One pot Bio-facile Fabricationand Characterization of Pullulan based Ag-FeO Bimetallic Nanocomposite

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Abstract

The bimetallic nanoparticles of FeO-Ag were prepared by co-precipitationtechnique.The synthesized bimetallic nanoparticles were characterized by FTIR, UV–Vis Spectrophotometry,Scanning Electron Microscopy (SEM), Energy Dispersive X-ray spectrometry (EDAX), antimicrobial activities and Bio film formation. The average diameter was found using DLS characterization. From EDAX studies the percentage of the present elements and morphology of the NP was found using SEM studies. In both anti bacterial and anti fungal study the higher concentration gives good potential activity then the other concentrations. These techniquesconfirmed the formation of the bimetallic nanocomposite and showed that the sizedistribution of the synthesized bimetallic nanoparticles.

Key Words: Nano particles, FTIR, SEM, Bio film, Antimicrobial studies.

1. Introduction

In recent years nanotechnology plays key role in every field and it tracks down application in innovative scientific research. Nanoscience and Nanotechnology are solely interdisciplinary in nature and have applications in divers fields of science, such as chemistry [1-2], physics [3-4], biology [5-6], materials science [7-8], designing and medication [9]. Nanoscience is the investigation of peculiarities on the nanometer scale [10-15].The nanometer scale is for the most part demonstrated as 1-100 nm.In this regard, polymers are one of the most effectively taken advantage of classes of materials because of the fantastic assortment of substance structures accessible and their resulting summary of properties, alongside their somewhat minimal expense, easy handling, and their conceivable recyclability and pertinence as sustainable materials[16-20]. These crossovernano-materials are typical to show a few synergistic properties between the polymer andthe metal nanoparticles, making them potential candidates for application in few fields,for examples, catalysis[21], biosensors [22], memory gadgets, sensors [23], super capacitor, semiconductor,photovoltaic gadgets [24]

and solar cells [25] etc. EMI shielding materials [27], electrodematerials, solar cells, flame-retardant [27], photovoltaic cells, corrosion inhibitorsand conducting paints [28] and so on.

2. Materials and Methods

For nanoparticle synthesis, analytical grade Silver nitrate, Ferric chloride, Sodium hydroxide were used. Nutrient Agar (HiMedia), potato influsion, dextrose and agar of analar grade has been used for antimicrobial studies.

2.1 Synthesis of bimetallic Ag-Fe nanoparticles

Initially,0.1mmol of silver nitrate and 0.1 mmol of ferric chloride was weighed and made up to 100 ml each separately.The bimetallic Ag–Fe solutions were prepared in the following ratio 2:1:1(pullulan: silvernitrate: ferric chloride). 25ml of silver nitrate solution and 25mlof ferric chloride solution was taken into a 250 ml beaker.It was then constantly stirred for 3 hours using magnetic stirrer at a constant temperature (80^0 C). While during that time, 50ml of the bio- reductant*pullulan* was added dropwise at regular time interval. In between the reaction time, approximately two pellets of sodium hydroxide were added to maintain its pH>7. As the reaction proceeds, the change in colour of the bimetallic Ag-Fe solution can be observed.The solution was allowed for evaporation by keeping it in hot air oven to get the dry salt. The dry salt was collected in a silica crucible and kept in muffle furnace for incineration at (600^0 C) for about 6 hours. The bimetallic nanocompositethus, formed was ground well to make it into nanopowder. The nanoparticles were stored and taken for further studies.

2.2 Anti-Bacterial Activity (Agar- Well Diffusion Method)

a. Nutrient Agar Medium

The medium was prepared by dissolving 2.8 g of the commercially available Nutrient Agar Medium (HiMedia) in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

b. Nutrient broth

Nutrient broth was prepared by dissolving 2.8 g of commercially available nutrient medium (HiMedia) in 100ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

c. Agar- Well Diffusion Method

Petri plates containing 20 ml nutrient agar medium were seeded with 24hr culture of bacterial strains *(P.acnes, S. mutans, S. pyogenesandFusobacteriumnucleatum)* Wells were cut and different concentration of samplePul.Ag.Fe (500µg/ml, 250µg/ml,100µg/ml and 50µg/ml) were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

2.3 Anti-Fungal Activity (Agar- Well Diffusion Method)

a. Potato Dextrose Agar Medium

The potato dextrose agar medium was prepared by dissolving 40 gm of potato influsion, 4 gm of dextrose and 3.5 gm of agar in 200 ml of distilled water. The dissolved

medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petri plates (25-30 ml/plate) while still molten.

b. Agar- Well Diffusion Method

Petri plates containing 20ml potato dextrose agar medium were seeded with 72hr culture of fungal strain*(Aspergillususstus, Aspergillusfumigatus, andSporothrixschenckii)* wells were cut and different concentration of sample M1(500, 250,100 and 50µg/ml) were added. The plates were then incubated at 37°C for 48-72 hours. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B (100 units) was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

2.4 Anti-Biofilm Assay

To evaluate the efficacy of drug in interrupting biofilm formation, MTP assay was carried out accordingly by Christensen *et al.*(1985) using 96 well-flat bottom polystyrene titre plates. Individual wells were filled with 180 µL BHI broth followed by inoculation with 10 µL of overnight pathogenic bacterial culture. To this 10 µL sample LP was added from the prepared stock solution of 500, 250, 125, 62.5 and 31.25 µg/mL respectively along with control (without test sample) and incubated at 37°C for 24 h. After incubation, content in the wells were removed, washed with 0.2 mL of phosphate buffer saline (PBS) pH 7.2 to remove free floating bacteria. The adherence of sessile bacteria was fixed with sodium acetate (2%) and stained with crystal violet (0.1%, w/v). Excessive stain was removed by deionized water wash and kept for drying. Further, dried plates were washed with 95% ethanol and optical density was determined using a microtitre plate reader (Thermo) at 600 nm. The percentage of biofilm inhibition was calculated using the below formula

$$\% \text{ Biofilm inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

3. Results and Discussion

3.1 XRD Analysis

Figure 1 shows the XRD patterns of Fe oxide (Fe_3O_4) nanoparticles the XRD diffraction peaks appeared at 2θ of 35.0° and 77.2° which corresponds to (110) and(211) Bragg reflection respectively.The XRD patterns of silver oxidenanoparticles the peaks appeared at2θ of 37.8, and 64.2° attributable to the indices (111), and (220) indicates existence ofAg nanoparticles. From XRD graph the nanoparticles was confirmed [29].

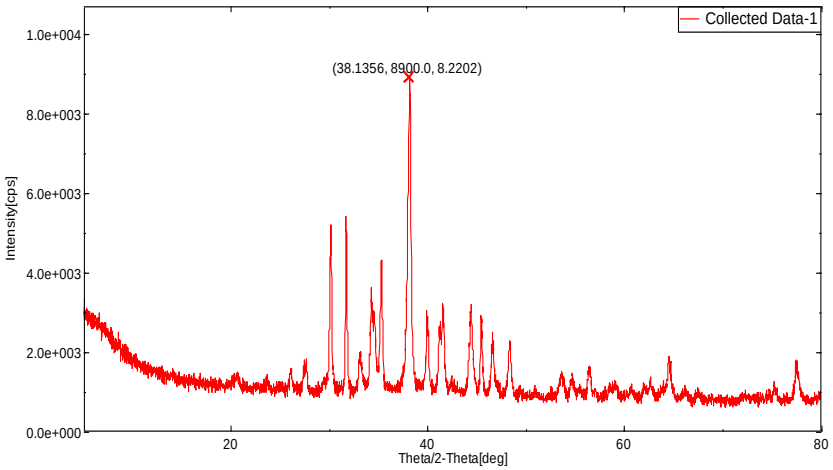


Fig. 1 XRD Analysis graph

3.2Fourier Transform Infrared (FTIR) analysis

FTIR measurements were carried out to determine the functional groups in nanostructures. Figure 2 shows the FTIR spectra of the synthesized FeO-Ag NPs in theregion of 400 – 4000 cm^{-1}. The broad peak at 3437.06 cm^{-1} indicates the O-Hstretching vibrations. The peak at1439.23 cm^{-1} depicts the C=C stretching aromatic group. The peak at439.77 cm^{-1} confirms the presence of FeO nanoparticle [30].

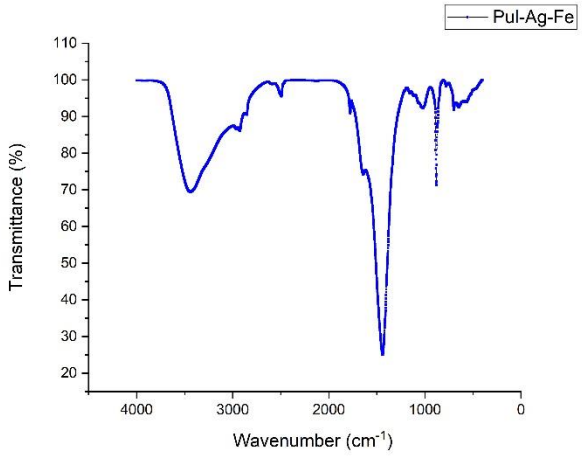


Fig. 2 FTIR Spectrum of FeO-Ag

3.3UV-Visible Spectral Study

Nanoparticles and bimetallic nanoparticles arrangement wereaffirmed by UV–Vis measurements. The absorption spectra of FeO nanoparticles and FeO-Ag bimetallic nanoparticles is shown in Figure 3.The range of the FeO-Ag bimetallic nanoparticlesshowed two different absorption bands at 282 nm and369 nm corresponding to both FeO and Ag. The existenceof the two surface bands refer to both FeO and Ag indicated the formation of bimetallic nanoparticles[31].

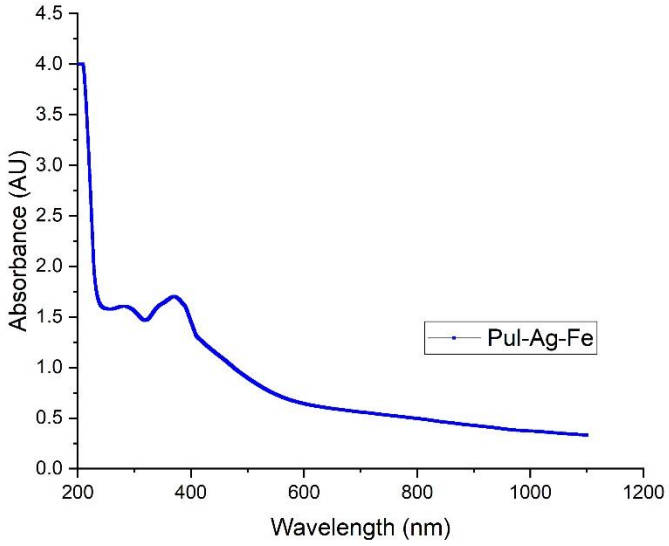


Fig. 3 UV-Visible Spectrum of FeO-Ag

3.4DLS

DLS is concerned with estimation of particlessuspended within a liquid. The typical sizes ofNPs were found as 183 nmDLS estimations [32,33]. Theseoutcomes are in lined up with the outcomes got bySEMexamination.DLS range displayed in Figure 4.

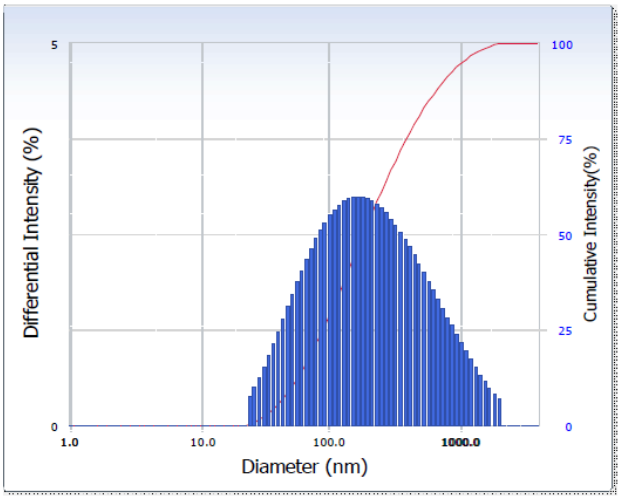
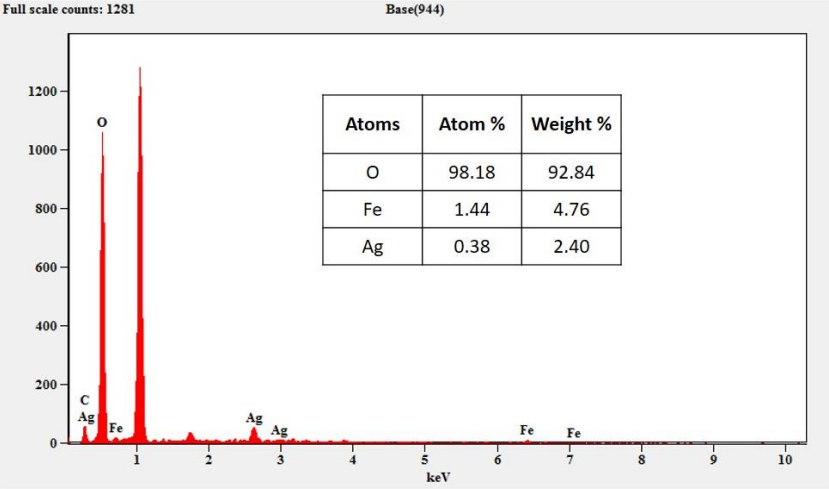


Fig. 4DLS Spectrum of FeO-Ag

3.5 EDAX

EDAX is a tools used to determine the elemental percentage of thesample. It was performed to confirm the elements present in the synthesized metaloxide nanostructures of FeO-Ag. The peaks observedfor the prepared samples indicate the presence of the elements of the samples. The atomic weight percent the synthesized metal oxide nanostructures reveals thepurity of the samples. The element peaks of Fe, O and Ag were observed as 4.76, 92.84 and 2.40 respectively; this indicates that FeO and Ag were successfullysynthesized [34].The data and the spectrum FeO-Ag nanoparticle are displayed in Figure 5.



Atoms	Atom %	Weight %
O	98.18	92.84
Fe	1.44	4.76
Ag	0.38	2.40

Fig. 5EDAX Spectrum of FeO-Ag

3.6SEM Analysis

SEM was used to investigate the morphology of FeO-Ag in Figure 6. As can be observed from the SEMimages ofFeO-Ag is in nanosized morphology.The synthesized FeO-Aghave a diameter of ~73.5 nm [35].

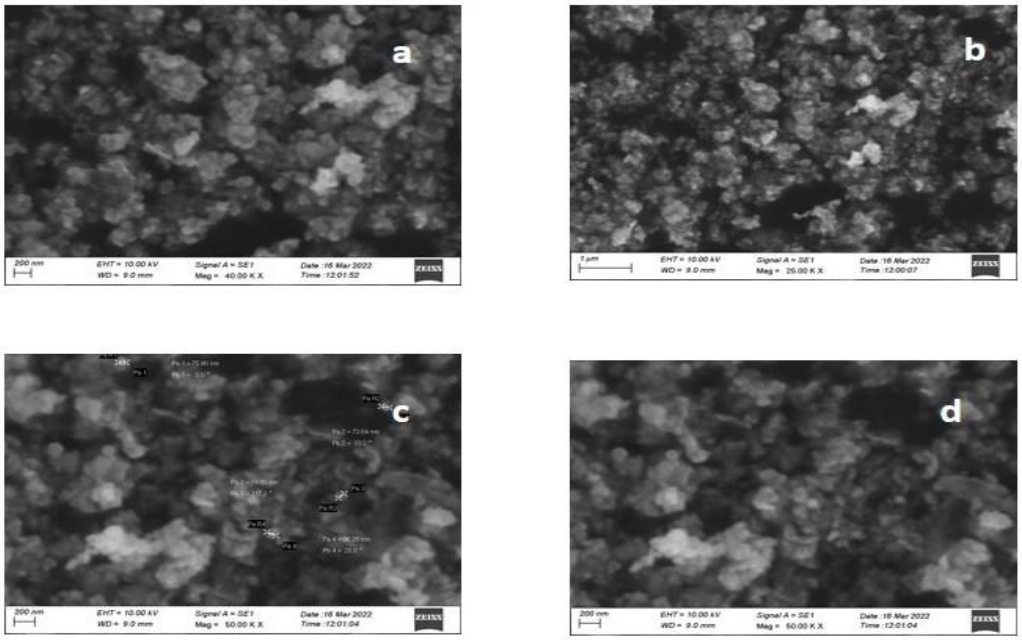


Fig. 6 SEM Morphology of FeO-Ag

3.7 Anti-Bacterial Activity

The newly synthesized FeO-Ag NPwasevaluated for their antibacterial activity against the microorganisms of *Propionibacterium acnes*, *Streptococcus mutans*, *Streptococcus pyogene* and *Fusobacteriumnucleatum* bacterial strains by the welldiffusion method. The plates were inverted andincubated for one day at 37 °C. Ciprofloxacin was utilized as a standard drug. Growth inhibition zones weremeasured in millimetre and compared with the positive controls. The bacterial inhibition zone values are summarized in Table 1Minimum inhibitory concentrations (MIC) were determined. This procedure was performed in triplicates.The screening test was carried out four different concentrations. Among all the concentration the

higher concentration gives good results then the other concentrations [36]. The correlation graph and anti bacterial activity was shown in Figure 7.

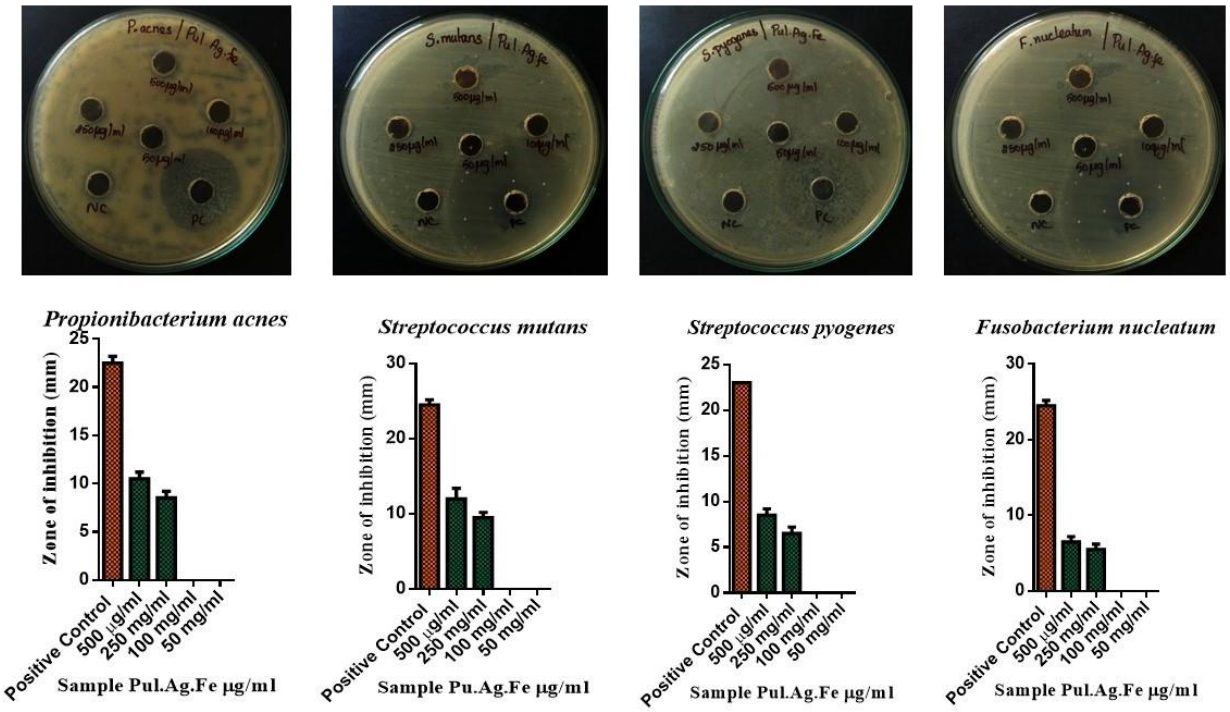


Fig. 7 Antibacterial activity *P.acnes, S. mutans, S. pyogenes* and *F.nucleatum* of FeO-Ag and its correlation graph

Table 1. Antibacterial activity tested against the organisms

S.No	Name of the organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			PC	500 µg/µl	250 µg/µl	100 µg/µl	50 µg/µl
1	*P. acnes*	Pul-Ag-Fe	22.5±0.7	10.5±0.7	8.5±0.7	0	0
2	*S. mutans*		24.5±0.7	12±1.4	9.5±0.7	0	0
3	*S.pyogenes*		23±0	8.5±0.7	6.5±0.7	0	0
4	*F. nucleatum*		24.5±0.7	6.5±0.7	5.5±0.7	0	0

3.8 Anti-Fungal activity

The antifungal activity of FeO-Ag against four strains of fungus (*Cryptococcus neoformans*, *Aspergillus fumigates*, *Sporothrixschenckii*, and *Aspergillususstus*) was studied. The results are shown in Table 2 and Figure 8.The MIC was measured against the control. In Figure 8 the medium and higher concentration gives good results then the lower concentration [37].

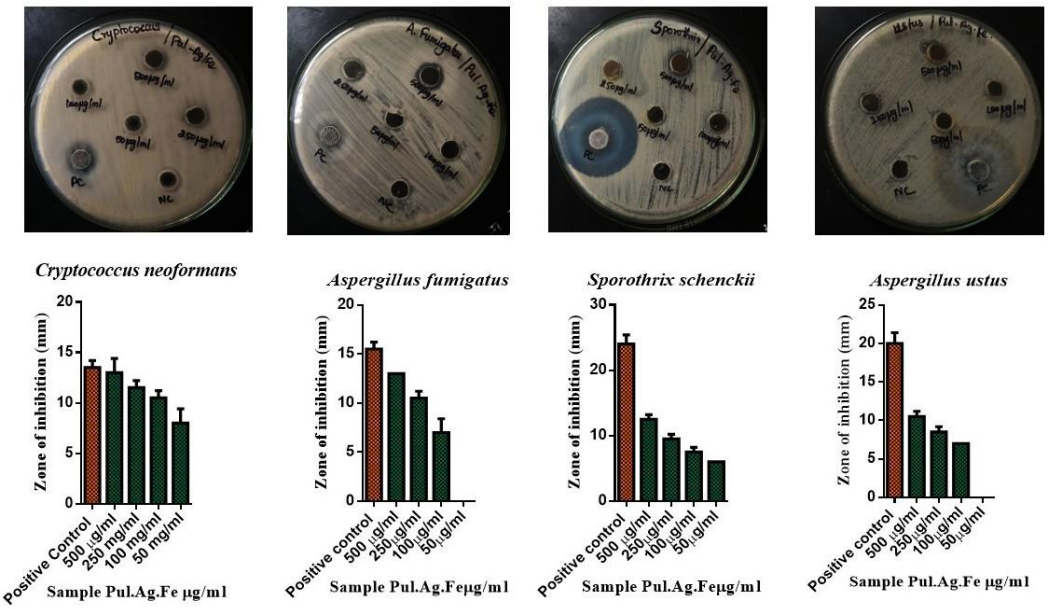


Fig. 8Antibfungal activity *C.neoformans, A.fumigatus, S. schenckii* and *A.ustus* of FeO-Ag and its correlation graph

Table 2. Antifungal activity tested against the organisms

S.No	Name of the organism	Name of the test sample	Zone of inhibition (mm) SD ± MEAN				
			PC	500 µg/µl	250 µg/µl	100 µg/µl	50 µg/µl
1.	*A.ustus*	Pul.Ag.Fe	20±1.4	10.5±0.7	8.5±0.7	7±0	0
2.	*A.fumigatus*		15.5±0.7	13±0	10.5±0.7	7±1.4	0
3.	*Cryptococcus neoformans*		13.5±0.7	13±1.4	11.5±0.7	10.5±0.7	8±1.4
4.	*Sporothrixschenckii*		74±1.4	12.5±0.7	9.5±0.7	7±0	0

3.9Anti-Biofilm Assay

A. Percentage of inhibition

Table 3 : Inhibition of Biofilm Assay

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Control	100	100	100	100
2.	500 µg/ml	92.18	87.76	86.32	88.75
3.	250 µg/ml	48.22	26.61	18.40	31.07
4.	125 µg/ml	17.93	17.35	11.01	15.43
5.	62.5 µg/ml	10.74	8.32	5.74	8.27
6.	31.25 µg/ml	0	0.93	1.28	0.73

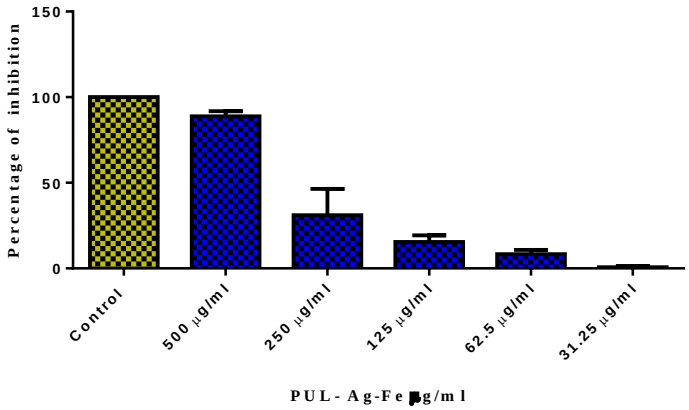


Fig. 9 Graphical Representation of Anti-Biofilm Assay

C. IC50 Value of tested sample: 278.5 µg/ml

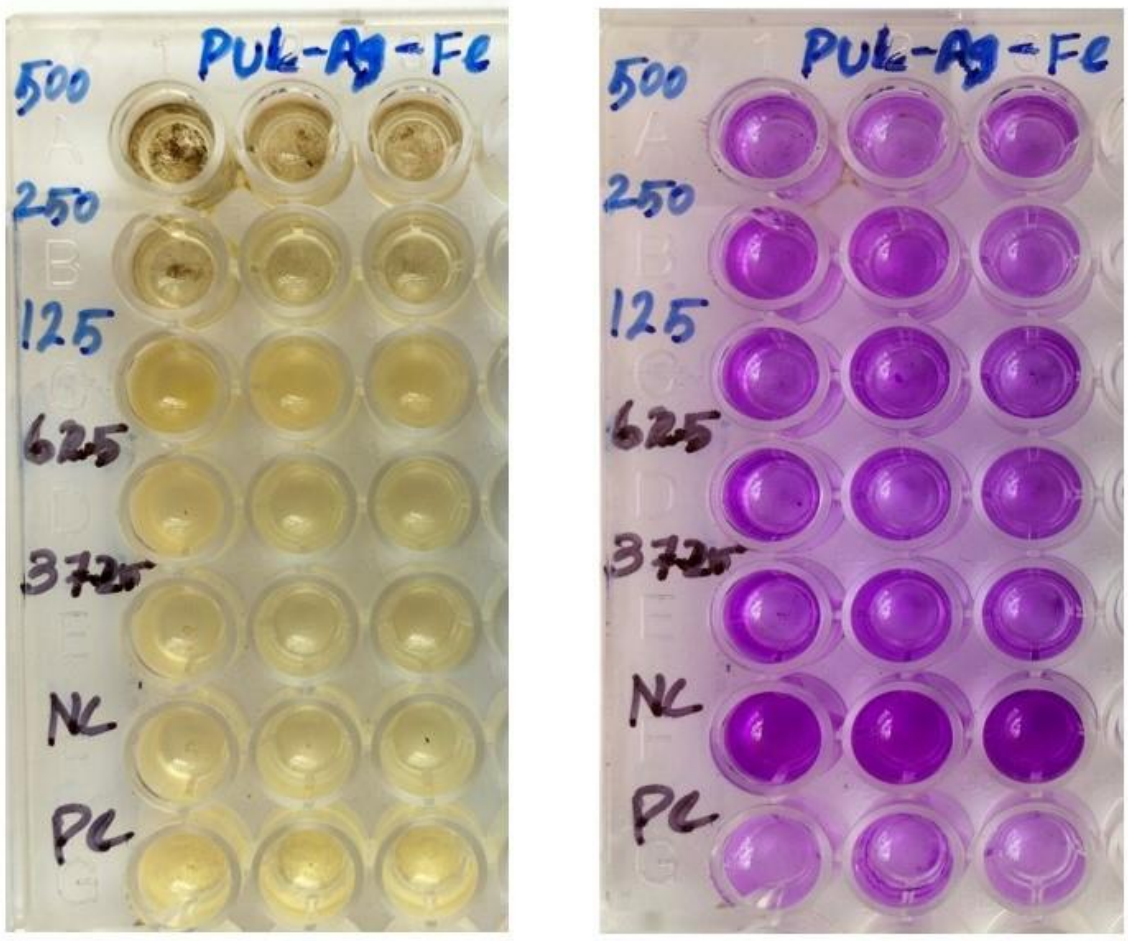


Fig.10Anti-Biofilm Assay

4. Conclusion

FeO-Ag bimentalic nanoparticles were prepared successfully byCo-precipitation technique. The sysenthizedbimetallic nanoparticles have been investigated by different techniques like FTIR, UV, SEM, EDAX, DLS.These techniques verified the formation of the

ZnO-Ag bimetallic nanocompiste. The antimicrobial activity of the ZnO-Ag bimetaic was validated by determined.

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