 however, *Salmonella typhi* was inactivated at the 100 mg/cm^3 concentration highlighting the potential of these Fe_2O_3 nanoparticles synthesized using *Funaria hygrometrica* as antibacterial agents. Therefore, the synthesized Fe_2O_3 nanoparticle can serve or can be used as an antibacterial agent against *Semolina typhi* in the treatment of wastewater and drug development.

Declarations

Author contribution statement: All authors listed have significantly contributed to the development and the writing of this article.

Conflict of Interests: The authors declare no conflict of interests.

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