



BIOSYNTHESIS AND CHARACTERIZATION OF SILVER AND IRON NANOPARTICLES FROM *SPINACIA OLERACEA* AND THEIR ANTIMICROBIAL STUDIES

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ABSTRACT: Nanoscale Silver and Iron particles have biological applications because of their unique properties, as antimicrobial activity. The aim of the work was to test effects of Silver and Iron nanoparticles from *Spinacia oleracea* using suggested bacterial colonies. We performed biosafety and biocompatibility tests for Nanoparticles and conjugate with *Spinacia oleracea*. UV-visible spectroscopy is important technique for analyzing the formation of silver and iron nanoparticles in aqueous solution AgNPs and iron has free electron, which gives rise to plasma resonance absorption band, due combined vibration of metal nanoparticles in resonance with the light wave. The presence of the elemental silver and iron can be seen in the graph presented by EDX, which indicates the reduction of silver ions to elemental silver and iron ions to elemental iron ions. Screening of ethanolic extracts of *Spinacia oleracea* resulted in moderate antibacterial activities against different bacteria. The silver and iron nanoparticles of average size have been synthesized using dried leaves of plant *spinacia oleracea*. Characterizations from UV-Vis, SEM, EDX support the stability of biosynthesized nanoparticles. The silver and iron nanoparticles using *spinacia* proved excellent antibacterial activity.

Key words: Nano medicine, Silver nanoparticle, Iron nano particle, *Spinacia oleracea*.

INTRODUCTION

Nanotechnology is an enormously powerful technology, which holds a huge promise for the design and development of many types of novel products with its potential medical applications on early disease detection, treatment, and prevention. Nanoparticles, because of their small size, have distinct properties compared to the bulk form of the same material, thus offering many new developments in the fields of biosensors, biomedicine, and bio nanotechnology. Silver is the metal of choice as they hold the promise to kill microbes effectively. Silver nanoparticles have been recently known to be a promising antimicrobial agent that acts on a broad range of target sites both extracellular as well as intracellular. Silver is widely used as a catalyst for the oxidation of methanol to formaldehyde and ethylene to ethylene oxide [1]. Ag NP highly antimicrobial to several species of bacteria, including the common kitchen microbe, *E. coli*. According to the mechanism reported, silver nanoparticles interact with the outer membrane of bacteria, and arrest the respiration and some other metabolic pathway that leads to the death of the bacteria. Nano-sized silver have already provides a more durable antimicrobial protection, often for the life of the product. The two most commonly studied iron oxides have been magnetite (Fe₃O₄) and maghemite (γ -Fe₂O₃) [2]. IONPs are found naturally in the environment as particulate matter in air pollution and in volcanic eruptions. Either Fe₃O₄ (magnetite) or γ -Fe₂O₃ (maghemite), particles can be generated as emissions from traffic, industry and power stations but can also be specifically synthesised chemically for a wide variety of applications [3]. Due to their magnetic properties SPIONs have been extensively used in a number of bioapplications including magnetic drug and gene delivery [4], tissue repair, cell separation, magnetic resonance imaging and magnetic fluid hyperthermia. Antibody-targeted SPIONs can be used for diagnosis and targeted therapy of cancer [5].

Tumour cells have shown a greater sensitivity to heat treatments compared to healthy cells. This has led to the use of thermo-ablation and hyperthermic therapies in the clinic, often in combination with other treatments. The first clinical study of magnetic fluid hyperthermia by [6] showed that direct injection of 12.5 ml SPION suspension into the prostate at concentrations of 120 mg/ml was well tolerated. The *Verticillium* sp. fungal biomass when exposed to aqueous AgNO_3 solution resulted in the intracellular formation of silver nanoparticles, while *Fusarium oxysporum* biomass resulted in the extracellular silver nanoparticles [7]. The use of microorganisms such as bacteria, yeast, fungi and actinomycetes has been described for the formation of nanoparticles and their applications [7] Silver is a non-toxic, safe inorganic antibacterial agent being used for centuries and is capable of killing about 650 microorganisms that cause diseases Bactericidal behavior of nanoparticles is attributed to the presence of electronic effects that are brought about as a result of change in local electronic structure of the surface due to smaller sizes. Metal oxides NPs are finding increasing application in a wide range of fields and represent about one-third of the consumer products nanotechnology market [8]. Aluminium nanomaterials act as drug delivery systems, by encapsulating the drugs the drugs to increase solubility for evading clearance mechanisms and allowing the site-specific targeting of drugs to cells [9].

MATERIALS AND METHODS

Chemicals

Pure and analytical grade chemicals were used in all experiments including synthesis of iron and silver nanoparticles, media preparation for growth of bacterial cells. Ferric chloride (FeCl_3) and silver nitrate (AgNO_3), were purchased. The bacterial cultures of *E.coli*, *E.coli(d)*, *E.coli(M)* *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Klebsilla pneumonia*, *Staphylo coccus* were obtained from DST-purse centre, SV university, tirupati, india. Antibiotic Amoxyclav(Himedia SD063).

Synthesis of nanoparticles from *Spinacia oleracea* extracts

Preparation of the Extract

Fresh leafy vegetable are collected, cut into fine pieces and dried at room temperature. Dried leaves are powdered and 3g of powder was weighed into 60 ml of Ro water and boiled for 10 min at 100°C. the extract was filtered through Whatman No.1 filter paper. The extract was stored at 40 C for further experiments.

Synthesis of Silver nanoparticles from *Spinancia oleracea* extract

The aqueous solution of 1mM silver nitrate (AgNO_3) was prepared and used for the synthesis of silver nanoparticles. 5 ml of *spinancia oleracea* extract was added into 5ml of aqueous solution of 1 mM silver nitrate for reduction into Ag^+ ions. Here the filtrate acts as reducing and stabilizing agent for 1mM of AgNO_3 .

Synthesis of iron nanoparticles from *spinancia oleracea* extract

The aqueous solution of 1mM ferric chloride (FeCl_3) was prepared and used for the synthesis of iron nanoparticles. 5 ml of *spinancia* extract was added into 5ml of aqueous solution of 1 mM ferric chloride for reduction into iron ions. Here the filtrate acts as reducing and stabilizing agent for 1mM of FeCl_3 .

Characterization techniques:

UV-Vis Spectroscopy-

The Iron and Ag nanoparticles were characterized in a Nanodrop 8000 UV-VIS spectrophotometer, to know the kinetic behavior of Iron and Ag nanoparticles. The scanning range for the samples was 200-800 nm at a scan speed of 480 nm/min. The spectrophotometer was equipped with "UV Winlab" software to record and analyze data. Baseline correction of the spectrophotometer was carried out by using a blank reference. The UV-Vis absorption spectra of all the samples were recorded and numerical data were plotted in the ".....".

Scanning electron microscope (SEM)

In this present work Scanning Electron Microscopy (SEM) and EDX was performed by oxford Inca penta Fetx3 EDS instrument attached to Carl Zeiss EVO MA 15 Scanning electron Microscope (200kV) machine with a line resolution 2.32(in Å^0). These images were taken by drop coating AgNPs and iron nanoparticles on an aluminium foil. Energy dispersive Absorption Spectroscopy photograph of AgNPs were carried out by the SEM equipment, as mentioned above.

Particle size analyzer and Zeta potential measurement

Particle size and Zeta potential measurement experiments were carried out by using a Nanoparticle (HORIBA).

Antimicrobial activity

The antimicrobial activity of silver and iron nanoparticles was evaluated against Gram positive: *Staphylococcus aureus*, *Bacillus megaterium*, Gram negative *Escherichia coli* (M&D), *Pseudomonas aeruginosa*, *klebsiella pneumonia* by disc method. The 24h old cultures were prepared in nutrient broth (composition (g/l) peptone, yeast extract, sodium chloride, and D(+)-glucose). two replicas of respective microorganisms were prepared by spreading 100ul of revived culture on the nutrient agar plate (composition(g/l) pepton, yeast extract. Sodium chloride, D (+)-glucose and agar-agar), with the help of spreader. Discs were prepared by using Whatman No.1 filter paper. The discs were placed on agar plates and sample of synthesized silver and iron nanoparticles were added on the disc with the help of micropipette. The plates were incubated at 37^oc overnight. Amoxyclav (Himedia SD063) disc was used as reference drug. The Bacterial strains of Microorganisms used for the determination of antibacterial activities of silver and iron nanoparticles synthesized were obtained from Department of Microbiology, S.V.University, Tirupathi. Different bacterial strains maintained on nutrient agar and subcultures were freshly prepared before use. Bacterial cultures were prepared by transferring two to three colonies into a tube containing 20 ml nutrient broth and grown overnight at 37^oC.

RESULTS AND DISCUSSION

Synthesis and characterization of nanoparticles

Syntheses of Ag nanoparticles from plant extract

Nanoparticles are synthesized according to the protocol discussed in “methods and materials” (section3). On mixing the extract with aqueous solution of the Ag ion complex for silver synthesis and FeCl₃ for iron, a white precipitate was formed then the color changes for silver and for iron color changes immediately on addition of FeCl₃ to extract. For silver nanoparticles color changes from colorless to yellowish color where as for iron color changes from colorless to yellowish brown color. It was due to the reduction of Ag⁺ and iron ions which indicates the formation of Ag and iron nanoparticles shown in Figure 1.

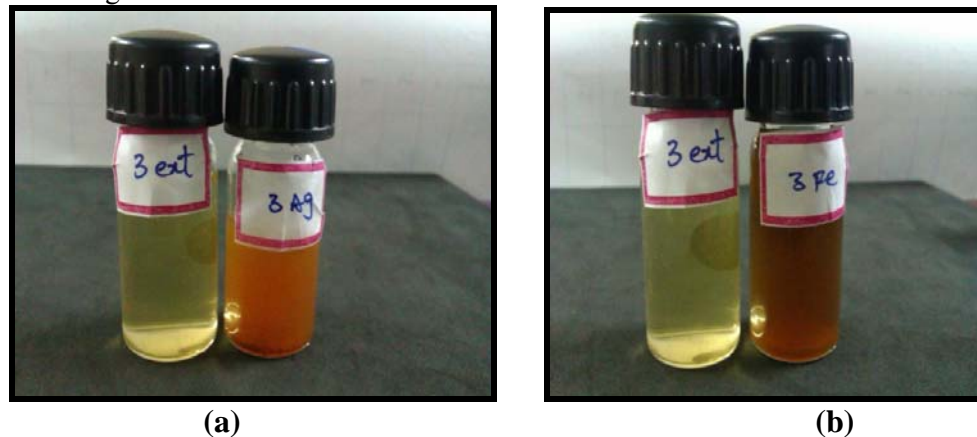
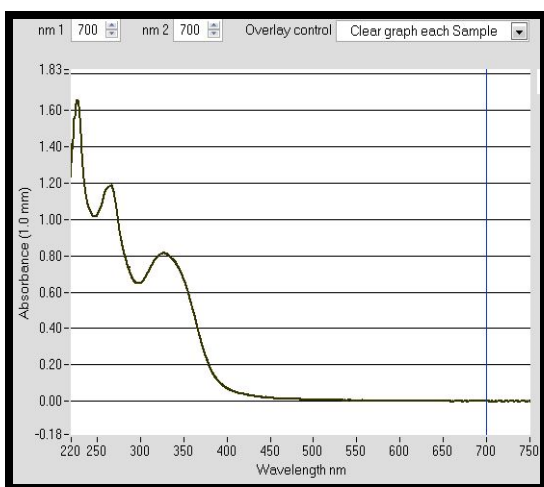


Figure 1: Colour changes of leaf extract before and after synthesis of (a) silver (b) iron nanoparticles

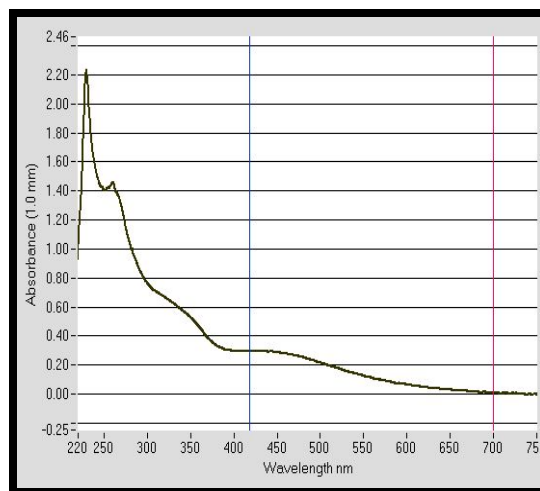
Characterization of nanoparticles

UV–Vis spectral analysis

UV-visible spectroscopy is important technique for analyzing the formation of silver and iron nanoparticles in aqueous solution AgNPs and iron has free electron, which gives rise to plasma resonance absorption band, due combined vibration of metal nanoparticles in resonance with the light wave. A surface plasma resonance spectrum of AgNPs and iron nanoparticles was obtained at 422nm and 261 after 5min color changing to light yellowish color. in figure the surface plasma AgNPs and iron nanoparticles at increasing concentration was taken and the color changes were observed for both nanoparticles. For silver color changes from colorless to light yellowish brown color and for iron colorless to yellowish brown color respectively. Metal nanoparticles can be synthesized by reducing metal ions using some chemical molecules. in green synthesis, it is observed that natural material extract act as reducing agent for generation of metal nanoparticles.



(a)



(b)

Figure 2: Spectral analysis of *Spinacia oleracea* (a) plant extract (b) silver nanoparticles

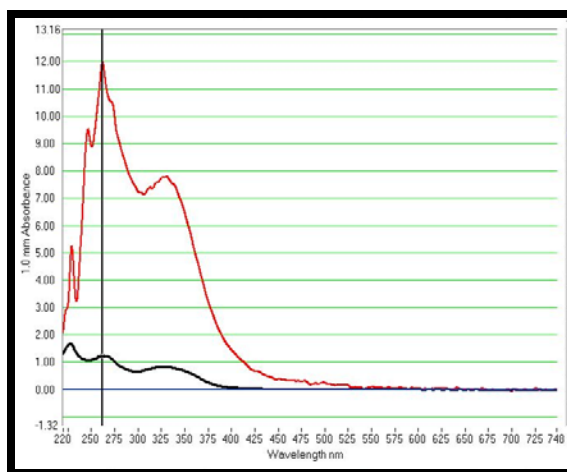


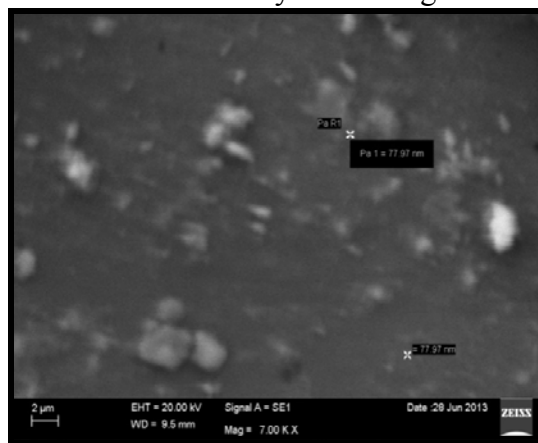
Figure 3: Spectral analysis of *Spinacia oleracea* and Iron nanoparticles

SEM analysis of Ag and iron nanoparticles

The Morphology and size of nanoparticles in solution is determined by SEM images



(a)



(b)

Figure 4: SEM images of (a) Silver and (b) Iron nanoparticles (Mag.7.00 KX)

EDX studies of Ag and iron nanoparticles

The presence of the elemental silver and iron can be seen in the graph presented by EDX, which indicates the reduction of silver ions to elemental silver and iron ions to elemental iron ions.

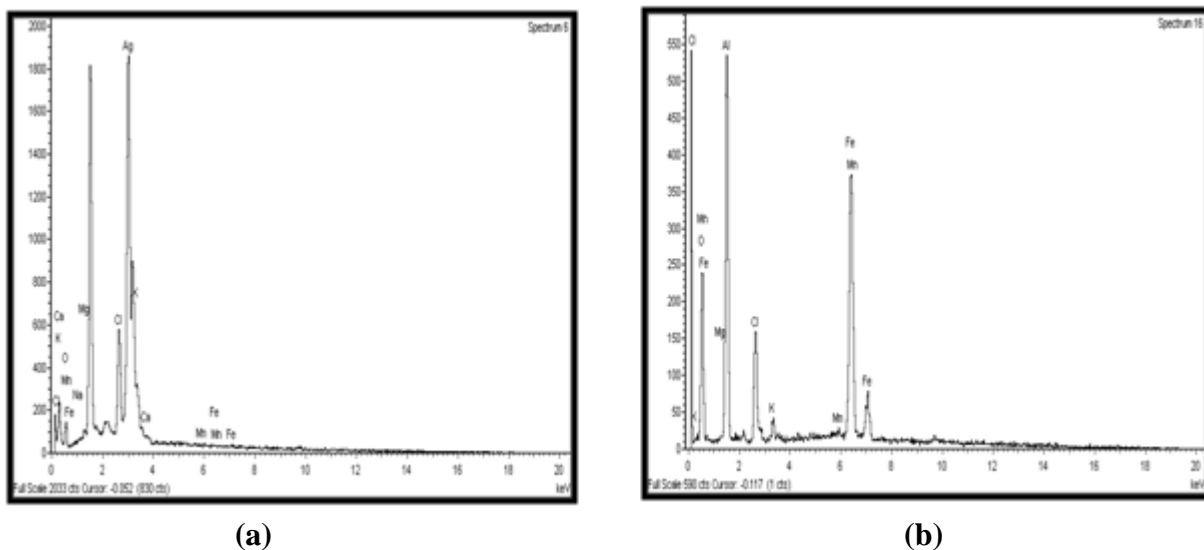


Figure 5: EDX spectrum of synthesized silver nano particles and Iron nano particles

Zeta potential

The zeta potential of the synthesized silver and iron nanoparticles is determined in water as a dispersant. The zeta potential is found to be -21.6 mV and -114mV.

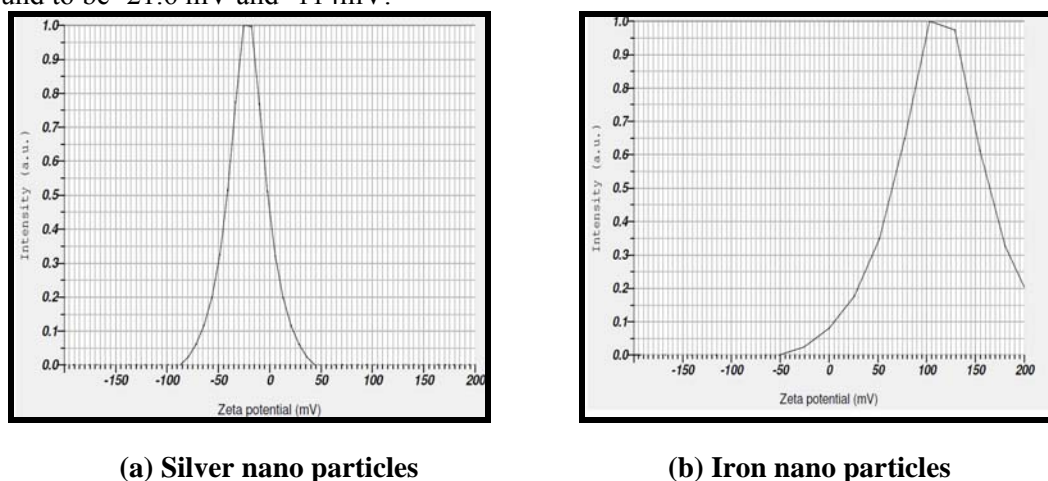


Figure 6: Zeta potential of synthesized silver and iron nanoparticles

Antimicrobial activity

The nanoparticles synthesis by green route was found extremely against 6 bacterial species at a concentration of 20 μ l Ag and iron nanoparticles, Gram positive..*Bacillus megaterium*, *staphylococcus aureus*, Gram negative..*Escherichia coli (M)*, *Escherichia coli (D)*, *pseudomonas aeruginosa*, *klebsila pneumonia*. The results are shown in table 1. The cultures shown zone of inhibition which was about 1.6,2.2,2.8,3.2,2.6,2.7cm for Ag nanoparticles and 1.7,1.2,1.8,2.3,1.6,1.4 for iron nanoparticles in diameter respectively. The culture of *Escherichia (D)* shows maximum zone of inhibition for both nanoparticles. The *spinancia oleracea* leaf extract is found suitable for simple and rapid extraction of Ag and iron nanoparticles by green synthesis within 5- 10min. The spectroscopy characterization from UV-Vis, SEM, and EDX support the formation and stability of the biosynthesized AG and iron nanoparticles. This is a very simple and rapid method of green synthesis of Ag and iron nanoparticles which can be useful in various biomedical and biotechnological applications.

Table1: Inhibitory activity of Silver and Iron nano particles on different bacteria

Microorganisms	Zone of inhibition(cm)		
	Control(AC)	Silver	Iron
<i>Bacillus megaterium</i>	2.4	1.6	1.7
<i>Staphylococcus aureus</i>	0.9	2.2	1.2
<i>Pseudomonas aeruginosa</i>	2.4	2.6	1.6
<i>E.coli(D)</i>	2.4	3.2	2.3
<i>E.coli(M)</i>	2.3	2.8	1.8
<i>Klebsiella pneumonia</i>	1.5	2.7	1.9

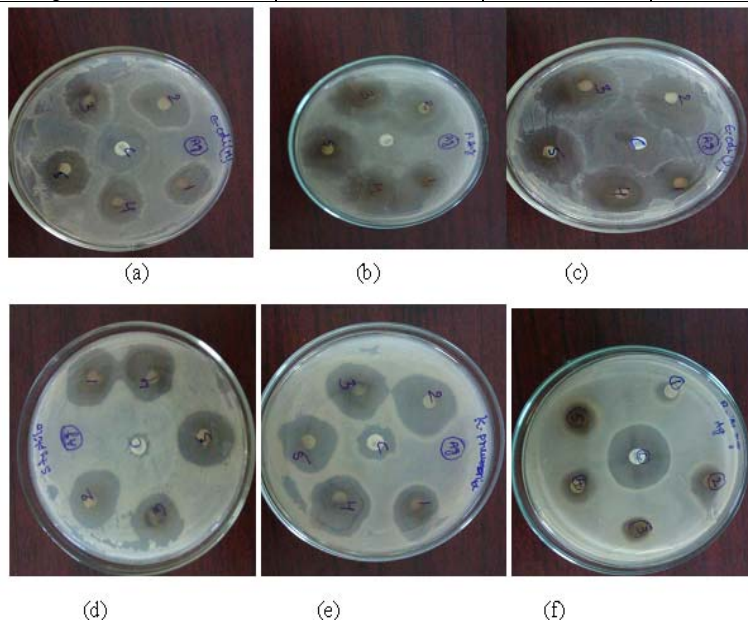


Figure 8:antimicrobial activity of silver nanoparticles(zone 3 for spincia oleracea) against 6 microorganisms (a) pseudomonas aeruginosa,(b) Escherichia coli (M),(c) Escherichia coli(D),(d)staphylococcus aures,(e)klebsila pneumonia,(f) Bacillus megaterium.

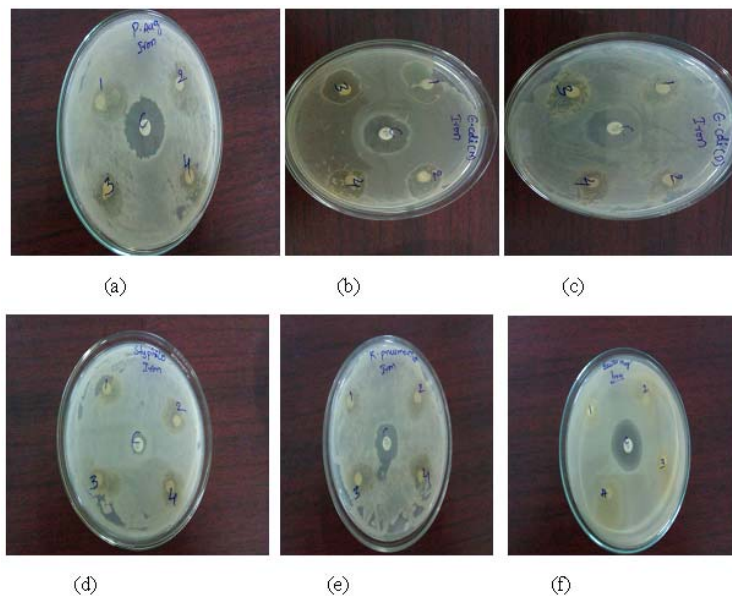


Figure 9: Antimicrobial activity of iron nanoparticles(zone 3 for spincia oleracea) against 6 microorganisms (a) pseudomonas aeruginosa,(b) Escherichia coli (M),(c) Escherichia coli(D),(d)staphylococcus aures,(e) klebsila pneumonia,(f) Bacillus megaterium.

CONCLUSION

The silver and iron nanoparticles of average size have been synthesized using dried leaves of plant *spinacia oleracea*. Characterizations from UV-Vis, SEM, and EDX support the stability of biosynthesized nanoparticles. The silver and iron nanoparticles using *spinacia* proved excellent antimicrobial activity. These silver and iron nanoparticles may be used in food and pharmaceutical industries.

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