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Green Synthesis and Characterization of Zinc Nanoparticle Using Eclipta Prostrate Leaf Extract and Its Application for Environmental Protection

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ABSTRACT

Nanotechnology is a developing interdisciplinary field of research interspersing material science, bionanoscience, and technology. Nanoparticles are studied extensively for their specific catalytic, magnetic, electronic, optical, antimicrobial, wound healing and anti-inflammatory properties. The main aim of the present study was to synthesize Zn nanoparticles using the extract EcliptaProstrata leaves and to evaluate their antimicrobial efficacy against some selected microbes. The synthesis Zn nanoparticles were characterized by UV/VIS spectroscopy, particle size analyzer and Scanning Electron Microscopy. The synthesized Zn nanoparticles showed significant antimicrobial activity against Gram positive and Gram negative bacteria. The maximum zone of inhibition had been found against Pseudomonas aeruginosa (2000 ± 50.5) whereas the minimum is found against Escherichia coli (5 ± 4.5). Thus from this study it can be concluded that EcliptaProstrata leaf extracts can be effectively used for synthesizing Zn nanoparticles . This study also suggests that green synthesized Zn nanoparticles can be used as an alternative to existing antimicrobial agents. The method is eco-friendly and provides bio-compatibility in pharmaceutical, biomedical and cosmetic applications as they do not use toxic chemicals for the synthesis protocol. Green synthesis

size. In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties. Particles are further classified according to diameter. Ultrafine particles are the same as nanoparticles and between 1 and 100 nanometers in size, fine particles are sized between 100 and 2,500 nanometers, and coarse particles cover a range between 2,500 and 10,000 nanometers. Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields. The National Nanotechnology Initiative has led to generous public funding for nanoparticle research in the United States

Nanoparticle Applications in Medicine

- The use of polymeric micelle nanoparticles to deliver drugs to tumors.

has also proved to be cost-effective.

Introduction:

Eclipta prostrate commonly known as False Daisy , and Bhringraj, is a plant belonging to the familyAsteraceae. It is widely distributed throughout India, China, Thailand, and Brazil. In ayurvedic medicine, the leaf extract is considered a powerful liver tonic, rejuvenative, and especially good for the hair. A black dye obtained from Ecliptaalba is used for dyeing hair and tattooing. Ecliptaalba also has traditional external uses, like athlete foot, eczema and dermatitis, on the scalp to address hair loss and the leaves have been used in the treatment of scorpion stings. It is used as anti-venom against snakebite in China and Brazil. It is reported to improve hair growth and color. Nanoparticles are particles between 1 and 100 nanometers in up the nanoparticle to absorb more free radicals.

- Researhers are developing ways to use carbon nanoparticles called nanodiamonds in medical applications. For example nanodiamonds with protein molecules attached can be used to increase bone growth around dental or joint implants.
- Researchers are testing the use of chemotherapy drugs attached to nanodiamonds to treat brain tumors. Other researchers are testing the use of chemotherapy drugs attached to nanodiamonds to treat leukemia.
- More about Nanotechnology in Medicine

MATERIALS AND METHODS

1- Collection of the extracts:

Selected plant *EcliptaProstrata, Hydnocarpuslaurifolia,symplocosracemosa*was washed and the cleaned herbal parts

- The use of polymer coated iron oxide nanoparticles to break up clusters of bacteria, possibly allowing more effective treatment of chronic bacterial infections.
- The surface change of protein filled nanoparticles has been shown to affect the ability of the nanoparticle to stimulate immune responses. Researchers are thinking that these nanoparticles may be used in inhalable vaccines.
- Researchers at Rice University have demonstrated that cerium oxide nanoparticles act as an antioxidant to remove oxygen free radicals that are present in a patient's bloodstream following a traumatic injury. The nanoparticles absorb the oxygen free radicals and then release the oxygen in a less dangerous state, freeing

5- Microscopy Observation :

Surface morphology of silver nanoparticles was demonstrated by microscopy. The sample was prepared by centrifuging colloidal solution after 6 h of reaction at14000 rpm for 4 min. The pellet was re-dispersed in deionized water and again centrifuged. The process was repeated three times and finally washed with acetone. The purified nanoparticles were sonicated for 10 min for making the suspension and then a drop from the suspension was placed on the glass slide and observed on Microscope.

***6*-Antimicrobial activity :**

***a*-Standard Preparation:**

Anti microbial drug Ofloxacin was selected for standard purchased from the local pharmacy, weight accurately 10mg of the standard equivalent weight of the tablet powder dissolved in 10mlof sterile distilled water to obtain the concentration of 1000µg/ ml which is consider as stock solution. From the stock solution 10,50,100µg/ ml were prepared.

***b*- Sample preparation:**

10mg of the Synthesized Zinc Nano particles was weighed accurately dissolve in 5 ml of water the concentration of the sample is 2000µg/ ml. various dilutions were prepared from the stock solution like 1000, 500, 100, 50, 25,10,5µg/ ml.

***c*-Test Solution Preparation**:

Four pathogenic organisms were selected for Antimicrobial activity of Zinc nano particles . One loopful amount of organism was taken from the freshly prepared sub culture , diluted with sterile water(5ml). were dried with water absorbent paper (wet filter paper). Then it was cut into small pieces, dispensed in 100 ml of sterile distilled water and boiled for one hour at 80ºC. Then the herbal extracts were collected in separate conical flasks by standard filtration method.

2- Microorganisms used:

1. *Bacillus substilis*
2. *Escherichia coli*
3. *Pseudomonas aeruginosa*
4. *Staphylococcus aureus*

3- Synthesis of Zinc nanoparticles:

The 10^{-3} M Zinc solution was prepared and stored in brown bottles. 10ml of herbal extracts was taken separately and to this 90 ml of zinc chloride solution was added .The same protocol was followed for all the three herbal extracts. The samples were incubated at room temperature. The color change of the leaf extracts from pale yellow to dark brown was checked periodically. The brown color formation indicates that the silver nano particles were synthesized from the herbs and they were centrifuged at 5000 rpm for 15 minutes in order to obtain the pellet which is used for further study.

***4*- Ultraviolet-Visible Spectrophotometer :**

The reduction of zinc ions in the colloidal solution was confirmed by UV–Visible spectroscopy. A small aliquot of sample was taken in a quartz cuvette and observed for wavelength scanning between 300-800 nm with distilled water as a reference.

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- The agar medium should be allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8°C).
- Plates should be used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of the agar.
- A representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35°C for 24 hours or longer.

e- Preparation of dried filter paper discs

Whatman filter paper no. 1 is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in a hot air oven. The loop used for delivering the antibiotics is made of 20 gauge wire and has a diameter of 2mm.

f- Antimicrobial activity by Agar diffusion test:

The cooled media was poured into the sterile petri plate by adding 0.1ml cell suspension of the selected microorganism, mixed well for complete mixing of the spores and allowed to solidify. Then the filter paper disks were immersed in the prepared three different dilutions of the synthesized nano particles and then the discs were placed in the petri dish. Then it was incubated at 35^0c in a sterile incubator. The inhibition in the growth of the microorganisms by the synthesized nano particles were observed after 24h. The inhibition zone of inhibition was measured The prepared test solution was poured in the plates nearly 1-2ml before adding the agar medium.

_d_-Nutrient agar :

Nutrient agar is a microbiological growth medium commonly used for the routine cultivation of non-fastidious bacteria. It is useful because it remains solid even at relatively high temperatures. Also, bacteria grown in nutrient agar grows on the surface, and is clearly visible as small colonies. In nutrient broth, the bacteria grow in the liquid, and are seen as a soupy substance, not as clearly distinguishable clumps. Nutrient agar typically contains

0.5% Peptone
0.3% Beef extract/yeast extract
1.5% agar
0.5% NaCl
Distilled water
pH adjusted to neutral (6.8) at 25 °C.

- After the preparation of the Nutrient Agar medium, autoclave the medium at 125°c, 15lbs pressure for 20min. Immediately after autoclaving, allow it to cool in a 45 to 50°C water bath.
- Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm.
- This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm.

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cultures. It can also be suitably scaled up for large-scale synthesis of nanoparticles

Here green synthesis of Zinc nanoparticles were studied using three different plants *EcliptaProstrata, Hydnocarpuslaurifolia*and *symplocosracemosa*as reducing agent. Different types of metal solutions containing Zinc solutions including Zinc sulphates, Zinc chloride and Zinc Oxide were used as metal source.

Different concentrations of metal solution and different volumes of biological reducing agent were used for the synthesis process. The procedure gives successful results were used for the determination of anti-microbial activity.

Zinc nanoparticles appear yellowish brown in color in aqueous medium as a result of surface plasmon vibrations. As the different extracts were added to Zinc chloride solution, the color of the solution changed from faint light to yellowish brown to reddish brown and finally to colloidal brown indicating nano particles formation. Similar changes in colour have also been observed in previous studies and hence confirmed the completion of reaction between extract and Zinc metal. The color change of the various combinations of the plant extracts according to the time intervals are given in the table below.

and was compared with the standard antibiotic inhibition.

RESULTS AND DISCUSSIONS

Current research in inorganic nanomaterials having good antimicrobial properties has opened a new era in pharmaceutical and medical industries. Zinc is the metal of choice as they hold the promise to kill microbes effectively. Zinc 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 intra-cellular. Zinc 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. Among the synthetic methods used for the preparation of Zinc nanoparticles, some toxic chemicals such as NaBH4, citrate, or ascorbate are most commonly used as a reducing agent. Considering that such reducing agents may be associated with environmental toxicity or biological hazards, the development of a green synthesis approach for silver nanoparticles is desired Plant or plant extract have been suggested as possible eco-friendly 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

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Table 5.2: Indication of color change in the plant extracts nanoparticle solution (ZnO)

S.NO	Name of the part	Color Observed after 8Hours	pH	Time In Hours				
				0	2	4	6	8
1	*EcliptaProstrata,*	Dark Brown	5	+	+	++	++	++
2	*Hydnocarpuslaurifolia,*	light Brown	5		+	+	+	++
3	*symplocosracemosa*	Dark Brown	5		+	+	++	++

+ Light color
++ Dark color
+++Very Dark color

Table 5.3: Indication of color change in the plant extracts nanoparticle solution ($ZnSO_4$)

S.NO	Name of the part	Color Observed after 8Hours	pH	Time In Hours				
				0	2	4	6	8
1	*EcliptaProstrata,*	Dark Brown	5	+	+	++	++	+++
2	*Hydnocarpuslaurifolia,*	light Brown	5	+	+	+	++	++
3	*symplocosracemosa*	Dark Brown	5	+	+	+	++	+++

+ Light color
++ Dark color
+++Very Dark color


Figure 5.e:Color change after 18HFigure5.d : Color change after 12H

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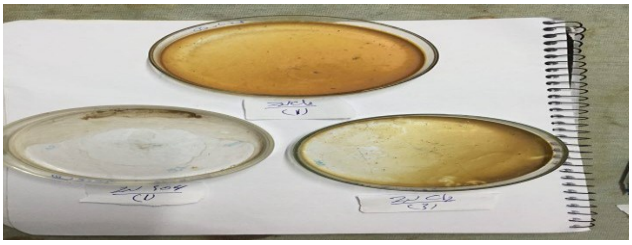
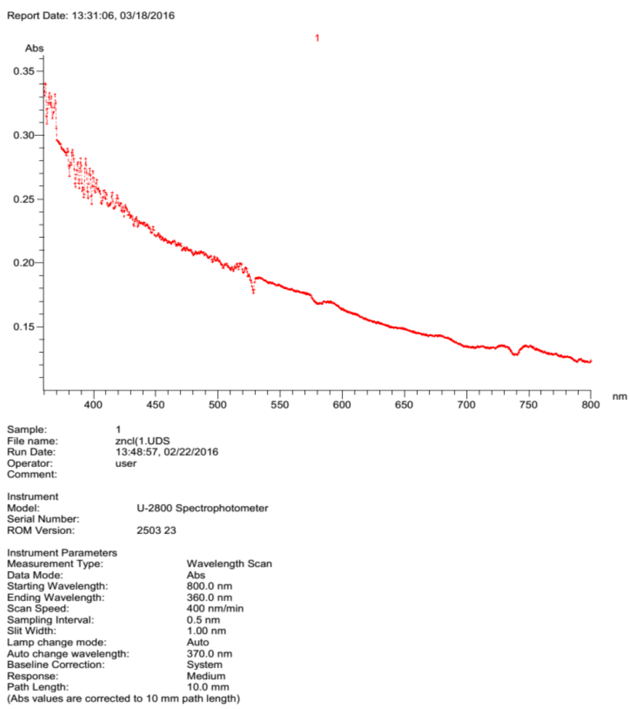
UV-Vis Spectra analysis:

The reduction of pure zinc ions was monitored by measuring the UV-Vis spectrum by diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer at the range of 300-700 nm and observed the absorption peaks in the visible regions, which are identical to the characteristics UV-visible spectrum of metallic silver and it was recorded. The wavelength scanning spectra was shown in figure

The formed color solution due to the bio-reduction of Zinc using the selected plant extracts was centrifuged separately and the supernatant was discarded and the pellet was collected and dried. The dried zincnano-particles were preserved and the characterization of the particles was carried. The synthesized nano-particles were given in figure





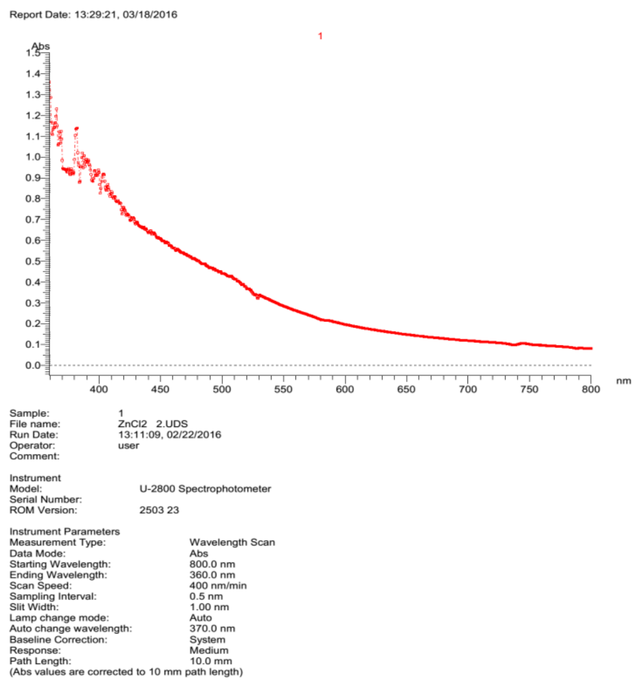


Figure 5.h : Wavelength scanning spectra for ***Figure 5.i : Wavelength scanning spectra for HydnocarpusEcliptaProstratalaurifolia***

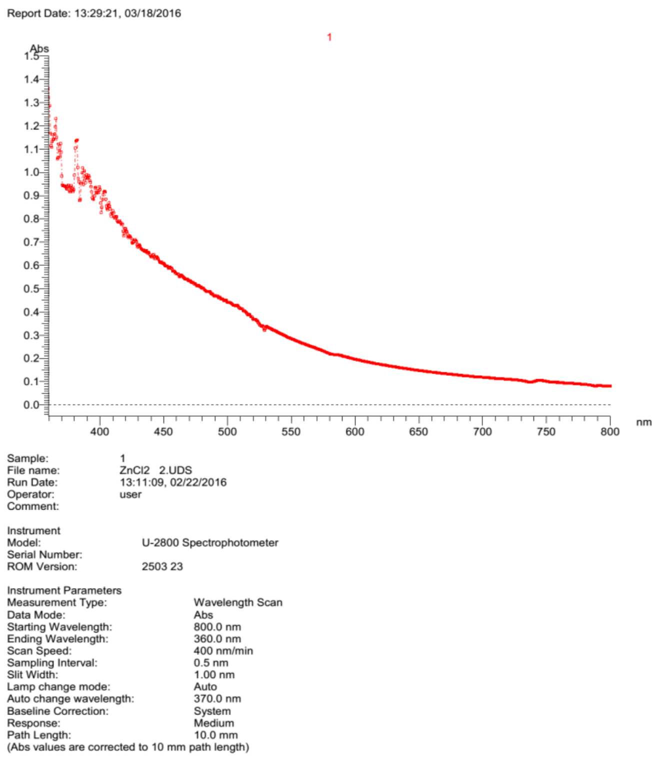


Figure:5.j Wavelength scanning spectra for symplocosracemosa

***5.2* Microscopic observation:**

The microscopic observation of synthesized Zn nanoparticles was studied using Nikon Eclipse (K 1000) microscope. The microscopic observation roves that the nanoparticles were in clustrus form and the shape was found to be irregular. The surface was observed to be rough in nature. The microscopic images were in figure.

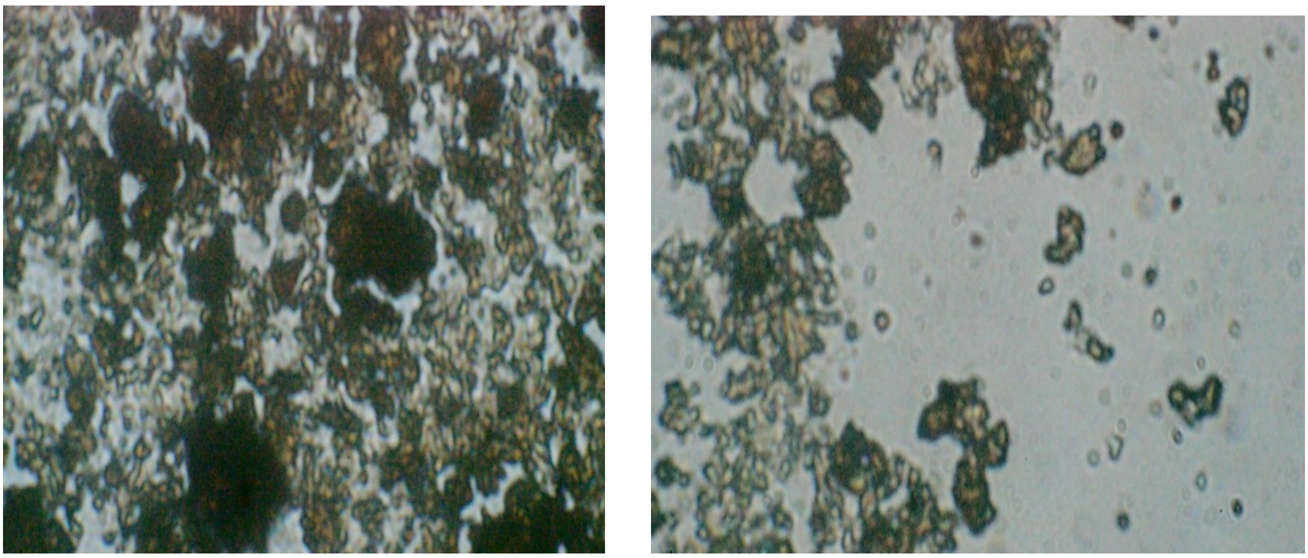


Figure 5.k: Microscopic Observation of Synthesized Zn nanoparticles

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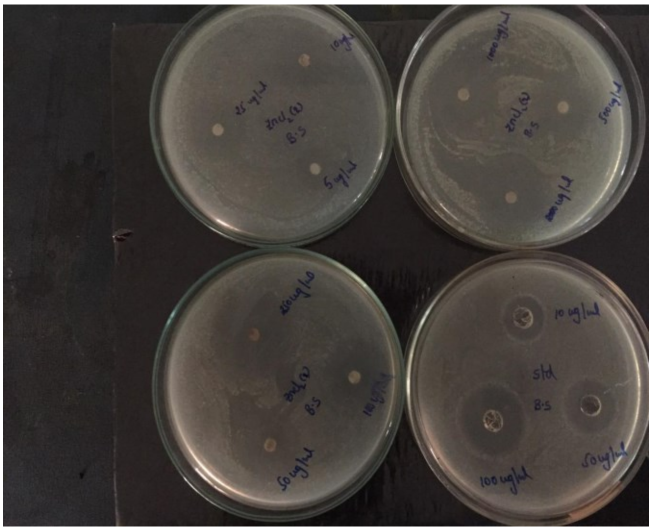
Three plants were used for the synthesis of Zinc nanoparticles. *EcliptaProstrata, Hydnocarpuslaurifoliasymplocosracemosa*was used for the synthe\isis of Zinc nanoparticles. The color change of the reaction mixture was studied and the particles obtained in the reaction mixture were centrifused and the wavelength maximum was determined. The results proved that the Zinc nanoparticles were formed in using the pant *EcliptaProstrata*using Zinc chloride as metal source. The microscopic observation of the plant proved that the surface of the nanoparticles was found to be irregular.

By using disc method anti microbial activity was studied.The results were observed by zone inhibition method by measuring the zones near the discs in mm

Anti microbial activity of Synthasised Zinc Nano particles

S. No	Test Organism	Size of zones (in mm) observed for different concentrations (in µg/ml)								
		2000	1000	500	250	100	50	25	10	5
1	*Bacillus substilis*	40.6	33.4	25.1	19.4	12.3	9.8	6.7	5.9	4.8
2	*Escherichia coli*	39.1	30.7	21.3	19.7	11.9	10.5	5.3	4.9	4.5
3	*Pseudomonas aeruginosa*	50.5	41.5	30.2	20.1	15.5	10.3	6.4	5.3	4.7
4	*Staphylococcus aureus*	49.3	38.6	29.9	17.4	11.3	9.6	7.1	5.8	4.9



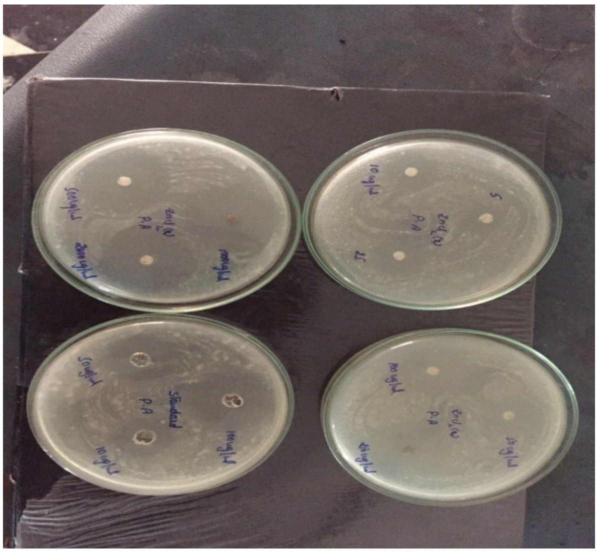


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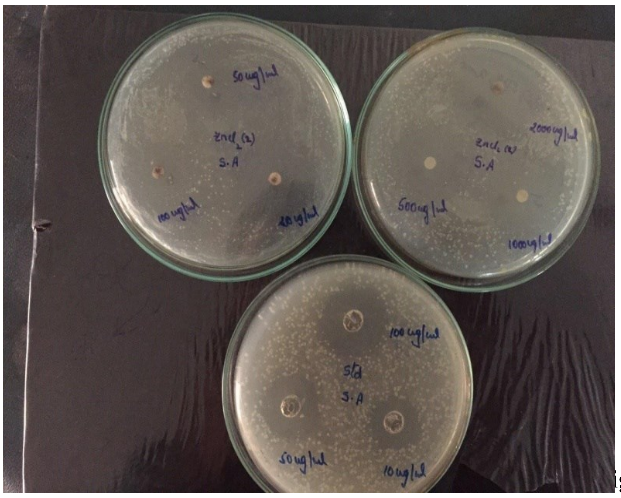
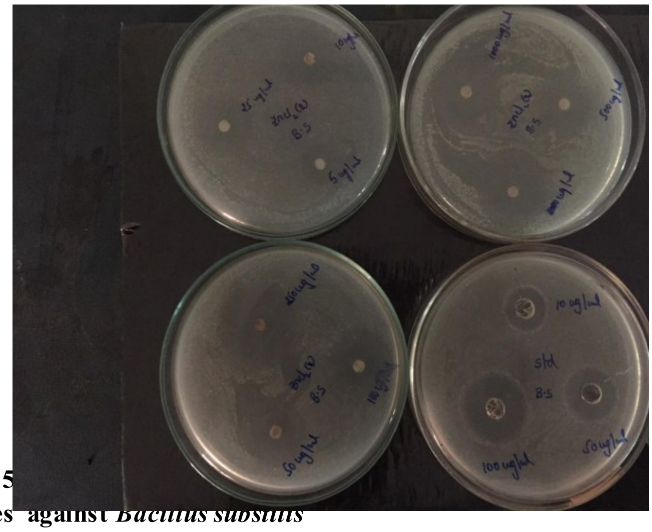
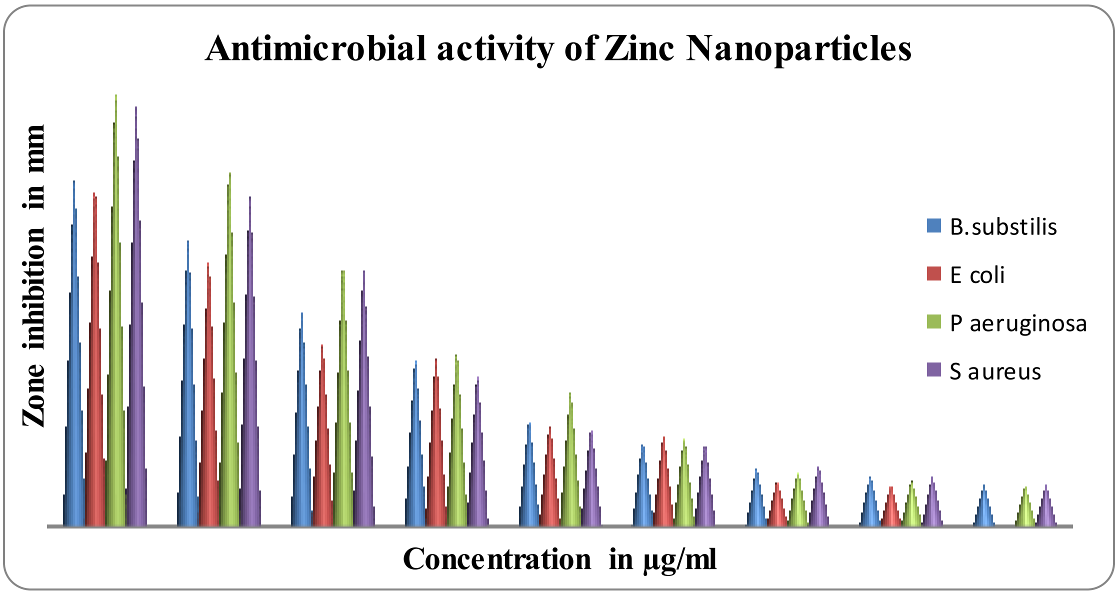
Figure 5.m: Antimicrobial activity of synthesized Figure 5.n: Antimicrobial activity of nanoparticles against *Escherichia coli*synthesized nanoparticles against *P aeruginosa*





...igure 5... nanoparticles against *Staphylococcus aureus*nanoparticles against *Bacillus substilis*



varied form of basic elements derived by altering their atomic and molecular properties of elements. Biological methods for nanoparticle synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible eco-friendly

CONCLUSION

Nanotechnology research has gained momentum in the recent years by providing innovative solutions in the field of biomedical, materials science, optics and electronics. Nanoparticles are essentially a synthesized by biological method are as efficient as that synthesized by chemical method making the former competent enough to replace the latter. Green synthesized zinc nanoparticles could be a potential antibacterial agent to treat diseases caused by bacteria. Green synthesis offers a number of advantages over other methods. The method is eco-friendly and provides bio-compatibility in pharmaceutical, biomedical and cosmetic applications as they do not use toxic chemicals for the synthesis protocol. Chemical synthesis generally leads to formation of by-products that get adsorbed onto the surface of nanoparticles that prove to be toxic in medical applications. Green synthesis has also proved to be cost-effective.

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alternatives to chemical and physical methods. Zn nanoparticles have been prepared using green synthesis method. The medicinal plant *EcliptaProstrata*was used as a green reducing agent for the preparation of Zn nanoparticles.

The synthesized ZnO nanoparticles exhibit the UV absorption peak at 410.0 nm The microscopic observation proves that the nanoparticles were in irregular in shape and the surfaces were found to be rough. The antimicrobial activity of synthesized nano-particles were studied using five different pathogenic microorganisms (*Bacillus substilis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*). The results of the anti-microbial activity proved that the nanoparticles were found to be having high growth inhibition activity against the pathogenic strains in the study. What is evident from this study is that concluded that nanoparticles

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