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Antibacterial activity of silver and zinc nanoparticles loaded with *Enicostemma Littorale*

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Abstract
Nanotechnology is important in the major fields of biology, chemistry, physics, and material sciences. Nanotechnology deals with the study of materials at the nanometers. The green synthesis of silver and Zinc nanoparticles using aqueous extract of *Enicostemma littorale* was also investigated *E. littorale* is a glabrous perennial herb belongs to family Gentianaceae. It possesses anti-oxidant, hypolipidemic, anti-microbial, anti-nociceptive, anti-edematologinic and anti-tumor activities. The UV-Vis spectrum of the prepared nanoparticles can be measured in the wavelength range of 200-700 nm, The Ultraviolet (UV) region scanned is normally from 200-400 nm and the visible portion is from 400-600 nm Complete removal of water has been confirmed by recording the FTIR spectrum of pure KBr, The synthesized Silver and Zinc nanoparticles by Characterization studies in Scanning Electron Microscope (SEM), The antibacterial activities of synthesized AgNPs and ZnONPs were carriedout by well diffusion methods. 5gm of *Enicostemma littorale* leaf powder, in dried form, was added into 100 mL of distilled water and magnetically stirred for 2 h at 80 °C. After cooling to room temperature and filtering through Whatman No. 1 paper, to remove particulate matter and to get clear solutions then refrigerated (4 °C) in 250 mL Erlenmeyer flasks for further experiments.10 mL of *Enicostemma littorale* plant leaf extract was mixed with 90 ml of 1 mM $AgNO_3$ and Zinc aqueous solution in 250 mL conical flask. The reaction mixture was heated at 80 °C for one hour. The light yellowish brown coloured solution was changed into dark brown, which shows the reduction of Ag+ ions to metallic Ag. The present study systematically evaluated the antibacterial effect of *Enicostemma littoreale* nanoparticles against a Gram Positive and Gram negative bacteria. The Ag NPs and Zinc effectively inhibited the growth of both Gram Negative and Gram Positive bacteria. Our results show that Ag NPs and Zinc synthesized using an aqueous extract of *Enicostemma littorale* have potential antibacterial activity against both Gram Negative and Gram Positive bacteria.

Keywords: Silver nanoparticles, Zinc nanoparticles *and Enicostemma littoreale*

Introduction

The progression of green methods for the synthesis of nanoparticles is developing into significant branch of nanotechnology. The study on synthesized nanomaterials and its characterization is an emerging field of nanotechnology from the past few decades, due to their vast application in various fields majorly physics, chemistry, biology and medicine (Ponarulselvam *et al*., 2012) [6]. Many scientists demonstrated the green synthesis of silver nanoparticles (AgNPs) together with plants or plants extract, bacteria, fungi and enzymes (Ahmed *et al*., 2016) [1]. The plant resources have been effectively used for nanoparticle synthesis, because of their potential therapeutic property (Singh *et al*., 2016) [11]. In past few years research showed the metal nanoparticles have been developed its antimicrobial effects and may locally cleave pathogenic organism and without harm to the surrounding tissues (Figure-1).

Fig 1: *Enicostemma littorale* Blume

Number of studies reported on the synthesis of metal nanoparticles and silver nanoparticle has their own significance among all. Plant based green synthesis of nanoparticle synthesis is rapid, cost efficient, environmental safe and easy to synthesis (Natarajan *et al*., 2012) [4]. Number of studies reported the green synthesis of AgNPs using various plant extracts, such as *Azadirachta indica*, *Pelargonium graveolens, Medicago sativa, Emblica officinalis* (Ponarulselvam *et al*., 2012) [6]. Synthesis of zinc oxide nanoparticles (ZnoNps) has received significant interest because of their antimicrobial, UV blocking, high catalytic and phytochemical activities (Senthilkumar and Sivakumar, 2014) [10].
Plants phytochemicals are employed to synthesize the nanoparticles. *Enicostemma littorale* is a glabrous perennial herb belongs to family Gentianaceae (Sanmugarajah *et al*., 2013) [8]. It is traditionally used as antidiabetic, urinary astringent, antiperiodic, anthelmintic, anti-inflammatory, laxative and carminative. It possesses anti-oxidant, hypolipidemic, anti-microbial, anti-nociceptive, anti-edematologinic and anti-tumor activities (Rajasekaran *et al*., 2015; Saranya *et al*., 2013; Vishwakarma *et al*., 2010) [7, 9, 12]. The present study is focused to green synthesis of silver and zinc nanoparticles using *Enicostemma littorale* and assesses the antibacterial activity.

Materials and methods

Materials

Silver nitrate, Zinc acetate and all Glassware's were obtained from the Department of Biochemistry and Biotechnology, Annamalai University. All chemicals used were of analytical grade with 99% purity.

Preparation of Leaf Extract

Fresh and healthy leaves *Enicostemma littorale* were collected locally and rinsed thoroughly first with tap water followed by distilled water to remove all the dust and unwanted visible particles, cut into small pieces and dried at room temperature and then grind at mortar to get powder form. 5gm of *Enicostemma littorale* leaf powder, in dried form, was added into 100 mL of distilled water and magnetically stirred for 2 h at 80 °C. After cooling to room temperature and filtering through Whatman No. 1 paper, to remove particulate matter and to get clear solutions (Figure-2a) which were then refrigerated (4 °C) in 250 mL Erlenmeyer flasks for further experiments. In each and every steps of the experiment, sterility conditions were maintained for the effectiveness and accuracy in results without contamination (Figure-2A).

Synthesis of AgNPs

10 mL of *Enicostemma littorale* plant leaf extract was mixed with 90 ml of 1 mM $AgNO_3$ aqueous solution in 250 mL conical flask. The reaction mixture was heated at 80 °C for one hour. The light yellowish brown (Figure-2B) coloured solution was changed into dark brown (Figure-2B), which shows the reduction of Ag+ ions to metallic Ag.

E. Littorale

A B C D E

Fig 2: Digital photographs of (a)Plant leaf extract (b) AgNO3 with *Enicostemma littorale* before synthesis (c) AgNO3 with *Enicostemma littorale* after synthesis (d) ZnO with *Enicostemma littorale* before synthesis (e) ZnO *Enicostemma littorale* after synthesis

Synthesis of Zinc Nanoparticles

30 mL of *Enicostemma littorale* plant leaf extract was mixed with the 70 mL of 0.2 M zinc acetate solution in 250 mL conical flask. The reaction mixture was kept in oven at 60 °C for overnight. The brown (Figure-2C) coloured solution was changed into dark reddish brown (Figure-2D,2E) ZnO nanoparticles, which were finally calcined at 100 °C for 1 h and preserved in air-tight vials for further studies.

UV-visible spectrophotometer analysis

The synthesized samples were dispersed in de-ionized water and then filled in a cuvette. The absorbance and fluorescence spectra of the samples contained in 1 cm^2 cuvette were scanned immediately after preparing the solution. The UV-Vis spectrum of the prepared nanoparticles can be measured in the wavelength range of 200-700 nm. The spectra were recorded by taking pure solvent used as reference (Figure-3). The Ultraviolet (UV) region scanned is normally from 200-400 nm and the visible portion is from 400-600 nm.

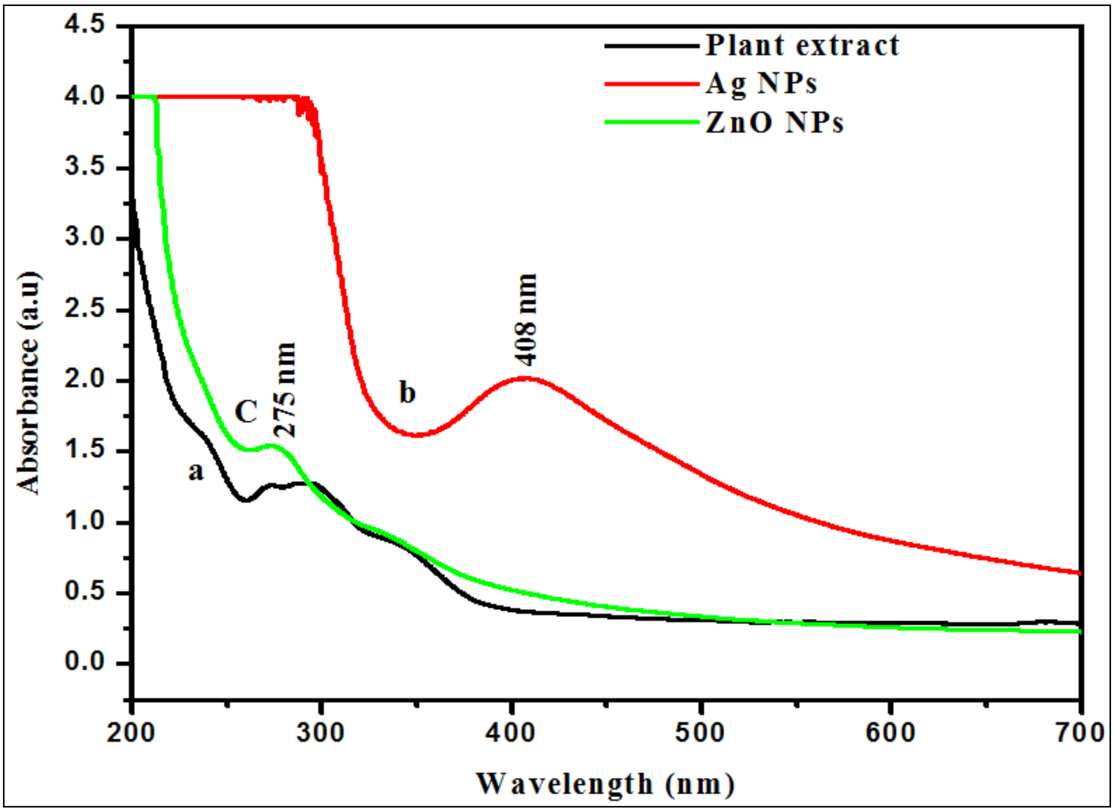

Fig 3: UV–Vis spectra of (a) plant extract, (b) Ag NPs, (c) ZnO NPsynthesized by *Enicostemma littorale* leaf extract.

The optical absorption spectra of all the samples in deionized water were recorded from CISL in Department of physics, Annamalai University, in the wavelength range of 200-800 nm using SHIMADU-UV 1800 spectrometer.

FT-IR

The standard Potassium Bromide (KBr) pellet technique is most commonly employed as a sampling technique for FTIR absorption measurements of solid material. The same technique has been adopted for the present work. Materials required for KBr pressed pellet method are Potassium Bromide (KBr), Acetone, die for making KBr pellets, laboratory hydraulic press for confined sample, small hand agate mortar and pestle mechanical vacuum pump. To remove the moisture absorbed by KBr powder required spectrum pure has been dried at 125 °C-150 °C for about 6 hours. Complete removal of water has been confirmed by recording the FTIR spectrum of pure KBr (Figure-4, 5, 6). Then fine grained samples were mixed with pure KBr in the ratio of 1:30 and then the mixture was pressed under Vacuum at about 10 tones by placing the mixture in 13 mm diameter die to get a disc of about 13 mm diameter and 1 mm thickness. All the spectra were recorded in the region 4000-400 cm^{-1}.

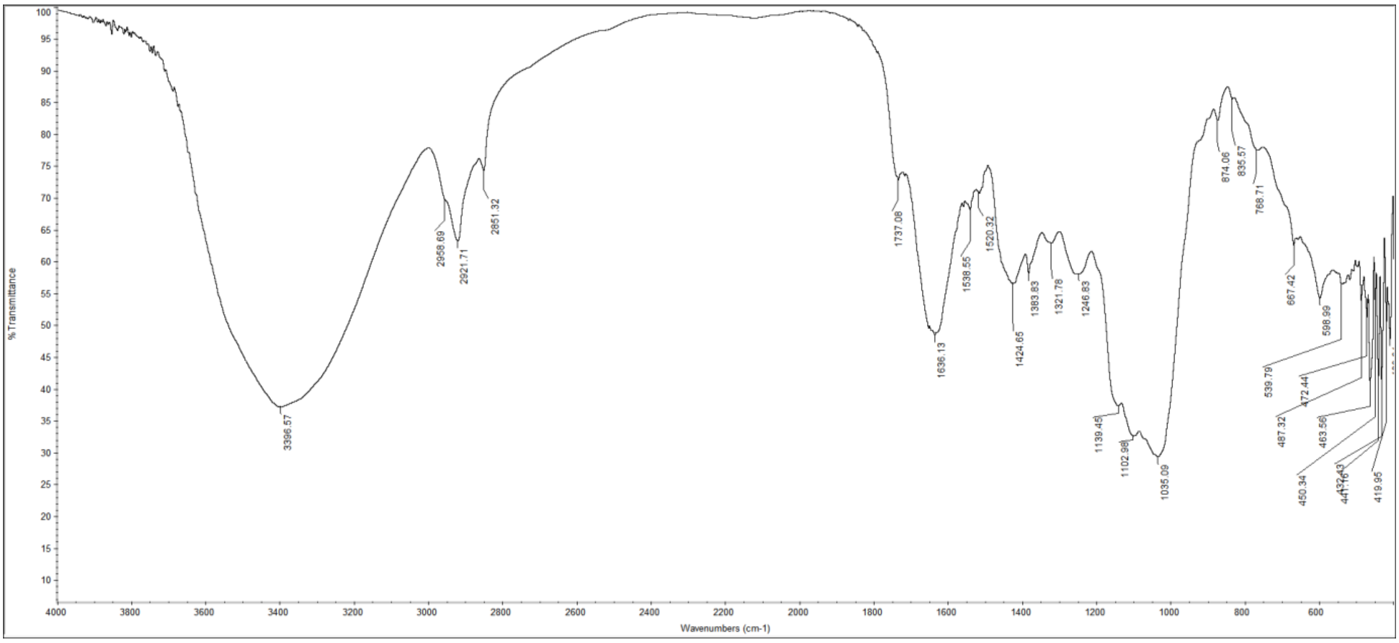

Fig 4: FTIR Spectra of Plant leaf extract

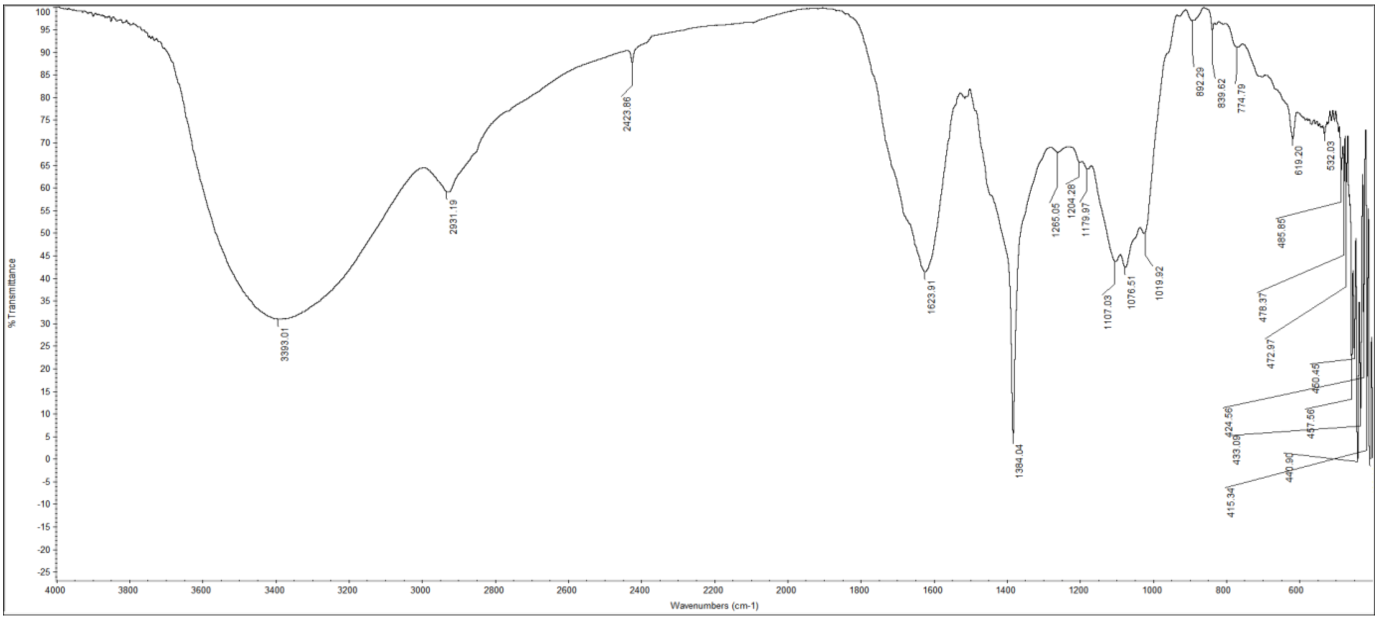

Fig 5: FTIR Spectra of Silver Nanoparticles (AgNPs)

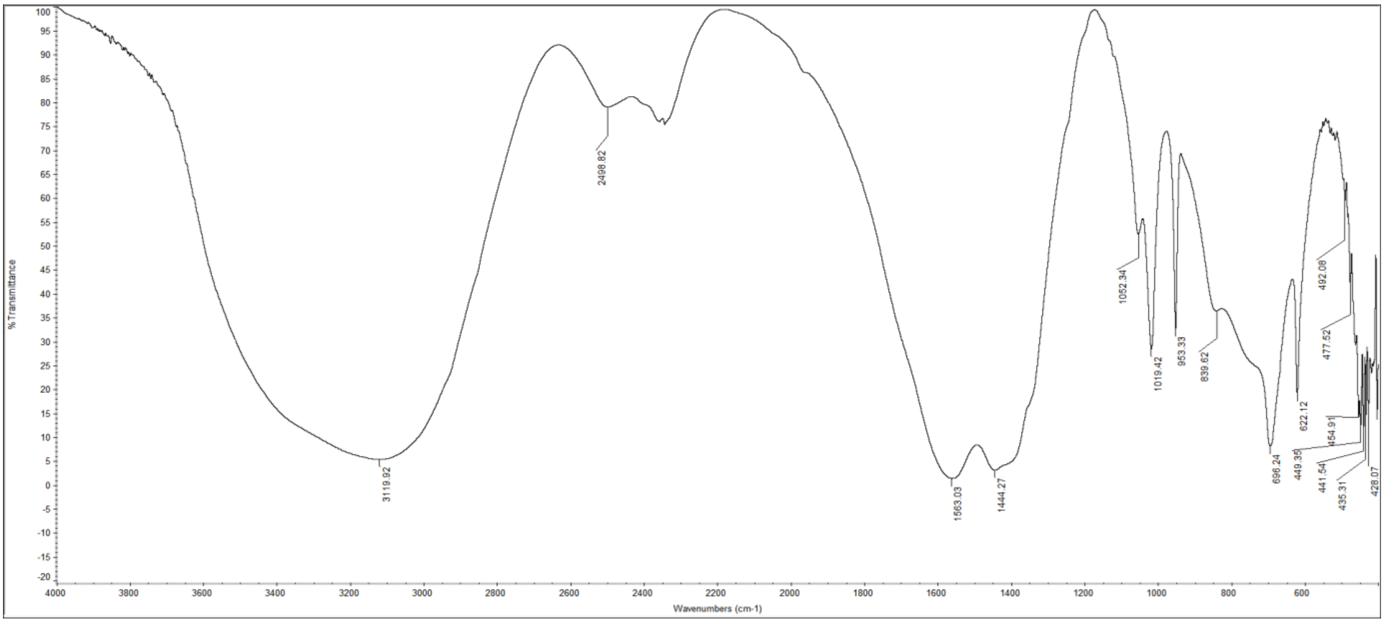

Fig 6: FTIR Spectra of Zinc Oxide Nanoparticles (ZnO NPs)SEM analysis

Powder samples of nanoparticles are studied by FE-SEM. The surface of the samples has been stubbed using the double-side adhesive carbon tape. Samples are coated with the help of good coater (JEOL-JSM-56100) and deposited with thin layer gold (heavy metal) on the sample (Figure-7a, 7b). The morphological studies of the products were recorded from department of manufacturing engineering, Annamalai University, Tamil Nadu, India, using JEOL-JSM-56100 scanning electron microscope (SEM).

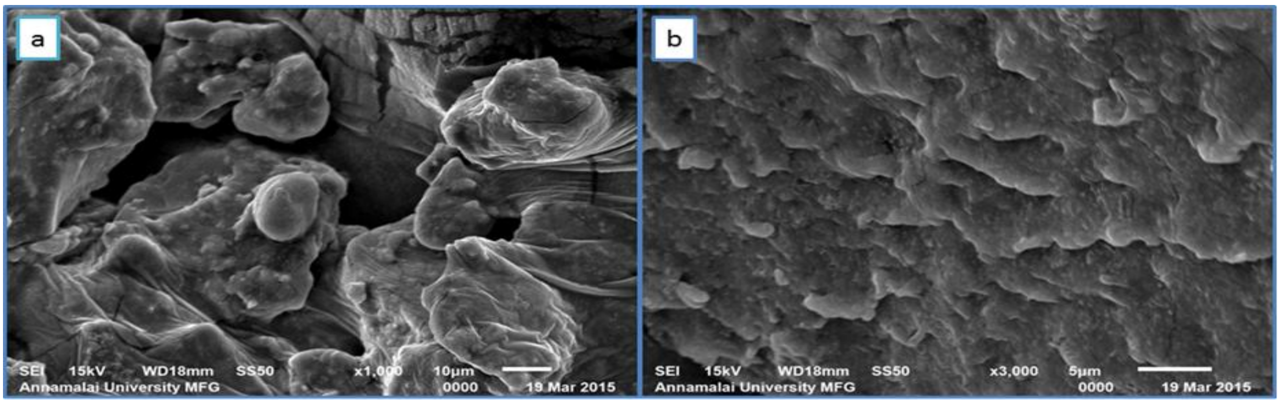

Fig 7: SEM micrographic image of synthesized Ag NPs at various resolutions (a). 1000, (b). 3000.

Antibacterial activity

The antibacterial activities of synthesized AgNPs and ZnONPs were carriedout by well diffusion method. Nutrient agar plates were prepared, sterilized and solidified. After solidification, various bacterial cultures namely *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis* were swabbed on plates. Holes of 6 mm in diameter were made by using Gel puncture. Aliquot of 50 µl from plant extract and silver (10µl, 20µl, and 30µl) and zinc nanoparticle (10µl, 20µl, and 30µl) was added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at 37 °C for 24 h. The resulting zones of inhibition were measured in millimeters (mm).

Result and Discussion

The green approach to synthesis nanoparticles is easy, trustworthy and ecofriendly, various methods are used to synthesize nanoparticles but now researchers are focused on green synthesis of metal nanoparticles (Vankar *et al.,* 2010). Past two decades plant extracts and natural resources have been found to be useful in the reduction of silver ions to AgNPs. AgNPs show a dark reddish-brown color in aqueous solution due to the surface plasmon resonance phenomenon (Krishnaraj *et al.,* 2010) [2].

The bio-reduction of silver nitrate into silver and zinc nitrate into zinc oxide in nano size are confirmed by UV-Visible absorption measurements since absorption measurement can give optimum information regarding the shape and stability of the particles. Figure-6 depicts the absorption results of the plant extract, Ag and ZnO nanoparticles recorded in the range of 200 – 700 nm after 24 h of incubation. As shown in figure, the absorption curve of plant extract exhibits a absorption peak at 294 nm (a). On the other hand, the bio-reduced Ag nanoparticles show an intense absorption at 408 nm (b), revealing a characteristic SPR of Ag nanoparticles. However, ZnO shows the absorption feature at 275 nm (c) corresponding to SPR peak of ZnO nanoparticles. These results confirm that the bio reduced particles are in their respective nanosize. The surface plasmon resonance band of AgNPs can be monitored through the ultraviolet-visible spectrum (UV-Vis) ranging from 400 nm to 450 nm, indicating formation of AgNPs (Mulvaney *et al.,* 1996) [3]. The stretching at the wave number 3117 cm-1 shows the presence of O-H functional group with H bonded and the bending at wave number indicates the presence of NH functional groups in Enicostemma littarole (Figure 6). According to Niraimathi *et al*., (2013) the FT-IR spectra suggest that the proteins present in the plant extract acting as a capping agent.

The UV-VIS absorption spectrum of *Enicostemma littorale* zinc nanoparticles, an excitonic absorption band at 275 nm. It is also evident that significant sharp absorption of ZnO indicates the monodispersed nature of the nanoparticle distribution (Ponarulselvam *et al*., 2012) [6]. To get an idea regarding the functional groups existing in the plant extract which have played a vital role in the bio-reduction process, FT-IR spectra were recorded for plant extract, synthesized Ag and ZnO nanoparticles using KBr pellet technique in the mid infra red region. FT-IR results of plant extract (Figure-7), Ag nanoparticles (Figure-8), and ZnO nanoparticles (Figure-9). As shown in figure, the plant extract shows the broad absorption band at 3398 cm^{-1} which can be ascribed to OH stretching vibrations, whereas, the absorption at around 1630 cm^{-1} indicates the presence of OH bending vibrations. The appearance of peak at 1620 cm^{-1} indicates the C = O stretching of amide I band of peptide linkage. The presence of peak at around 1246 cm^{-1} specifies the CN stretching and bending of peptides linkage. The presence of peaks at 874 and 835 cm^{-1} reveals the amide V band arising due to out of plane NH bending of peptide linkages. Methylene C – H asymmetric or symmetric stretch and primary amine C – N stretch were represented by the peaks at around 2965 and 1021 cm^{-1}, respectively. The results of FT-IR revealed that proteins are responsible for stabilizing the nanoparticles Ag and ZnO from their precursors. The formation of Ag nanoparticles is explained by the creation of intense metallic bands in the region of 400 – 700 cm^{-1}. In the same way the stabilized ZnO nanoparticles are also confirmed from the appearance of absorption bands in the lower wave number region. Senthilkumar and Sivakumar (2014) [10] reported that FT-IR absorption band at 682 and 457 cm^{-1} are the characteristic peaks of ZnO molecules.

The surface morphology of the synthesized Ag NPs of *Enicostemma littorale* was studied by using SEM. The results obtained from SEM showed that the nanoparticles are crystalline in nature of spherical with wave like shape at higher magnification, having an average particle size of 80–100 nm. (Table-1) reveals that all Ag NPs of *Enicostemma littorale* are polycrystalline nanoparticles showing spherical and wave-like shapes with narrow particle size distribution and further no agglomeration was noticed.

Table 1: *Enicostemma littorale* silver & zinc nanoparticles against

Zone of Inhibition in mm				
Organism	**Antibiotic Streptomycin (positive control)**	***Enicostemma littorale* leaf Extract (negative control)**	***Enicostemma littorale* AgNPs**	***Enicostemma littorale* ZnONPs**
Staphylococcus aureus	14±0.03	8±0.07	11±0.02	10±0.04
E. coli	11±0.04	7±0.05	9±0.04	8±0.03
Pseudomonas aeruginosa	16±0.03	9±0.08	14±0.03	12±0.02
Bacillus subtilis	12±0.02	8±0.06	11±0.02	10±0.04

Conclusion

The present study systematically evaluated the antibacterial effect of *Enicostemma littoreale* nanoparticles against a Gram Positive and Gram negative bacteria. The Ag NPs and ZnO NPs effectively inhibited the growth of both Gram Negative and Gram Positive bacteria. Our results show that Ag NPs and ZnO NPs synthesized using an aqueous extract of *Enicostemma littorale* have potential antibacterial activity against both Gram Negative and Gram Positive bacteria.

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