Biosynthesis of iron nanoparticles using *Ageratum conyzoides* extracts, their antimicrobial and photocatalytic activity

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Abstract

Metallic nanoparticles synthesized using aqueous plant extracts are environment-friendly, biocompatible, and highly stable. The aim of this study was to synthesize iron nanoparticles using aqueous *Ageratum conyzoides* extracts and evaluating their antimicrobial and photocatalytic properties. The particles were analysed using UV–Vis spectrophotometer, FT-IR Spectrophotometer, X-ray diffractometer and Scanning electron microscope. GC–MS profile of the extracts revealed presence of secondary metabolites which were further quantified to determine the total phenolic and total flavonoids content of the extracts. The antibacterial activity of the plant extract and the synthesized iron oxide nanoparticles was evaluated against five microorganisms using agar well diffusion method. Iron nanoparticles synthesized in a one step process observed using visible spectra and the functional groups present such as C=O were identified from IR spectrum. SEM–EDX profile identified presence of iron, oxygen, chlorine, calcium in the particles while XRD data revealed the particles synthesized were composed oxides of iron which had moderate activity against the selected microorganisms as compared to the antibiotic ciprofloxacin. The particles were able to photocatalytic degrade methylene blue with a degradation efficiency of 92%. The results obtained in this study confirms that *Ageratum conyzoides* can play an important role in the bioreduction of Fe ions to FeNPs which have moderate activity against microorganisms and can act as photocatalyst to degrade methylene blue.

Keywords Iron nanoparticles · XRD · Antimicrobial · Photocatalytic activity

1 Introduction

In exploring various options to address microbial resistance to antibiotics, metal oxide nanoparticles, have emerged as a promising alternative as they possess lower toxicity, higher stability, higher selectivity and greater durability as compared to organic nanomaterials [53]. Several metal oxides nanoparticles have been reported to exhibit antibacterial activity allowing eradication of various bacterial strains [4, 14, 53]. Biosynthesized metallic nanoparticles have received much attention due to their unique physical, chemical, and biological properties

in fields such as medicine [4, 14, 53], environmental remediation [39], and agriculture [60]. Their relatively low toxicity against human cells, low cost, size-dependent effective inhibition, ability to prevent biofilm formation and the ability to eliminate spores makes them suitable anti-bacterial agents in a variety of industries [53]. Many biological systems such as plants [35], algae [16], diatoms [48], bacteria [29], yeast [26], fungi [43], and human cells [3] have been used to synthesize metal nanoparticles via the reductive capacities of the metabolites found in these organisms. For iron nanoparticles, several physical and chemical methods including mechanical milling

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[22], sodium borohydride reduction [8, 59], solvothermal method [7], and carbothermal synthesis [51] have been employed for their preparation [32]. Iron nanoparticles synthesized by these methods rapidly agglomerate to form clusters due to interparticle Van der Waals and magnetic forces and may further undergo rapid oxidation in the presence of oxidants thereby limiting their reactivity [19, 53]. Iron nanoparticles synthesized using plant extracts have been reported to be environment-friendly, biocompatible, highly stable, and hence they can be applied in various fields [32, 60]. The plant biomass serve not only as a reducing agent but as a support material for template assisted synthesis of the nanoparticles [18] and they are low-cost, eco-friendly, renewable, easily removable and may aid in nanoparticle synthesis [18, 62]. Secondary metabolites having –OH functional groups such as terpenoids, alkaloids, phenolic acids, polyphenols, sugars and proteins present in the plant extract play a significant role in the bioreduction of metal ions [32, 49]. Growth of the magnetite nanoparticles has been reported to proceed via rapid accretion or gradual growth of primary clusters of ferric and ferrous ion hydroxides. Therefore, formation of hydroxides and oxyhydroxides of Fe^{2+} and Fe^{3+} cations via phenolic mediated reduction of Fe^{3+} is considered as the critical step involved in the formation of iron oxide nanoparticles [38]. These reactions occur spontaneously, the procedure does not require heating and the active biological component such as polyphenols acts as a reducing and capping agent protecting the iron nanoparticles from premature oxidation, thereby reducing the overall cost of the synthesis process [18, 32, 38, 49]. The aim of this study was to obtain water-soluble phytochemical extracts from *Ageratum conyzoides* and use these extracts to reduce ferric chloride into iron nanoparticles. The plant species used in this study is novel in terms of iron nanoparticle synthesis and it has diverse and varying phytochemical profiles. Furthermore, its use as therapeutics and antimicrobials agent indicates that it has interesting phytochemical compositions, which may lead to novel interactions between biological and inorganic species.

2 Materials and methods

2.1 Collection of plant extracts

Dry *Ageratum conyzoides* plant materials were collected from Juja, Kambu County in Kenya washed with distilled water and dried in the oven for 48 h at 70 °C. After cooling, the biomaterial was finely ground, washed with double distilled water, and oven dried at 65 °C. The ground material (100 g) was then mixed with 1000 ml of distilled water, boiled for 5 min and after cooling, the solution

was filtered thrice through Whatman filter paper no. 1 to get a clear extract which was then stored in the refrigerator at 4 °C to await further studies [28].

2.2 Quantitative phytochemical screening

2.2.1 Total phenolic content

The total phenolic content of the crude extracts was evaluated using Folin–Ciocalteu reagent. In brief 0.1 g plant material was extracted with 4.9 mL 80% methanol and filtered through a Whatman no.1 filter paper to make the stock sample. The stock sample (50 μL) was made to 1 mL with distilled water, then 0.5 mL of 1 N Folin–Ciocalteu reagent and incubated for 5 min. After incubation, 2.5 mL of 5% Na_2CO_3 was then added and the total volume made to 4 mL using distilled water followed by incubation for 40 min at room temperature. The same quantity of the reagents was used to prepare the calibration standards. Both distilled water and gallic acid (0–75 $\mu\text{g}/\text{mL}$) were used to produce standard calibration curve and the total phenolic content was expressed in mg of gallic acid equivalents (GAE)/g of dry weight extract (DW). Absorbance was measured at 769 nm using a Shimadzu 1800 UV–Vis spectrophotometer [5, 30, 31, 55].

2.2.2 Total flavonoid content

Aluminium chloride method was used to determine the total flavonoid content as described in literature [5, 33, 55]. One gram of plant material was extracted with 4.9 mL 80% methanol and filtered with Whatman no. 1 paper to make the stock sample. 100 μL of the stock sample was made to 1 mL with distilled water. Then to this solution, 150 μL of 5% sodium nitrite (NaNO_2) was added and the contents vortexed and incubated for 5 min at room temperature. One hundred and fifty microliter of 10% aluminium trichloride (AlCl_3) was then added, the solution vortexed and then incubated for 6 min at room temperature. 2.0 mL of sodium hydroxide was then added, the solution made to 5.0 mL and incubated at room temperature for 15 min. The same quantity of the reagents was used to prepare the calibration standards. Both distilled water and rutin (0–75 $\mu\text{g}/\text{mL}$) were used to generate the standard calibration curve and the total flavonoid content was expressed as mg of rutin equivalents (RE)/g of Dry weight (DW) extract. Absorption readings were carried out at 511 nm using Shimadzu 1800 UV–Vis spectrophotometer [30, 31, 55].

2.3 GC–MS analysis

GC–MS analysis of crude *A. conzyoides* was evaluated using a Shimadzu GC–MS. 5 g of the powdered plant samples were extracted with Acetonitrile, then solvent exchanged with 2, 2, 4-Trimethylpentane before GCMS analysis. GC–MS technique was used for identification of the chemical compounds present in the extracts and it was carried out on Agilent 5975 GC–MS operating in EI mode at 70 eV with a mass range of 40 – 400 m/z. A capillary column 30 m × 0.25 mm (id) and Helium gas was used as carrier gas with flow rate of 1.2 ml/min and oven temperature of 60 °C.

2.4 Synthesis of iron nanoparticles (ZVIN)

The aqueous plant extract was mixed with 0.1 M $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ prepared in double distilled water in ratio of 1:1. For the reduction of Fe^{3+} ions, equal volume of aqueous plant extract was added slowly to aqueous ferric chloride solution and vortexed (Vortex Genie 2, scientific industries, USA) for 3 min at room temperature. The formation of iron nanoparticles was indicated by the colour change of solution from light yellow to black [27]. The black precipitate was obtained through centrifugation at 6000 rpm (Hettich zentrifugen, Andreas Hettich group, Tuttlingen, Germany) and washed several times with 1:1 ethanol and water followed by drying at 60 °C in the oven [49].

2.5 UV–Vis analysis

UV–Vis absorption analysis was carried out using a Shimadzu UV–Vis 1800 spectrophotometer in the range of 200–800 nm [4].

2.6 FT-IR analysis

The functional groups present in the reaction product were analyzed by a Shimadzu Fourier Transform Infrared spectrophotometer, Model FTS- 8000. The KBr pellets of samples were prepared by grinding 10 mg of samples, with 250 mg KBr (FT-IR grade). The 13 mm KBr pellets were prepared in a standard device under a pressure of 75 kN cm^{-2} for 3 min. The spectral resolution will be set at 4 cm^{-1} and the scanning range from 400 to 4000 cm^{-1} [30, 31, 40].

2.7 XRD analysis

The powder XRD profile of the nanoparticles was carried using STOE STADIP P X-ray Powder Diffraction System (STOE and Cie GmbH, Darmstadt, Germany). The X-ray generator was equipped with a copper tube operating at

40 kV and 40 mA and irradiating the sample with a monochromatic $\text{CuK}\alpha$ radiation with a wavelength of 0.1545 nm. XRD spectra was acquired at room temperature over the 2θ range of 2°–90° at 0.05° intervals with a measurement time of 1 s per 2θ intervals [23].

2.8 SEM analysis

Morphological analysis of iron nanoparticles was performed using Scanning electron microscope FEI XL30 Sirion FEG (Oxford Instruments Plc, Abingdon, United Kingdom) operated at an accelerating voltage of 30 kV and view field of 6 kV. The system was equipped with an Energy Dispersive x-ray Spectrometer (EDS) system from EDAX having a lithium doped silicon detector [58].

2.9 Agar disc diffusion assay

The plant extracts were screened for their activity against *Staphylococcus aureus* (ATCC-25923), *Bacillus subtilis*, *Pseudomonas aeruginosa* (ATCC-27853), *Escherichia coli* (ATCC-25922) and *Candida albicans* (ATCC 90028) using disc diffusion method [61]. Inoculum suspension was spread over the nutrient agar surface by sterile collection swab, and 6 mm discs sterilized at 120 °C for about 15 min were then loaded with ciprofloxacin (20 mg/mL) as positive controls. The paper discs were prepared by dipping Whatman filter paper in plant extracts and placed onto the surface of inoculated plates with sterilized flamed forceps. The plates were labelled and incubated at 37 °C for 24 h. The zones of inhibition (ZOI) were measured using a ruler in millimeters around the disc and interpreted as low activity (1–6 mm), moderate activity (7–10 mm), high activity (11–15 mm), very high activity (16 mm–) and no activity (–) [30, 31, 37, 61].

2.10 Methylene blue degradation

Photocatalytic activity of the synthesized iron nanoparticles were studied using UV–Vis spectrophotometer and methylene blue as a model organic molecule. In brief, catalytic activity of iron nanoparticles was evaluated by studying the decrease of methylene blue absorption band at 665 nm before and after addition of 0.02 g of iron nanoparticles [21, 24].

3 Results and discussions

3.1 Quantitative phytochemical screening

The total phenolic and total flavonoid content of *A. conzyoides* extracts are depicted in Table 1 below as gallic acid

Table 1 Quantitative total phenolic and total flavonoid content of *A. conyzoides* extracts

Sample	Total flavonoids (mg RE/g DW)	Total phenols (mg GE/g DW)
Roots	36.64 ± 1.32	83.27 ± 0.05
Stalks	23.69 ± 2.20	103.32 ± 0.07
Leaves	26.236 ± 0.01	229.65 ± 0.26

equivalent (mg GE/g DW) and rutin equivalent (mg RE/g DW) per dry weight respectively.

From the results obtained the concentration of total phenolic content was highest in all samples as compared to total flavonoid content. Of the three samples the leaves had the highest total phenols (229.65 ± 0.26 mg GE/g DW) while the roots had the highest total flavonoid content (36.64 ± 1.32 mg RE/g DW) respectively. Polyphenols in plant act as both a reducing agent and a capping agent during synthesis of metallic nanoparticles, resulting in stable green nanoscale particles with unique properties [46, 60]. For instance, the transformation of the flavonoids luteolin and rosmarinic acid present in *Ocimum basilicum* extracts from the enol- to the keto-form has been reported to play a role in the synthesis of silver nanoparticles from Ag ions [1]. Moreover, the internal mechanism of the conversion of ketones to carboxylic acids in flavonoids is likely to be involved in Au³⁺ ion reduction [32]. The presence of such mechanisms may indeed explain the ability of flavonoids to be adsorbed onto the surface of a nascent nanoparticle implying that they are involved in the nucleation and aggregation stages of nanoparticle synthesis, as well as in the bio reduction stage [32, 41].

3.2 GC–MS profile of *A. conyzoides*

Secondary metabolites present in *A. conyzoides* extracts were identified with the help of GC–MS and the results are depicted in Table 2 below.

From the data obtained, a number of volatile compounds belonging to alcohols, aldehydes, ketones, esters, terpenoids, and acids were identified in the *A. conyzoides* extracts (Table 2). These volatile compounds belong to various chemical classes: alcohols, ethers or oxides, aldehydes, ketones, esters, amines, amides, phenols, heterocycles, and mainly the terpenes [13]. Lupeol is a novel anticancer and anti-inflammatory dietary triterpene that helps in stabilizing phospholipid bilayers in plant cell membranes [47]. Amyrins are found in various plants and plant materials such as leaves, bark, and resins and they have been shown to exhibit various pharmacological activities both in vitro and in vivo against various conditions such as inflammation, microbial, fungal, and viral infections and

cancer cells [56]. Phytol is a diterpene alcohol obtained from the degradation of chlorophyll that is used in the synthesis of Vitamins E and K and it possesses promising antischistosomal properties in vitro and in a mouse model of *schistosomiasis mansoni* [10]. 4,8,12,16-tetramethylheptadecan-4-olide originates from isoprenoids of greater chain length than phytol and it is a precursor of the autoxidation of vitamin E which constitutes a potential source of its presence in the environment [45]. Isolated flavonoids and flavonoid glycosides have been reported to have the ability to induce the formation of metal nanoparticles as in the case of Apiin (apigenin glycoside) extracted from *Lawsonia inermis*. This glycoside was used in the synthesis of anisotropic gold and quasi-spherical silver nanoparticles having an average size of 21–30 nm [32, 41].

3.3 Optical studies

UV–Vis absorption spectra of an aqueous solution of the *A. conyzoides* extracts observed in both the absence and presence of Fe³⁺ and the results are depicted in Figs. 1 and 2 below.

From Fig. 1 above, the two peaks at 210 nm and 298 nm were attributed to Fe(H₂O)₆³⁺ and Fe(OH)(H₂O)₅²⁺ species present in the aqueous solution of FeCl₃ [25]. Upon addition of the aqueous extracts into the Fe³⁺ ions aqueous solution, the shape of the spectra changed drastically as the intensity of the first peak (220 nm) decreased while the second peak (298 nm) disappeared indicating the formation of Fe-nanoparticles [25, 54]. The reaction between FeCl₃ and the extracts occurred almost instantaneously and the colour of the reaction mixture changed from light yellow to black (Fig. 1) due to the excitation of the surface plasmon resonance of the metal nanoparticle [23, 50]. Isolated particles had an absorption peak at 200 nm which is typical of magnetite (Fe₃O₄) while the surface plasmon peak of FeNPs has been reported to be appearing at approximately 390 nm [28]. The band gap energy of *A. conyzoides* synthesized FeNPs was calculated using formula $E = hc/\lambda$ (h —Planck's constant, c —velocity of light and λ —wavelength and was found to be 3.11 eV [12, 20].

3.4 FTIR characterization of iron nanoparticles

FTIR spectra of iron nanoparticles synthesized using *A. conyzoides* extracts are depicted in Fig. 3.

From Fig. 3, the peaks at 686.0, 1068.5, 1030, 1124.4, 1309.6, 1400.2, 1596.9 and 3442.7 cm⁻¹ were observed and have been reported to be as a result of metal oxygen, C–O–C and OH vibrational frequencies [49]. The vibrational frequency at 686.0 parallel has been associated with metal–oxygen (M–O) vibrational frequency, the band observed at 1064.5 and 1065.0 cm⁻¹

Table 2 Some secondary metabolites identified using GC–MS with the help of a NIST 17 spectral data base

RT	Compound	MF	MW	Class
7.3628	Alfa-copaene	C ₁₅ H ₂₄	204.357	Tricyclic sesquiterpenes
7.6815	1H-2-Benzopyran-1-one, 3,4-dihydro	C ₉ H ₈ O ₂	148.161	Ketone
8.7371	2-propenoic acid 3-(2-hydroxyphenyl)- (E)-	C ₉ H ₈ O ₃	164.16	Hydroxycinnamic acid
8.9708	Precocene 1	C ₁₂ H ₁₄ O ₂	190.242	Alkene
9.5505	Lycopodan-8-one,5,12-dihydroxy-15-methyl-(5a,15S)-	C ₁₆ H ₂₅ NO ₃		Ketone
10.2581	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	C ₁₁ H ₁₆ O ₃	180.244	Furanone
10.3529	Cadala-1(10)3,8-triene	C ₁₅ H ₂₂	202.341	Terpenoid
10.5850	1,6,10-Dodecantrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	C ₁₅ H ₂₆ O	222.366	Alcohol
10.8694	Fumaric acid, ethyl-2-methylallyl ester	C ₁₀ H ₁₄ O ₄	198.218	Carboxylic acid
11.0889	Caryophyllenyl alcohol	C ₁₅ H ₂₆ O	222.372	Sesquiterpene Alcohol
11.9943	Cubenol	C ₁₅ H ₂₆ O	222.372	Sesquiterpene Alcohol
12.2404	Isoaromadendrene epoxide	C ₁₅ H ₂₄ O	220.356	Oxygenated sesquiterpenes
12.6949	Globulol	C ₁₅ H ₂₆ O	222.372	Sesquiterpene alcohol
13.2363	4-(1,3,3-trimethyl-bicyclo[4.1.0]hept-2-yl-but-3-en-2-one	C ₁₄ H ₂₂ O	206.324	Ketone
15.1620	Rotundene	C ₁₅ H ₂₄	204.357	Terpene
17.3441	4,4,8-trimethyltricyclo[6.3.1.0(15)]dodecane-2,9-diol	C ₁₅ H ₂₆ O ₂	238.37	Alcohol
18.3041	Phthalic acid cyclohexyl pentyl ester	C ₁₉ H ₂₆ O ₄	318.413	Acid esters
20.6065	Terephthalic acid phenyl undecyl ester	C ₂₅ H ₃₂ O ₄	396.527	Acid esters
20.9910	Terephthalic acid 3,4-dichlorophenyl undecyl ester	C ₂₅ H ₃₀ Cl ₂ O ₄	465.411	Acid esters
21.2597	Phytol	C ₂₀ H ₄₀ O	296.539	Acyclic diterpene
23.9657	Pregnan-16-one	C ₂₁ H ₃₄ O	302.502	Steroid
24.4105	Tricosane	C ₂₃ H ₄₈	324.637	Acyclic hydrocarbons
24.6096	2-pentacosanone	C ₂₅ H ₅₀ O	366.674	Ketone
25.7570	4,8,12,16-tetramethylheptadecan-4-olide	C ₂₁ H ₄₀ O ₂	324.549	Isoprenoid γ-lactone
26.7570	4(1H)-Pyrimidinone 6-ethoxy-2-phenyl	C ₁₂ H ₁₂ N ₂ O ₂	216.24	Ketone
27.8517	2-Undecanone 6,10-dimethyl-	C ₁₃ H ₂₆ O	198.35	Ketone
31.8723	13-Docosenamide	C ₂₂ H ₄₃ NO	337.592	Amide
38.0458	Stigmasterol	C ₂₉ H ₄₈ O	412.3705	Steroid
39.3079	a-Amyrin	C ₃₀ H ₅₀ O	426.729	Pentacyclic triterpenoid
40.4425	Lupeol	C ₃₀ H ₅₀ O	426.729 g	Triterpene
41.4531	Friedelan-3-one	C ₃₀ H ₅₀ O	426.729	Triterpenoid

corresponds to C–O–C stretching vibrations respectively [42]. The bands appearing at 3407.6, 3435.5 cm⁻¹ and 1545.0 cm⁻¹ can be assigned to the O–H stretch-ing of water and C=C stretching vibrations respectively [4, 21, 23]. The bands at 1380.5 cm⁻¹, 1394.8 cm⁻¹ are due to C–N stretching vibration of aliphatic amines and C–N stretching vibration for aromatic amines respectively, which were collectively attributed to the presence of complex phenolic compounds [21]. Hence aqueous extracts of *A. conyzoides* consist of a complex mixture of natural organic chemicals and are therefore a rich source of both capping and reducing agents during Fe-NPs synthesis. Some authors report that –OH groups participate in the reduction process, oxidizing the –OH groups to carbonyl groups, and carbonyl and carboxylate groups are involved in particle stabilization [9].

3.5 Single crystal XRD of iron nanoparticles

Single crystal X-ray diffractogram of iron nanoparticles synthesized using aqueous extracts of *A. conyzoides* are depicted in the Fig. 4 (Table 3).

Phase identification of single crystal XRD data revealed that the synthesized nanoparticles comprised of oxides of iron. Comparison of the diffractogram of the particles with COD data base revealed that the diffractogram was similar to that of orthorhombic Fe oxides nanoparticles with an average crystal size of 85.98 nm [17]. The average crystal size of the nanoparticles obtained ranged between 11.45 and 614.03 nm with the average size estimated to be 85.98 nm. The peaks appearing at 2 theta (°) of 3.79, 7.40, 8.51, 9.74, 9.99, 13.04, 14.79, 14.97, 16.49 can be attributed to orthorhombic Fe particles revealed by COD

Fig. 1 UV-Vis spectra of iron oxide nanoparticles and Fe^{3+} . Inset color change during synthesis (a) FeCl_3 , (b) extracts and (c) iron nanoparticle

2310718 [2] while peaks appearing at 2 theta of 19.86, 25.90, 26.11, 28.31, 29.82, 29.99 and 30.04 correspond to orthorhombic $\text{FeO}(\text{OH})$ [17]. The diffractograms revealed the mixed phase nature of the ensuing iron nanoparticles synthesized by aqueous extracts of *A. conyzoides* which

have also been reported when *Platanus orientalis* extracts were used [11].

3.6 SEM micrographs of Iron nanoparticles

SEM micrographs and EDX profile of Iron nanoparticles synthesized using aqueous extracts of *A. conyzoides* are depicted in the Fig. 5.

From the SEM micrographs obtained (Fig. 5), the synthesized nanoparticles are cubic in nature and have variable sizes as compared to chemical synthesized nanoparticles [15]. This variability in size is due to the inability of the secondary metabolites to control the size of the particles formed as compared to chemical synthesis though phyto-mediated synthesis is a green approach. Since the plant extract contains various secondary metabolites, they act as reducing and stabilizing agents during bio-reduction reaction during the synthesis of metallic nanoparticles [15]. EDX spectrum showed that Fe-P NPs are composed primarily of Fe, O, C, and Cl. C and O are attributed to poly-phenols and other C-containing molecules in the plant extracts while Cl originated from the precursor FeCl_3 used in the synthesis of Fe-P NPs. These results can also explain why the iron nanoparticles have no metal properties such as magnetism due to presence of additional elements such

Fig. 2 Phyto-mediated synthesis of iron nanoparticles using *A. conyzoides* extracts

Fig. 3 FT-IR spectrum of iron nanoparticles synthesized using leaves (FeNPsL) and stalks respectively (FeNPsS)

Fig. 4 Single crystal pattern of iron nanoparticles obtained using *Ageratum conyzoides* extracts

Table 3 2 theta values, d spacing and Hkl indices for phases identified

No.	2 theta (°)	d (Å)	FWHM	Hkl (a)	Matched
1	3.69	11.0321	0.3744		B
2	7.40	5.5074	0.4215		B
3	7.40	5.5068	0.3744		A,B
4	8.51	4.7896	0.3744		
5	8.51	4.7885	0.4376		B
6	9.74	4.1863	0.2902		A,B
7	9.99	4.0819	0.2534		B
8	12.88	3.1683	0.3744		
9	13.04	3.1305	0.3744		A,B
10	13.04	3.1296	0.3837		
11	14.79	2.7601	0.1864		B
12	14.81	2.7565	0.3271		
13	14.97	2.7279	0.3172		A,B
14	16.38	2.4838	0.1721		
15	16.49	2.4782	0.1543	1 0 1	B
16	16.49	2.4782	0.1543		
17	16.49	2.4782	0.1543		
18	19.86	2.0603	0.4981		A
19	22.19	1.8468	1.0799	2 1 0	
20	25.90	1.5857	0.4160	2 1 1	
21	26.11	1.5733	0.1664	1 2 1	A
22	28.31	1.4530	0.8515	1 0 2	
23	29.82	1.3811	0.1664	3 0 0	
24	29.99	1.3736	0.4343		A
25	29.99	1.3736	0.3289		
26	29.99	1.3736	0.3289		
27	30.04	1.3712	0.4784		A
28	54.87	0.7713	0.0635	1 5 1	

A—Fe_{0.856}O, B—Goethite

as K and Ca which can be linked to impurities originating from the leaves [58].

3.7 Antimicrobial activity of zerovalent iron nanoparticles

Antimicrobial inhibition of synthesized zerovalent iron nanoparticles was evaluated using agar disc diffusion method and the results are depicted in Tables 4, 5 and 6.

Tables 4, 5 and 6 show the antimicrobial activity of iron nanoparticle synthesized using aqueous extracts from different parts of *Ageratum conyzoides*. From the results obtained, the highest activity was observed against *P. aeruginosa* as compared to *E. coli*, *B. subtilis*, *S. aureus* and *C. albicans* respectively. Bacterial destruction occurs due to the physical interaction between the cell wall and the nanoparticles and is more detrimental for gram negative bacteria due to absence of the thick peptidoglycan layer found in gram positive bacteria which acts as a protective layer [34, 52]. It is to be noted that the microorganisms have negative charge while iron nanoparticles are positively charged thereby creating an electromagnetic interaction [34]. Due to this interaction, bacteria is denatured as interaction of metallic nanoparticles with protein thiol groups releases ions which lead to breakdown of bacterial cell surface leading to death [6, 44, 52]. However, the toxicity of the particles was greatly affected by the size of the particles which was considerably large due to particle aggregation. Aggregated and clustered nanoparticles have a different motion from that of the primary nanoparticles and this results in the difference in the number of particles interacting with the cell's surface in vitro thereby affecting uptake and cytotoxicity [36, 57].

Fig. 5 SEM micrographs and EDX spectra of Iron nanoparticles synthesized using aqueous extracts of *A. conyzoides*

Table 4 Antimicrobial activity of zerovalent iron nanoparticles synthesized using *Ageratum conyzoides* roots extracts

Sample ID	Gram positive		Gram Negative		Fungus
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>C. albicans</i>
Roots extract	8.0 ± 0.0	9.3 ± 1.2	8.5 ± 0.7	9.0 ± 1.0	7.1 ± 0.7
1% INPs	8.0 ± 1.0	8.3 ± 1.5	8.0 ± 1.3	11.0 ± 1.0	7.0 ± 0.0
2% INPs	8.0 ± 1.0	9.0 ± 2.0	13.1 ± 1.2	8.0 ± 1.4	7.3 ± 0.6
4% INPs	9.5 ± 0.7	9.3 ± 1.2	9.5 ± 0.7	9.3 ± 1.2	7.3 ± 0.6
7% INPs	7.5 ± 0.7	8.7 ± 1.5	10.3 ± 1.2	10.0 ± 2.0	7.7 ± 0.6
10% INPs	8.3 ± 1.5	8.7 ± 1.5	9.7 ± 1.5	10.0 ± 2.0	7.0 ± 0.0
15% INPs	8.3 ± 0.6	9.0 ± 1.7	9.3 ± 1.5	9.7 ± 0.6	7.0 ± 0.0
DMSO	N.I	N.I	7	N.I	N.I
0.1 FeCl ₃	N.I	N.I	7	7	N.I
Cf (10 mg/g)	10	44	40	40	N.I

N.I no inhibition, DMSO dimethylsulfoxide, Cf ciprofloxacin, iron nanoparticles

Table 5 Antimicrobial activity of zerovalent iron nanoparticles synthesized using *Ageratum conyzoides* leaves extracts

Sample ID	Gram positive		Gram Negative		Fungus
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>C. albicans</i>
Leaves extract	9.0 ± 1.0	9.7 ± 1.5	9.3 ± 0.6	10.7 ± 1.2	8.3 ± 0.6
1% INPs	9.3 ± 0.6	9.3 ± 1.5	8.0 ± 1.7	8.3 ± 0.6	9.0 ± 1.7
2% INPs	8.0 ± 1.0	10.3 ± 0.6	10.3 ± 1.5	9.7 ± 2.5	8.0 ± 1.0
4% INPs	8.7 ± 1.5	9.0 ± 1.0	9.0 ± 2.6	9.7 ± 1.2	9.0 ± 1.0
7% INPs	9.3 ± 2.3	10.0 ± 1.7	8.3 ± 1.5	9.0 ± 2.0	8.3 ± 1.5
10% INPs	9.3 ± 1.2	8.3 ± 0.6	8.7 ± 1.2	8.3 ± 0.6	8.7 ± 0.6
15% INPs	9.0 ± 1.0	9.3 ± 2.3	8.5 ± 0.7	8.0 ± 0.0	8.0 ± 1.7
DMSO	N.I	N.I	7	N.I	N.I
0.1 FeCl ₃	N.I	N.I	7	7	N.I
Cf (10 mg/g)	10	44	40	40	N.I

N.I no inhibition, DMSO dimethylsulfoxide, Cf ciprofloxacin, iron nanoparticles

3.8 Methylene blue degradation

The catalytic activity of iron nanoparticles synthesized using *Ageratum conyzoides* extracts were evaluated and the results are depicted in Fig. 6.

Figure 6 shows the UV–Vis photocatalytic degradation spectra of MB solution after different time upon contact with hydrogen peroxide and iron nanoparticles. The results indicate that while addition of H₂O₂ resulted in a slight decrease in the absorption band of MB, addition of the iron nanoparticles resulted in an even greater decrease. The absorbance of

Table 6 Antimicrobial activity of zerovalent iron nanoparticles synthesized using *Ageratum conyzoides* stalks extracts

Sample ID	Gram positive		Gram Negative		Fungus
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>C. albicans</i>
Stalks extract	8.7 ± 2.1	9.3 ± 0.6	7.7 ± 0.6	10.3 ± 0.6	9.0 ± 1.0
1% INPs	8.7 ± 0.6	8.7 ± 1.5	8.7 ± 0.6	10.0 ± 0.0	8.0 ± 1.0
2% INPs	7.7 ± 1.2	8.0 ± 0.0	10.3 ± 0.6	9.3 ± 1.5	8.3 ± 1.2
4% INPs	10.0 ± 0.0	8.0 ± 1.0	9.3 ± 1.2	9.3 ± 1.5	7.3 ± 0.6
7% INPs	8.7 ± 0.6	8.0 ± 0.0	7.7 ± 0.6	10.0 ± 2.6	8.0 ± 1.0
10% INPs	8.3 ± 2.3	9.3 ± 2.3	9.3 ± 1.2	9.0 ± 1.7	8.7 ± 1.5
15% INPs	9.6 ± 3.1	9.3 ± 1.2	9.7 ± 0.6	10.0 ± 1.0	7.7 ± 0.6
DMSO	N.I	N.I	7	N.I	N.I
0.1 FeCl ₃	N.I	N.I	7	7	N.I
Cf (10 mg/g)	10	44	40	40	N.I

N.I no inhibition, DMSO dimethylsulfoxide, Cf ciprofloxacin, iron nanoparticles

Fig. 6 Degradation of methylene blue using iron nanoparticles

the solution was approaching zero with increase in the contact time between the solution and the nanoparticles after being activated upon exposure to UV light and the decolourization efficiency was calculated to be $92.10 \pm 0.05\%$. It has been reported that metal oxides upon activation with UV light leads to promotion of photoexcited electrons from the valence band to the conduction band forming an electron hole/pair which are able to reduce or oxidize compounds adsorbed on the photocatalyst surface. These activity is as a results of generation of $\text{OH}^\cdot$ radicals by oxidation of OH^- anions and generation of $\text{O}_2^\cdot$ radicals by reduction of O_2 which react with pollutants to degrade them [24].

4 Conclusion

Ageratum conyzoides extracts have been used to synthesize iron nanoparticles. The characterization results confirmed the formation of iron nanoparticles which were stabilized and capped with polyphenols from the extracts. The results obtained in this study confirms that *Ageratum conyzoides* can play an important role in the bioreduction of Fe^{3+} ions to FeNPs which have significant antimicrobial and photocatalytic degradation activity against selected microorganisms and methylene blue

respectively. Hence reduction of iron salt precursors with the plant extracts can generate iron nanoparticles with different antimicrobial, photocatalytic degradation activity, morphology and elemental composition. The morphology and size of the particles is greatly influence by aggregation which occurs during the synthesis process when secondary metabolites are used as a green alternative to chemical synthesis.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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