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Synthesis and Spectral Characterization of Silver and Iron Nanoparticles and Their Antimicrobial Activity of Amaranthus Viridis

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Abstract:

The synthesized nanoparticles were of spherical and sheet shaped and the estimated sizes were 160-180 nm. The size were bigger as the nanoparticles were surrounded by a thin layer of proteins and metabolites such as terpenoids having functional groups of amines, alcohols, ketones, aldehydes, etc., which were found from the characterization using UV-vis spectrophotometer, SEM, XRD, and FTIR techniques. All these techniques it was proved that the concentration of plant extract to metal ion ratio plays an important role in the shape determination of the nanoparticles.

Keywords: Nanoparticles, UV-vis spectrophotometer, SEM, XRD, and FTIR techniques

Introduction:

"Nano" refers to a length of a billionth of a meter (Williams and Wade 2007). The word "nano" from which this relatively new field derives its name is a prefix denoting 10-9. "Nano" comes from nanos, a Greek word meaning dwarf. In the case of nanotechnology, it refers to things in the ballpark that are one-billionth of a meter in size. When Albert Einstein was in graduate school in 1905, he took experimental data on the diffusion of sugar in water and showed that a single sugar molecule is about one nanometer in diameter.

As an extension of the above definition of nanotechnology, when materials and devices are used to control life processes or the ideas of nature are applied to develop new nano products, it is referred to as nanobiotechnology. It would be of interest to note that Feynman seeded the background of nanobiotechnology by believing that the organism itself is the most efficient macroscopic and microscopic machine. A few decades later, this idea opened the eyes of researchers to the fusion of nanotechnology and biotechnology. These characteristics of NPs result in remarkably different properties from those connected with the same material in the bulk state. Examples of properties that change when the particle size enters the nanometer range are

melting temperature, solubility, optical property and magnetic properties. For instance, the melting temperature of silver is 9600C, whereas silver nanoparticles can be melted at a substantially lower temperature. In the same way conventional gold is yellow, whereas gold NPs are red or blue depending on the particle size (Eustis and El-Sayed 2006). It is generally recognized that a large surface area per unit weight is a critical property of a nanomaterials.

Silver Nanoparticles

Silver nanoparticles are one of the promising products in the nanotechnology industry. The development of consistent processes for the synthesis of silver nanomaterials is an important aspect of current nanotechnology research. One of such promising process is green synthesis. Silver nanoparticles can be synthesized by several physical, chemical and biological methods. However for the past few years, various rapid chemical methods have been replaced by green synthesis because of avoiding toxicity of the process and increased quality.

Silver nanoparticles as an antimicrobial agent:

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. New technology advances in reducing silver compound chemically to nanoscale sized particles have enabled the integration of this valuable antimicrobial into a larger number of materials—including plastics, coatings, and foams as well as natural and synthetic fibers. Nano-sized silver have already provides a more durable antimicrobial protection, often for the life of the product. 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 nanoparticles shows very strong bactericidal activity against gram-positive as well as gram negative bacteria including multi resistant strains, and also it was found to be in few studies. However, the majority of these being energy and capital intensive processes deal with various toxic chemicals and non-polar solvents that hinder the efficacy of these NPs toward biomedical applications. The biogenic synthesis of metal NPs reduces these hazards through the elimination/minimization of generated waste and the implementation of sustainable processes.

On the other hand, the surface area derived from light scattering methods probes only that surface which is accessible to light. Intuitively, the outer surface area of nanoparticle is more important than the

inner one for stimulating a specific cell response, e.g. phagocytosis. However, biological fluids can enter pores as small as 1-2 nm, and the total surface area could still have a significant effect on biological responses to nanoparticles. In this context, the surface roughness of nanoparticles is often cited as an important aspect of the biological implications of their use. Because nanoparticles are so small, the distinction between a pore and a rough surface is rather vague. Biological methods of NPs synthesis using microorganisms, enzymes and plant or plant extracts have been studied as possible eco-friendly alternatives to chemical and physical methods. Jose Yacaman and co-workers first reported the formation of gold and silver NPs using living plants. Song and Kim (2009) prepared Ag NPs using five different leaf extracts (Pine, Persimmon, Ginkgo, Magnolia, and Platanus) and compared the extracellular synthesis of Ag NPs. They showed that among the five, magnolia leaf broth had the best reducing property in terms of synthesis rate and conversion to Ag NPs. Reaction kinetics showed that only 11 min was required for more than 90% conversion at the reaction temperature of 95 C using magnolia leaf broth. A simple technique using Sugar apple (Annona squamosa) peel extract was used by Kumar et al. (2012) and Roopan et al. (2015) to synthesize Ag NPs and SnO2 NPs. They have demonstrated the effect of temperature on the Ag NPs synthesis and also proposed a plausible mechanism for its reduction. The synthesized SnO2 NPs exhibited good efficiency on hepatocellular carcinoma cell line. Mollick et al. (2012) reported synthesis of Ag NPs through a reliable, eco-friendly and green route using Paederia foetida L. leaf extract as a reducing cum stabilizing agent. Shankar et al. (2004) carried out the synthesis of pure metallic NPs of silver and gold by the reduction of Ag+ and Au3+ ions using Neem (Azadirachta indica) leaf broth. Sastry and co-workers (Mukherjee et al., 2001a, 2001b) synthesized metal NPs using eukaryotic organisms such as Verticillium sp. They suggested that the processing and handling of the biomass was simpler in the case of fungi in comparison with the bacteria.

In another work, Ag NPs have been derived using green kondagogu gum as a template (Kora et al., 2010). Roopan and co-workers (Roopan et al., 2013; Roopan and Elango, 2015) have used extracts from different parts of Cocos nucifera to synthesize Ag NPs and Au NPs under different physical, chemical and biological conditions. It has also been reported that acacia gum (Mohan et al., 2007) can be utilized both as a reducing as well as stabilizing agent for the Ag NPs biosynthesis. To date, several approaches have been carried out for the biogenic synthesis of Ag NPs using various natural products such as latex from Alfalfa sprouts (Gardea-Torresdey et al., 2003), Jatropha curcas (Bar et al., 2009a), geranium leaves plant extract (Shankar et al., 2003), and the stem derived callus extract of bitter apple (Citrullus colocynthis) (Satyavani et al., 2011). Green synthesis of Ag NPs was carried out by Kumar et al. (2014) using

Alternanthera dentata leaf extract and the synthesized NPs showed good antimicrobial activity. Recently, Madhumitha et al. (2015) have nicely assimilated the different methods of synthesis of Ag NPs and their antimicrobial studies.

IRON NANOPARTICLES

Iron, the most ubiquitous of the transition metals and the fourth most plentiful element in the Earth's crust, is the structural backbone of our modern infrastructure. It is therefore ironic that as a nanoparticles, iron has been somewhat neglected in favor of its own oxides, as well as other metals such as cobalt, nickel, gold, and platinum. This is unfortunate, but understandable. Iron's reactivity is important in macroscopic applications (particularly rusting), but is a dominant concern at the nanoscale.

Metal oxide nanoparticles

Presently, the group of the most important nanomaterials includes simple metal oxides such as titanium oxide (TiO2), zinc oxide (ZnO), magnesium oxide (MgO), copper oxide (CuO), aluminium oxide (Al2O3), manganese oxide (MnO2) and iron oxide (Fe3O4, Fe2O3) (Pan et al, 2010, Fahmy and Cormier, 2009, Balasubramanyam et al, 2010, Sárközi et al, 2009, Oszlánczi et al, 2010, Singh et al, 2010). Metal oxides NPs are finding increasing application in a wide range of fields and represent about one-third of the consumer products nanotechnology market (Maynard, 2006). These materials are used as pigments in paints (TiO2), as sunscreens and cosmetics (TiO2, ZnO), as antimicrobial agents (MgO, CuO), in industrial operations (Al2O3, MnO2) and for medical purposes (Al2O3, Fe3O4, Fe2O3). 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. Previous toxicological studies on nanomaterials were conducted on TiO2, CdO2, C60, and carbon nanotubes only. The toxicity of iron oxide nanoparticles (IONPs), although they are the only metal oxide nanoparticles approved for clinical use, has been investigated only in a small number of studies.

Iron and iron oxides in nature

Iron and its compounds are widespread in nature and readily synthezised in the laboratory. Iron compounds present in the hydrosphere, the lithosphere and (as pollutants) in the atmosphere. Iron is a biogenic element, present in all biota, but some iron compounds can cause harmful effects to humans, animals, and environment. In occupational exposure of humans, iron and iron oxides are known to produce benign siderosis – but iron oxides have been implicated also as a vehicle for transporting high concentrations of carcinogens and sulfur dioxide deep into the lungs, thereby enhancing the activity of these pollutants. Iron oxides also cause damage by staining materials. Analyses of urban air samples showed that the probable sources of iron compounds are the iron and

steel industry and urban transport such as underground railways. Tunnel dust – generated by interaction of brakes, wheels and rails – contains about 90% iron, 1–2% quartz and the remnants of other metals in the underground rail system. There exist 6 iron oxides composed of Fe and O: hematite (α-Fe2O3), magnetite (Fe3O4), maghemite (γ-Fe2O3), β-Fe2O3, ε-Fe2O3.and Wüstite (FeO). In most of these compounds, iron is in the trivalent state, but FeO and Fe3O4 contain Fe(II). Hematite, α-Fe2O3, is the oldest known Fe oxide mineral and is widespread in rocks and soils. It is extremely stable and is often the final stage of transformations of other iron oxides. The blood-red-coloured hematite is an important pigment and a valuable ore. Other names for hematite include iron(III)oxide, ferric oxide, red ochre and kidney ore. Magnetite, Fe3O4, is a black, ferromagnetic mineral containing both Fe(II) and Fe(III). Magnetite is an important iron ore. Together with titanomagnetite, it is responsible for the magnetic properties of rocks. It is formed in various organisms in which it serves as an orientation aid. Other names for magnetite include black iron oxide, magnetic iron ore, iron(II,III)oxide and ferrous ferrite. Maghemite, γ-Fe2O3, is a red-brown, ferromagnetic mineral isostructural with magnetite, but with cation deficient site. It occurs in soils as a weathering product of magnetite or as the product of heating of other Fe oxides, usuallay in the presence of organic matter.

Silver Nanoparticles

The extraordinary optical properties of silver nanoparticles were used by glass founders as farback as in the time of the Roman Empire. This is evidenced by the so-called Lycurgus cup (4thcentury AD) now exposed in the British Museum. A detailed study of the composition of its bronze-mounted insets of stained glass, carried out in the late 20th century, revealed the presence of metal nanoparticles (with the average diameter of 40 nm) that consists of silver (70 %) and gold (30 %) alloy (Barber, 1990). This explained a remarkable feature of this bowl to change its color from red in transmitted light to grayish green in reflected light. In the preparation of this glass, nanosilver was formed institute. Before the 1980s, the scientific and practical interest in silver nanoparticles was exclusively caused by the possibility of their use as highly dispersed supports for enhancing the signals from organic molecules in the Raman spectroscopy. Fundamental studies carried out in the last three decade shows that silver nanoparticles exhibit a rare combination of valuable properties, namely, unique optical properties associated with the surface Plasmon resonance (SPR), well-developed surfaces, catalytic activity, high electrical double layer capacitance, etc.(Henglein, 1989). That's why they serve as a material in the development of new-generation electronic, optical and sensor devices. In the past 20 years, the trend miniaturization and the necessity of modernization of technological processes led to the substantial increase in the number of scientific publication devoted to the synthesis and

Properties of silver nanoparticles; at present, their synthesis is among the most actively developing trends of colloid chemistry.

Silver is widely used as a catalyst for the oxidation of methanol to formaldehyde and ethylene to ethylene oxide (Nagy et al., 1999). Colloidal silver is of particular interest because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activity (Frattini et al., 2005). For example, silver colloids are useful substrates for surface enhanced spectroscopy (SERS), since it partly requires an electrically conducting surface.

Biochemical Synthesis of Ag Nanoparticles

Chemical approaches are the most popular methods for the preparation of nanoparticles. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol. Since noble metal nanoparticles such as gold, silver and platinum nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals. Biological methods of nanoparticles synthesis using microorganism, enzyme, and plant or plant extract have been suggested as possible ecofriendly alternatives to chemical and physical methods. Using plant for nanoparticles synthesis can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures. It can also be suitably scaled up for large-scale synthesis of nanoparticles. It is well known that biological systems can provide a number of metal or metall8containing particles in the nanometer size range. The synthesis of magnetite nanoparticles by magneto tactic bacteria, siliceous materials by diatoms and gypsum and calcium carbonate layers by S-layer bacteria are some of the examples. The synthesis and assembly of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable ‘‘green chemistry’’ procedures and involving organisms ranging from bacteria to fungi and even plants. Hence, both unicellular and multicellular organisms are known to produce inorganic materials either intra-or extracellular. The Verticillium sp. fungal biomass when exposed to aqueous AgNO3 solution resulted in the intracellular formation of silver nanoparticles, while Fusarium oxysporum biomass resulted in the extracellular silver nanoparticles. The use of microorganisms such as bacteria, yeast, fungi and actinomycetes has been described for the formation of nanoparticles and their applications.

METARIALS 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 (FeCl3) and silver nitrate (AgNO3), were purchased. The bacterial cultures of E.coli, E.coli (d), E.coli (M), Bacillus megaterium, Pseudomonas aeruginosa, Klebsilla pneumonia, Stypilococcus were obtained from Global Institute of Biotechnology, Hyderabad, Telangana, India. Antibiotic Amoxyclav (Himedia SD063).

Synthesis of nanoparticles from Amaranthus viridis 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 100oC.the extract was filtered through WhatmanNo.1 filter paper. The extract was stored at 40 C for further experiments.

Synthesis of Silver nanoparticles from A. viridis extract

The aqueous solution of 1mM silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. 5 ml of A. viridis 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 AgNO3.

Synthesis of iron nanoparticles from A. viridis extract

The aqueous solution of 1mM ferric chloride (FeCl3) was prepared and used for the synthesis of iron nanoparticles. 5 ml of leaf 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 FeCl3.

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 "UVWinlab" 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.

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 A0). These images were taken by drop coating AgNPs and iron nanoparticals on an aluminium foil. Energy dispersive Absorption Spectroscopy photograph of AgNPs were carried out by the SEM equipment, as mentioned above.

Zeta potential measurement

Zeta potential measurement experiments were carried out by using a Nanopartica (HORIBA).

Antimicrobial activity

The antimicrobial activity of silver and iron nanoparticales was evaluated against Gram positive: Staphylococcus aureus, Bacillus megaterium, Gram negative Escherichia coli (M&D), Pseudomonas aeruginosa, Klebsilla 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 100µl 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 Whatmann No.1 filter paper. The discs were placed on agar plates and sample of synthesized silver and iron nanoparticals were added on the disc with the help of micropipette. The plates were incubated at 370c 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 nanoparticals synthesized were obtained from Global Institute of Biotechnology, Hyderabad, India. 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 370c.

RESULTS

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.

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(a) **(b)**

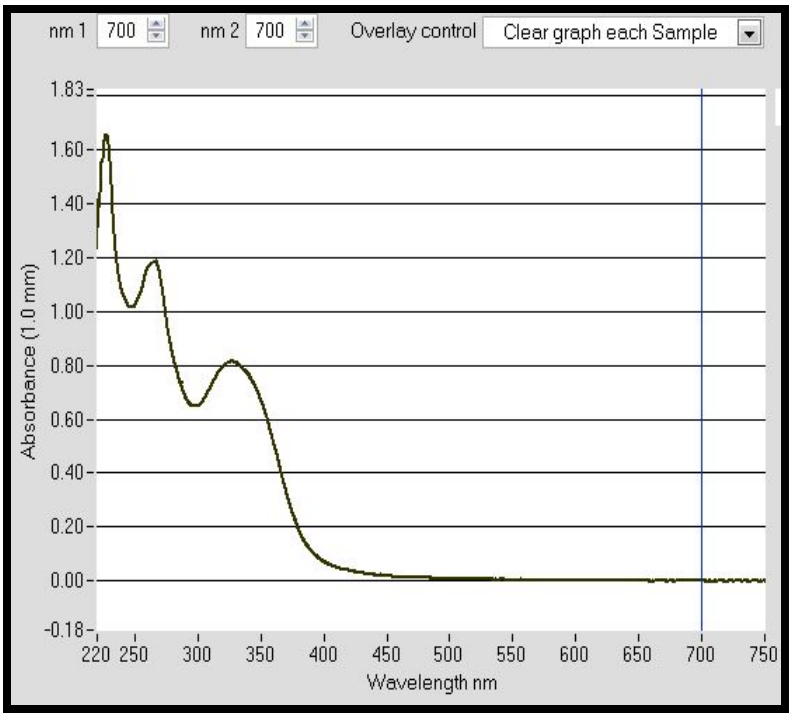
Figure 2: Color changes of leaf extract before and after synthesis of (a) silver (b) iron nanoparticles.

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



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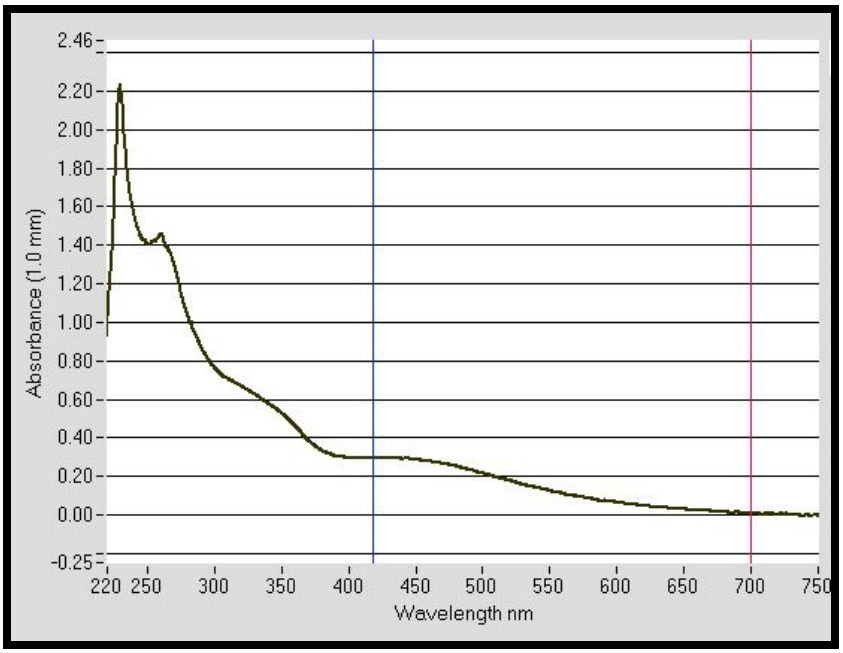

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Figure 3: Spectral analysis of *A.viridis* (a) plant extract (b) silver nanoparticles

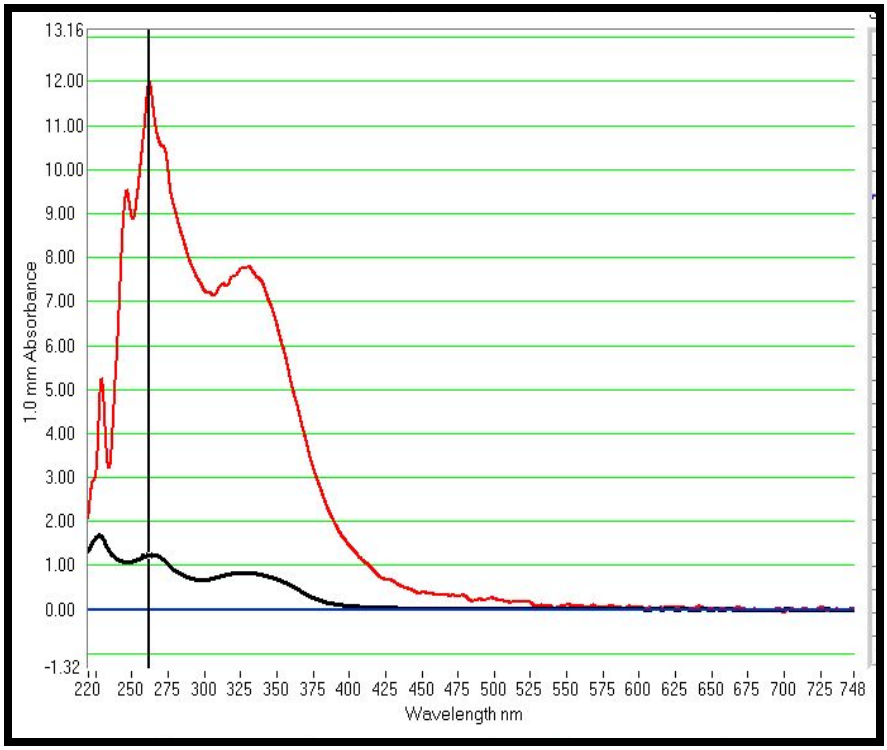

Figure 4: Spectral analysis of *A.viridis* and Iron nanoparticles

SEM analysis of Ag and iron nanoparticles

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

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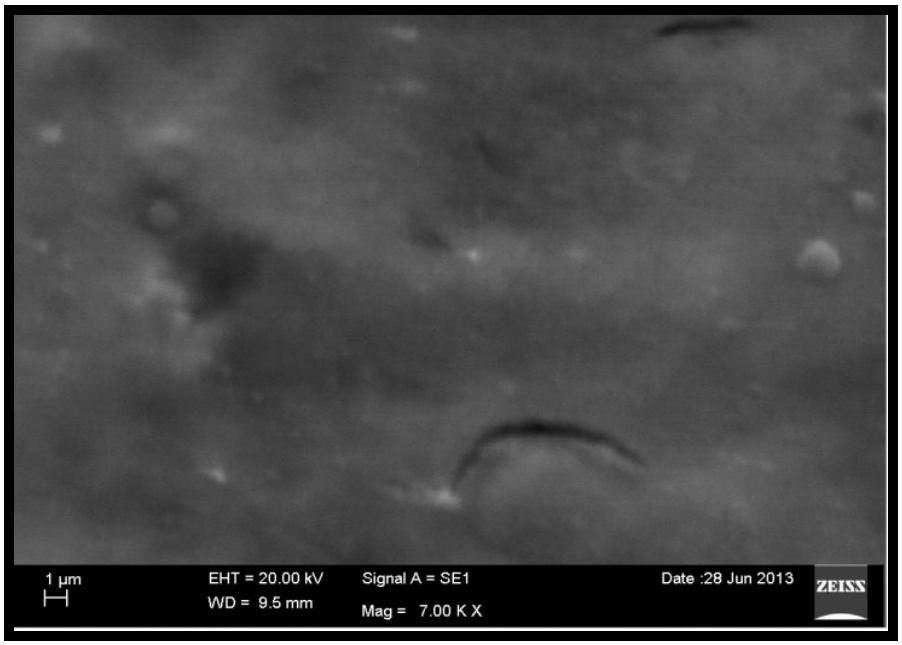

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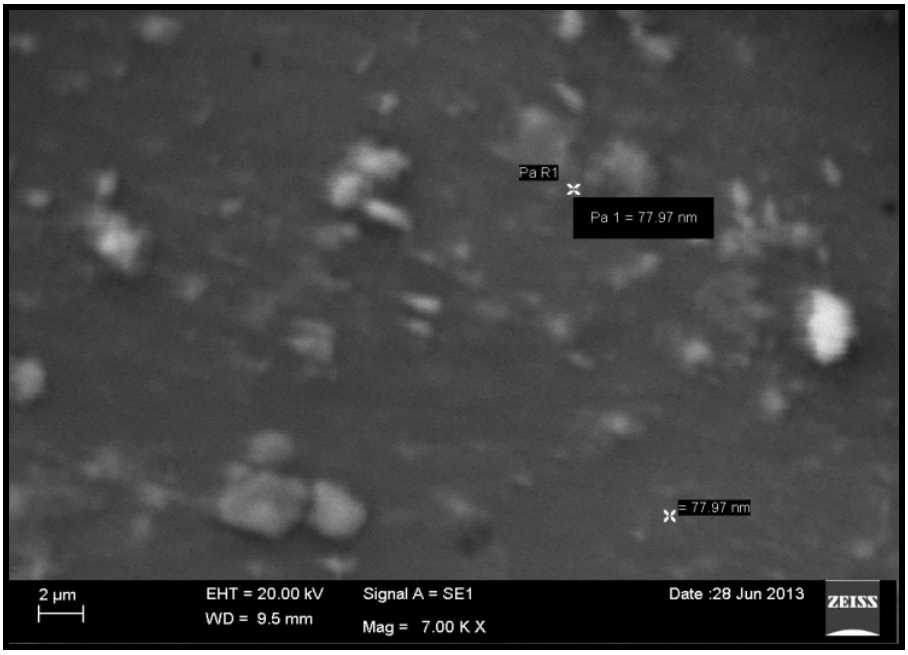
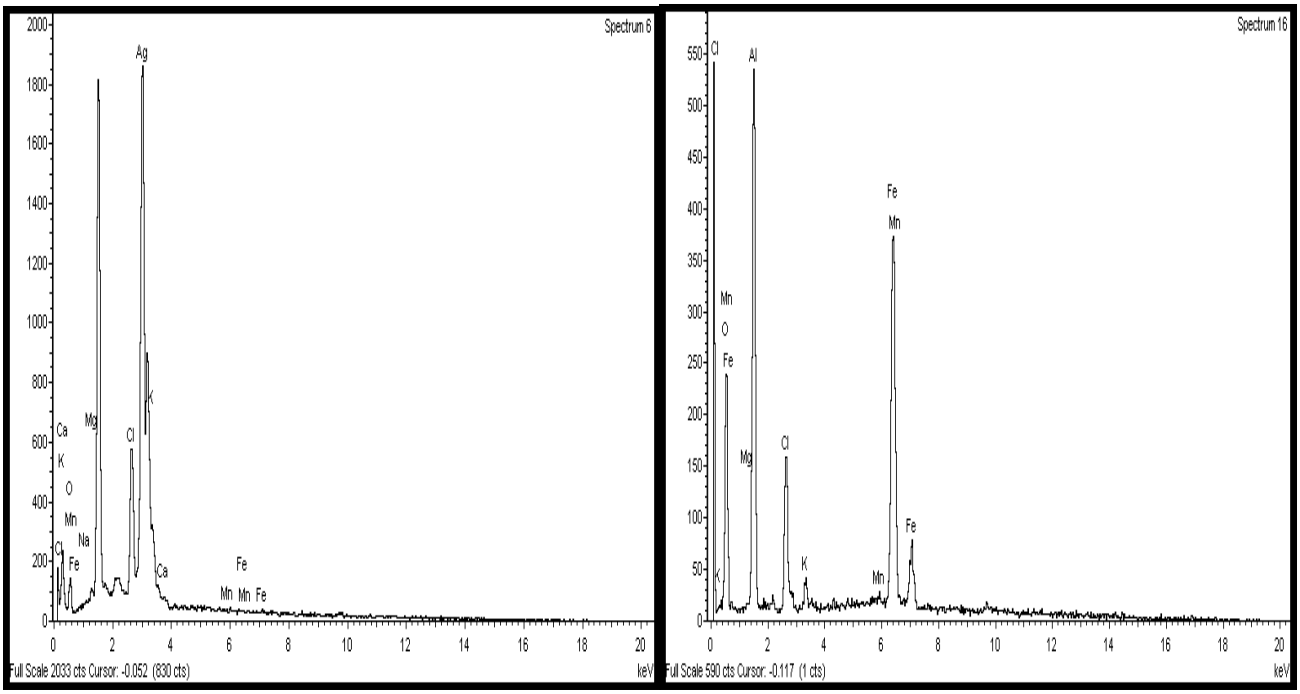


(b)

Figure 5: 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.

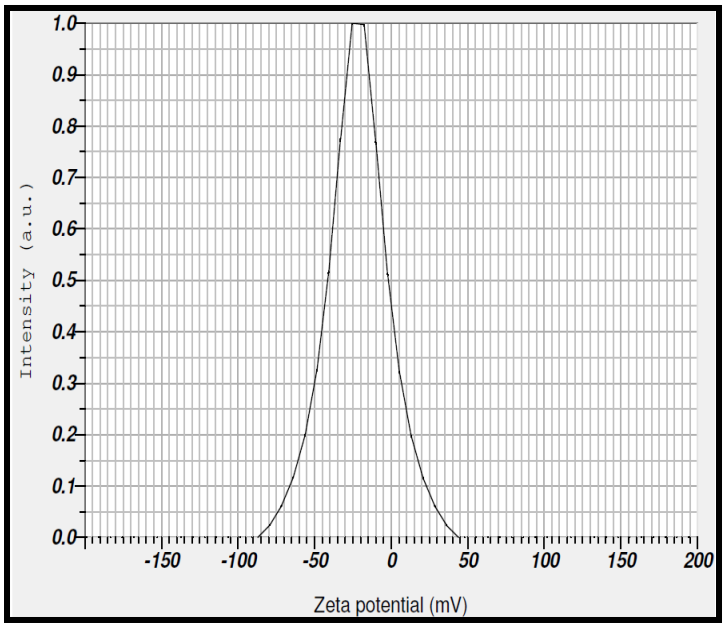


(a) **(b)**

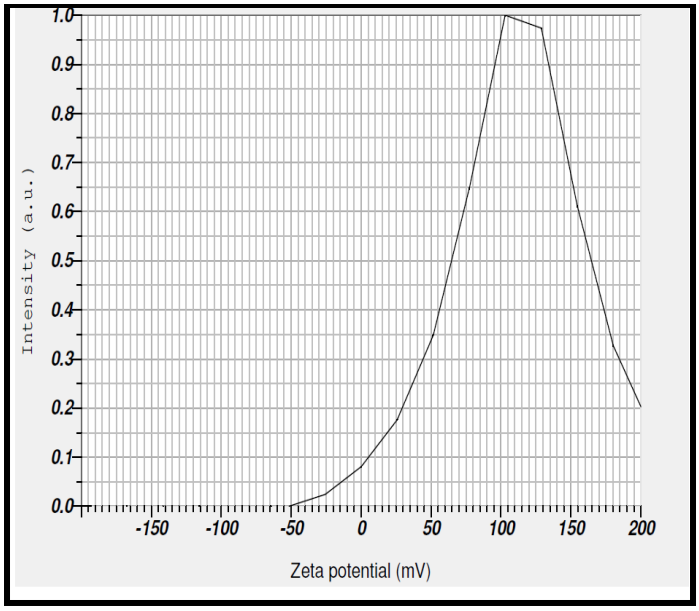
Figure 6: EDX spectrum of synthesized silver nanoparticles and Iron nanoparticles

Zeta potential.

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



(a) silver nano particles



(b) Iron nano particles

Figure 7: Zeta potential of synthesized silver and iron nanoparticles

Antimicrobial activity

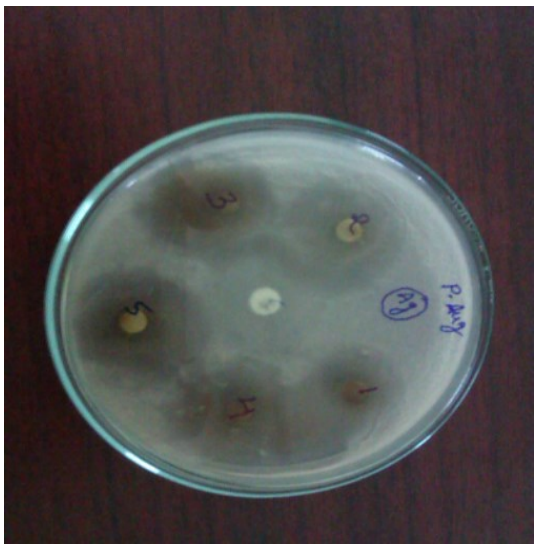
The nanoparticles synthesis by green route was found extremely against 6 bacterial species at a concentration of 20µl Ag and iron nanoparticles, Gram positive. *Bacillus megaterium*, *Staphylococcus aures*, Gram negative *Escherichia coli (M), Escherichia coli (D), Pseudomonas aeruginosa, Klebsila pneumonia*. 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 *A.viridis* 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.

Table 1: The cultures shown zone of inhibition

Microorganisms	Zone of inhibition(cm)		
	Control(AC)	Silver	Iron
Bacillus megaterium	2.4	1.6	1.7
Staphylococcus aureus	0.9	2.2	1.2
Pseudomonas aeruginosa	2.4	2.6	1.6

E.coli(D)	2.4	3.2	2.3
E.coli(M)	2.3	2.8	1.8
Klebsiella pneumonia	1.5	2.7	1.9



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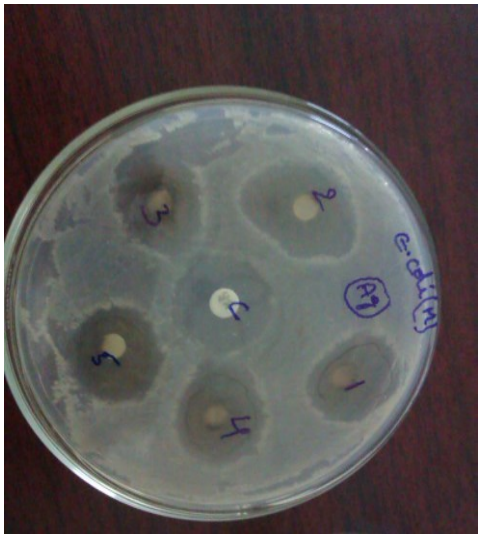

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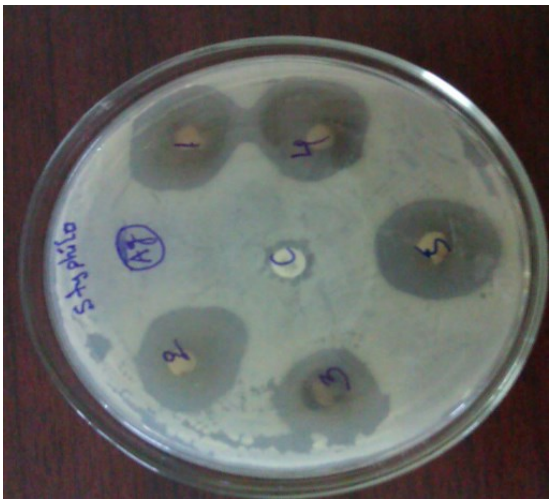

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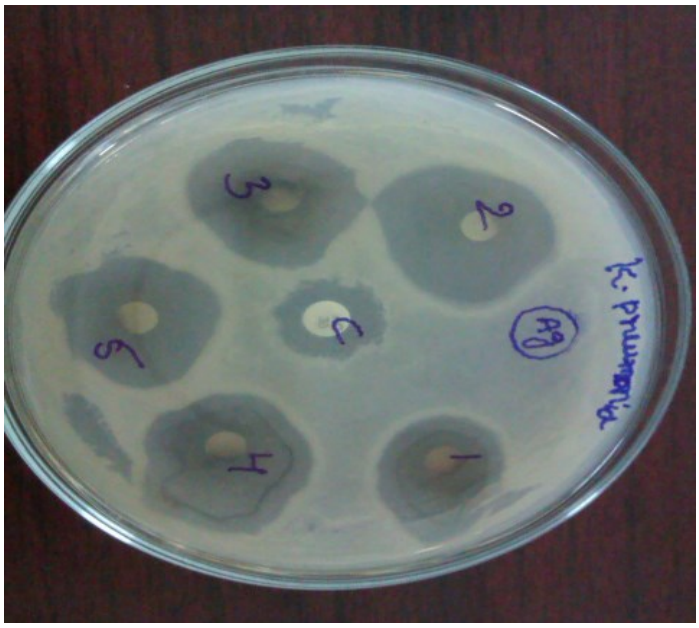

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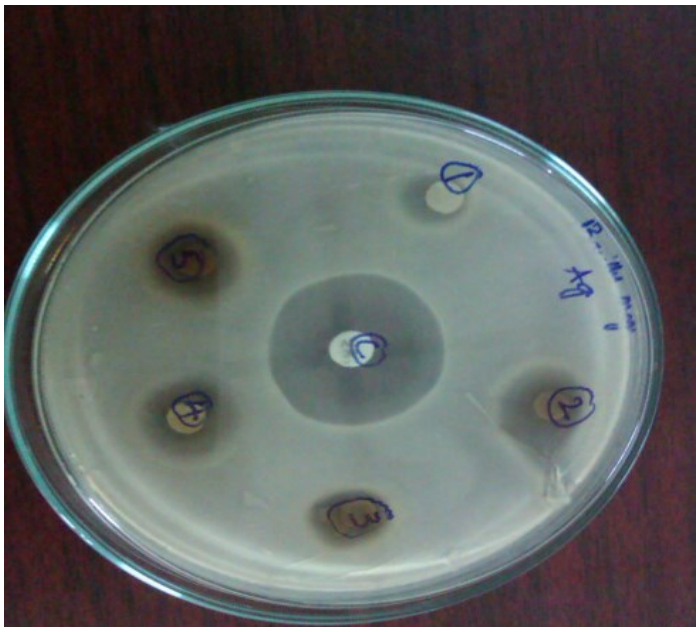

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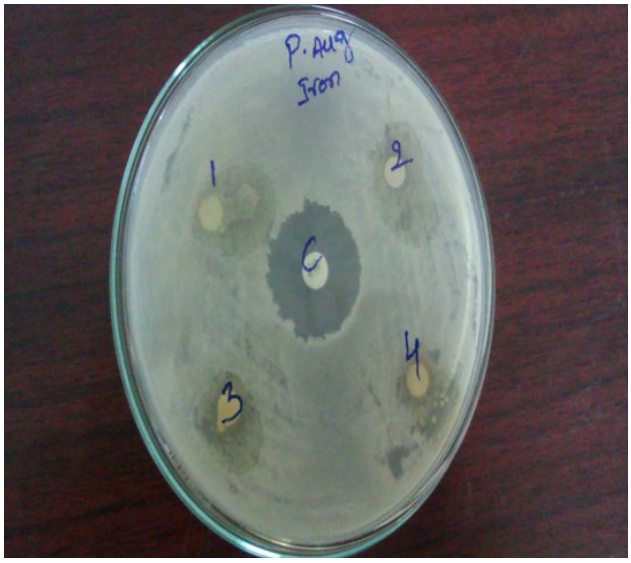
Figure 8: Antimicrobial activity of silver nanoparticles (zone 3 for *A.viridis*) against 6 microorganisms (a) *pseudomonas aeruginosa,(b) Escherichia coli (M), (c) Escherichia coli (D),* (d) *Staphylococcus aures, (e) Klebsila pneumonia, (f) Bacillus megaterium.*



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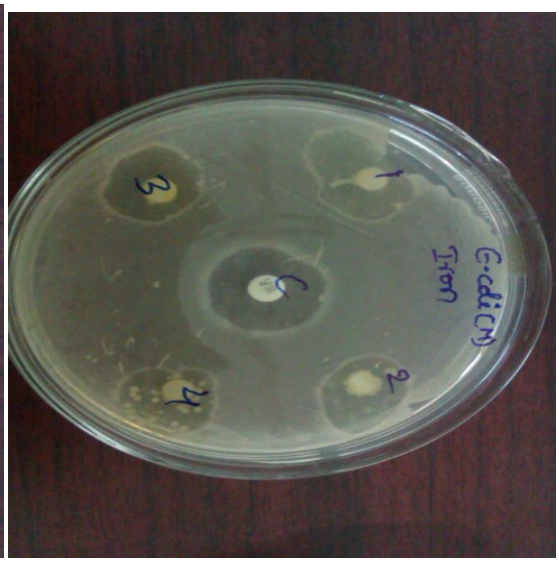

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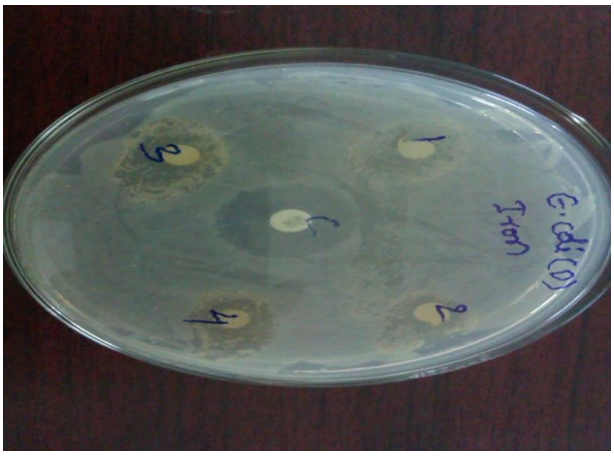

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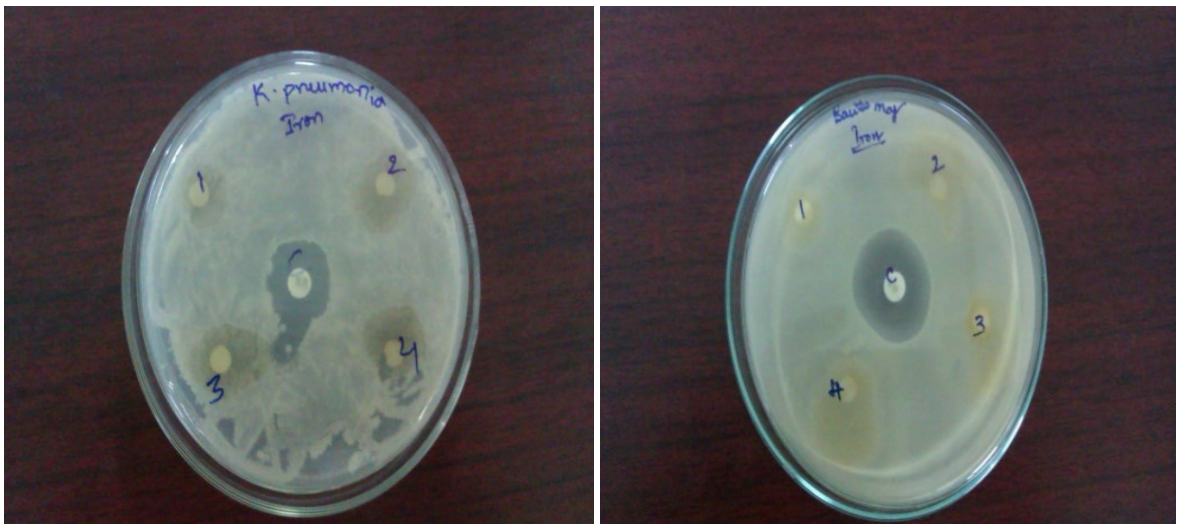

(e) (f)

Figure 9: Antimicrobial activity of iron nanoparticles(zone 3 for *A.viridis*) 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 synthesized nanoparticles were of spherical and sheet shaped and the estimated sizes were 160-180 nm. The size were bigger as the nanoparticles were surrounded by a thin layer of proteins and metabolites such as terpenoids having functional groups of amines, alcohols, ketones, aldehydes, etc., which were found from the characterization using UV-vis spectrophotometer, SEM, XRD, and FTIR techniques. All these techniques it was proved that the concentration of plant extract to metal ion ratio plays an important role in the shape determination of the nanoparticles. The higher concentrated nanoparticles had sheet shaped appearance where as the lower concentrations showed spherical shaped. The sizes of the nanoparticles in different concentration were also different which depend on the reduction of metal ions. From the data of DLS it was found that the 30:1 ratio solution had sharp

nanoparticles of around 5 nm and some has around 180 nm and the had the potential of around 15.5 mV. From the technological point of view these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production. The silver and iron nanoparticles of average size have been synthesized using dried leaves of plant A.viridis. Characterizations from UV-Vis, SEM, EDX support the stability of biosynthesized nanoparticles. The silver and iron nanoparticles using A.viridis proved excellent antimicrobial activity. These silver and iron nanoparticles may used in food and pharmaceutical industries.

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