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Research Article

PHYTO-ASSISTED SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING LEAF EXTRACTS OF A MEDICINAL MANGROVE PLANT *BRUGUIERA GYMNORHIZA* L. FOR ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY ASSESSMENT

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ABSTRACT

The present study involves green synthesis of ZnO nanoparticles (NPs) using aqueous leaf extract of *Bruguieragymnorrhiza*as green reducing agent. The ZnO NPs were characterized by X- ray diffraction (XRD), UV–visible studies and scanning electron microscopy (SEM). The NPs were evaluated for antimicrobial and antioxidant activities. The NPs were found to have a hexagonal wurtzite structure. UV-visible absorption of ZnONps showed absorption band at 320 nm which can be assigned to the intrinsic band-gap absorption of ZnO due to the electron transitions from the valence band to the conduction band. SEM image confirms the formation of nanoparticles and the average crystallite sizes were found to be 38-97 nm. Excellent bactericidal activity was shown by the NPs on *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*. The anti-oxidant activity was studied by % DPPH (*1, 1-Diphenyl-2-picrylhydrazyl*) inhibition in the concentration range of 5 µg/ml to 150 µg/mL and IC50 value was found to be 66.37, 73.93 and 92.35 µg/ mL for ascorbic acid, ZnO NPs and aqueous plant leaf extract. Hence the synthesized NPs were found to having potent anti-oxidant activity. Synthesis of multifunctional ZnO NPs using naturally occurring plant products has been advocated as a possible environment friendly alternative to chemical methods.

Keywords: Phyto-assisted synthesis, ZnOnano particles, Scanning Electron Microscopy, Anti-microbial activity, DPPH inhibition activity

1. INTRODUCTION

Nanotechnology involves the use of materials having nanoscale dimensions in the range of 1–100 nm. Operating with nanomaterials has allowed researchers to have a much better understanding of biology [1].Nanoparticles are being synthesized globally owing to various exciting and unique properties, which facilitate their exploitation in completely unrelated fields, such as, nanodiagnostics [2] and nanomedicine [3] and anti-microbial properties [4] in one hand and luminescence [5], photocatalytic potential [6] and photodiode response [7] on the other.

Zinc oxide nanoparticles (ZnO NPs) is a type of metal oxide nanoparticles with a band gap of 3.3 eV and excitation binding energy of 60 meV at room temperature [8]. It has gained considerable attention due to its unique catalytic, antibacterial, antifungal, photochemical, UV-filtering, anti-inflammatory properties owing to its large surface area to volume ratio [9]. Synthesis of metal and metal oxide nanoparticles through green routes and environmentally benign method has been focused by researchers to avoid the use of toxic chemicals making the process and the final product eco-friendly and non-toxic in nature. Use of plants as a green source for nanoparticles synthesis is most commonly practiced as plants are the hub of a wide range of phytochemicals which can act as reducing and stabilizing agent for nanoparticles synthesis [10].

The mangrove plant *Bruguiera gymnorrhiza* (L.) belongs to family *Rhizophoraceae* which is naturally distributed in high tidal zones and is a dominant species in the mangrove forest [11]. The available literature on the plant confirms that the roots of the plant *B.gymnorrhiza*was found to having Antinociceptive and Antidiarrheal activity [12]. The anti-microbial activity was reported in the fruit extract of *B.gymnorrhiza* [13]. The leaf and stem extract of *B.gymnorrhiza* was found to having anti-oxidant activity [14].

The plant having large source of bio active compounds, novel diterpenoids were isolated and characterized from the areal parts of the plant *B.gymnorrhiza* [15, 16]. The present work focusses on the biosynthesis of ZnO NPs using leaf extract of medicinal herb *Bruguieragymnorrhiza*. Further, the antimicrobial and antioxidant activity of synthesized nano particles was studied.

2. MATERIAL AND METHODS

2.1. Instrument, Chemicals and Standards

All the chemicals used were of laboratory reagent grade and were purchased from Merck chemicals, Mumbai. stable free radical DPPH (*2,2-diphenyl-1-picrylhydrazyl*) was purchased from Sigma Aldrich.

2.2. Collection of *Bruguieragymnorrhiza* plant material

The plant material *Bruguiera gymnorrhiza* was collected in the mangrove region, near to machilipatnam port, Krishna District, Andhra Pradesh, India. The Plant material was authenticated in department of Botany, SSN College, Narasaraopet, Guntur, AP. The leaves of the plant material were shade dried and were reduced to fine powder. The powdered leaf material was stored in an air tight amber color container and was used for the synthesis of nano particles.

2.3. Synthesis of ZnO nanoparticles

ZnOnano particles were synthesized using leaf extract of *Bruguiera gymnorrhiza* was carried as per the procedure described by *Agarwal et al.*, 2019 [17]. Briefly, 10 g of *Bruguiera gymnorrhiza* leaves were mixed with 100 mL of Milli-Q water. The mixture was boiled for 30 min at 60 °C. The mixture was cooled down to room temperature and double filtered using Whatman filter paper no. 1. Filtered mixture was used as the extract and used further for the synthesis of ZnO NP nanoparticles.

0.01 M Zinc acetate was used as precursor and 5 mL of leaf extract was used as a reducing agent. The mixture was stirred on a magnetic plate at 80°C for 20 min. pH of reaction mixture was adjusted to 12 using 2 M NaOH. The mixture was stirred for 2 h and UV-Visible readings were recorded, wherein a strong peak was observed at the end of 3 h. The mixture was centrifuged at 5000 rpm for 10 min. The sedimented pellet was double washed with Milli-Q water and dried overnight in a hot air oven operating at 80 °C. The white colored powder was obtained and used for characterization.

2.4. Characterization of ZnO nanoparticles:

The UV-visible absorption spectra of synthesized ZnO NPs were measured using UV-visible spectrophotometer (V 730, JASCO - United States). FTIR spectra of the as synthesized NPs were recorded using Fourier transform-infrared (FTIR) spectrometer (Bruker - Japan) in the wavelength range of 500-4000 cm^{-1}. The crystalline nature and pattern of powdered ZnO NPs were recorded by X-ray diffractometer (XRD, Bruker - Japan) at scan rate of 0.03°/s using Cu-Kα1 radiation (1.5406 Å, 45 kV, 40 mA. SEM (Scanning electron microscope) micrograph of the NPs was taken with Zeiss Ultra 60 Field Emission Scanning Electron Microscope. Energy-dispersive X-ray spectroscopy (EDS) was utilized to determine the elemental composition of ZnO NPs.

2.5. Measurement of antioxidant activity

The antioxidant potential of synthesized ZnO NPs was estimated as described by *Rajesh et al.*, 2018 [18]. The experiment was carried out using DPPH activity estimation. The deep violet color of DPPH turns yellow in the presence of an antioxidant compound. When DPPH is mixed with a hydrogen donor substance, free radicles are reduced and a color change occurs. The different volume of plant extract was added to 1 mL of 0.1 mM DPPH solution in methanol. The solution mixture was incubated for 30 minutes at room temperature in the dark. The absorbance was measured at 517 nm after the incubation period to estimate the reduction in DPPH free radical number. Methanol solution mixed with DPPH was used as a control, vitamin C was used as the standard and methanol plus plant extract solution was used as a blank. All the experiments were performed in triplicate. Origin pro 8.5 software was used for statistical analysis. DPPH free radical scavenging activity was calculated by the following formula;

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absobance of Sample}}{\text{Absorbance of control}} \times 100$$

2.6. Antibacterial activity

The anti-bacterial activity of the synthesized ZnO NPs was tested using agar well diffusion method on MH-nutrient agar (HI media) as per the procedure described by *Tura Safawo et al.*, 2018 [19] against two gram positive (*Bacillus subtilis*-MTCC 441 and *Staphylococcus aureus*-MTCC 96) and two-gram negative bacteria (*Salmonella typhi*-MTCC 733 and *Escherichia coli*-MTCC 443). About

100 µL of these organisms were introduced on the plates of MH-nutrient agar and spread uniformly. Wells were made on the agar plates with sterile borer (6 mm) and different concentrations of ZnO NPs (1, 10 and 100 µg/mL) were added in to well using micropipette followed by incubation at 37°C for 24 hrs. The bacterial activities were determined by measuring the diameter of zone of inhibition around the wells.

3. RESULT AND DISCUSSION

Zinc oxide nanoparticles received huge interest due to their good optical properties leading to various applications in biomedical sciences. Hence ZnO NPs were synthesized using aqueous leaf extract of *Bruguiera gymnorrhiza* as reducing agent and zinc acetate as precursor. The formation of ZnO NPs was monitored by visual exam. The color of solution altered from green to pale white color through the reaction, showing the formation of *B. gymnorrhiza* fabricated ZnO NPs.

The UV-Visible absorption spectrum of the *B. gymnorrhiza* fabricated ZnONPs were achieved after 3 h. *B. gymnorrhiza* fabricated ZnO NPs exhibited a characteristic UV absorption was found in the range of 300-350 nm and a strong peak was found at 320 nm (figure 1) elucidating the basic band gap absorption of ZnO nanocrystals.

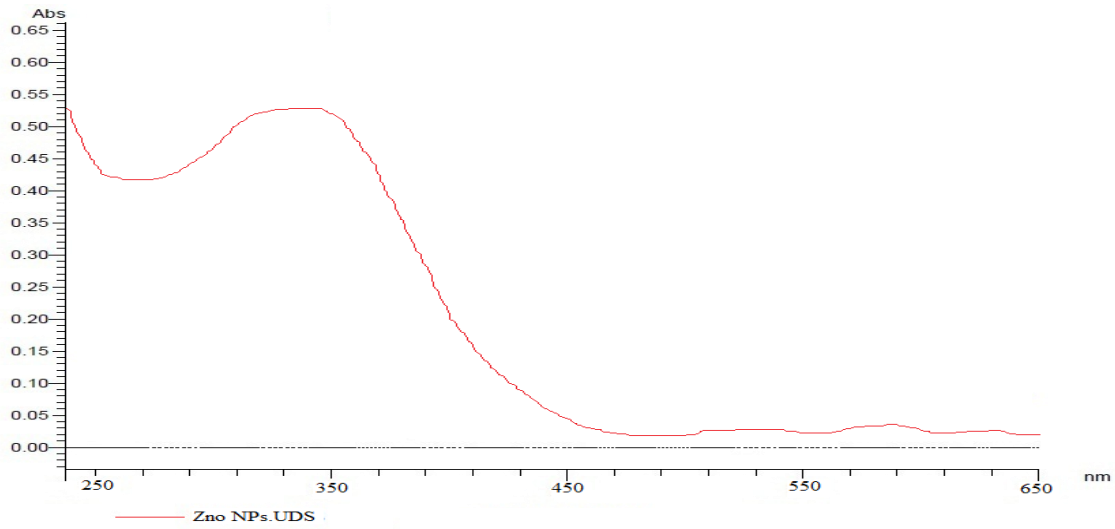

Fig. 1: UV-Visible absorption spectra of ZnO NP synthesized using *B. gymnorrhiza*

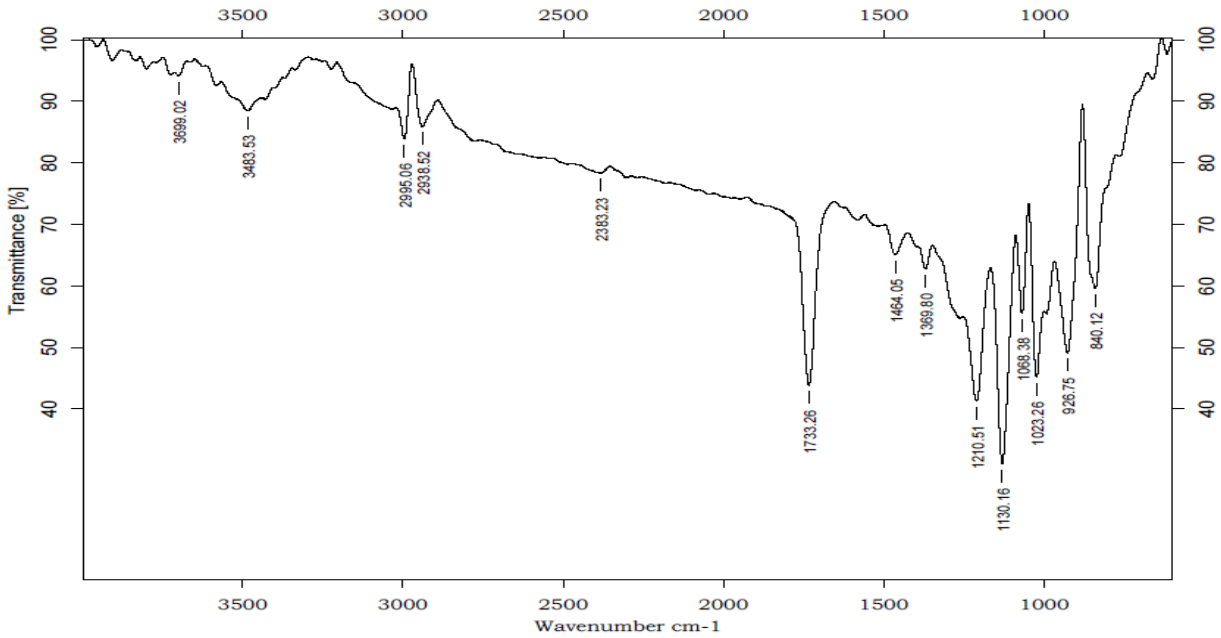

Fig. 2: FT IR spectra of ZnO NPs

FTIR analysis helped in identifying the possible functional groups involved in Zinc Oxide nanoparticles. FTIR spectrum of *B. gymnorrhiza* Zinc Oxide nanoparticles was in the range of 500-4000 cm^{-1} (figure 2).The peak at 3438 cm^{-1} attributed the O-H stretch, Hydroxy group andH-bonded. A peak at 2938 cm^{-1} is observed which is attributed to C-Hstretching and C-H Stretching. The band at 1735 cm^{-1} is attributed to be the C=O Stretching and bands at 1464 cm^{-1} is attributed to C-H bending respectively. The OH bending group in protein gives the bond at 1068 cm^{-1}. Therefore, it may be concluded that the main chemical reactions involved in the biosynthesis of ZnO NPs using the leaf extract of *B. gymnorrhiza* are either reduction or an oxidation mechanism. The biological materials possess phytocompounds and enzymes which were take part in the conversion of metal compound to specific nanoparticles. The leaf extract has variety of metabolites such as alkaloids, organic acids, flavonoids, terpenoids and aromatic dicarboxylic acid etc., which are responsible for the antioxidant or reducing property that aids in the immediate reduction of Zinc ions in to nanostructured ZnO.

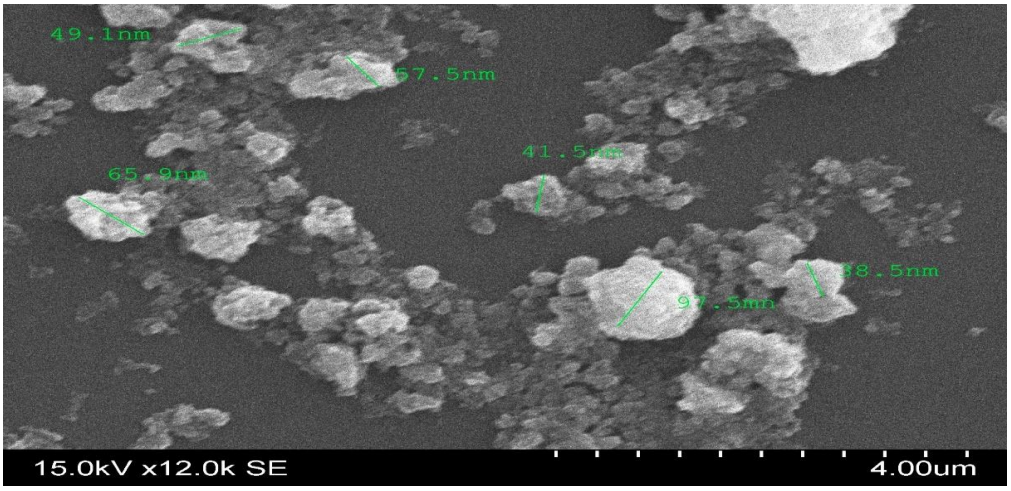

Fig.3: SEM image of ZnO

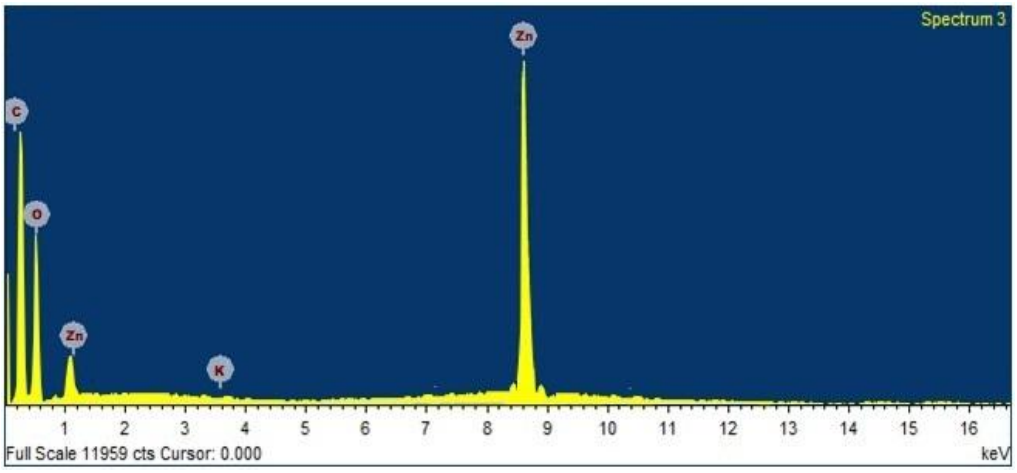

Fig. 4: EDS spectra of ZnO NPs

Morphological analysis and elemental composition of synthesized ZnONPs were studied by SEM and EDX studies. From the SEM image (figure 3), we found the synthesized Zno nanoparticles are sphere shape and its surface areir-regular. The EDX spectra (figure 4) of synthesized ZnO NPs composed of zinc, oxygen and carbon. The EDX pattern confirms that the zinc content in the synthesized Zinc Oxide nanoparticles are present at the weight percentage of 58.97%. The presence of other elements as identified by EDX attributes to the groups present in the heterocyclic rings of the biomolecules present along with the ZnO NPs.

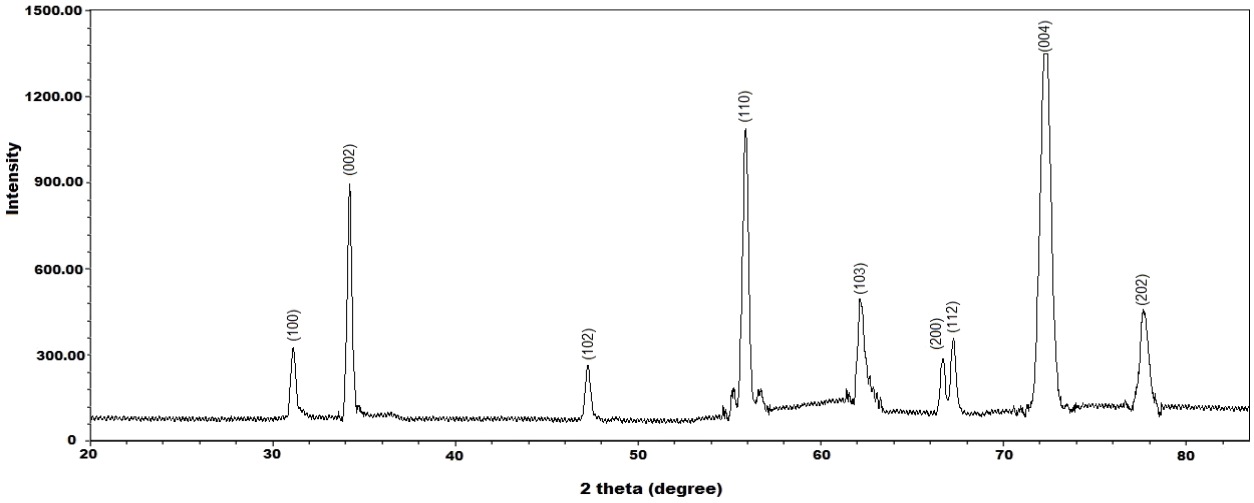

Fig. 5: XRD spectra of ZnO NPs

Figure 5 shows the XRD patterns of ZnO nanoparticles synthesized using *B. gymnorrhiza* leaf extract. All the peaks at 31.9, 34.8, 36.2, 47.5, 56.7, 62.3, 66.17, 67.2, 68.1, 72.4 and 77.6° corresponding to lattice planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) respectively, which indicated that the samples were crystalline Wurtzite ZnO structure (JCPDS Card no.89-1397). The presence of (100), (002) and (101) planesin XRD patterns indicates the formation of high purity of the ZnO nanoparticles. Further, no peaks were observed due to impurities. Strong intensity and narrow width of ZnO diffraction peaks indicate that the resulting product was highly crystalline in nature. Average crystallite size was estimated using Scherrer's formula, average crystallite size was estimated and it was found to be in the range of 38-97 nm. Hence, we can conclude that the bio active compounds present in the leaf extract of *B. gymnorrhiza* is primarily responsible for the reduction of the particle size

3.1.Anti-oxidant activity

Antioxidant capacity of green synthesized ZnONPs using *B. gymnorrhiza* leaf extract was measured by DPPH assay which is widely used to study the radical scavenging activity of green synthesized NPs. Deep violet color of DPPH solution gradually changed to pale yellow in the presence of ZnO NPs and ascorbic acid standard which indicate the antioxidant capacity of ZnO Nps which is further confirmed by the UV-Vis reading. Table1 depicts the % DPPH inhibition at 517 nm as the concentrations of ZnO NPs increase from 5 µg/ml to 150 µg/mL which indicated radical scavenging activity of ZnO Nps. The radical scavenging capacity of *B. gymnorrhiza* mediated synthesized ZnO NPs slightly lower than standard ascorbic acid in all concentration (Figure 6).The radical scavenging activities of the synthesized ZnO Nps were showed to have increase with increasing the concentration having IC50 value of 66.37, 73.93 and 92.35 µg/ mL for ascorbic acid, ZnO NPs and aqueous plant leaf extract.

Table 1: DPPH activity results of standard ascorbic acid, ZnO NPs and aqueous plant leaf extract of *B. gymnorrhiza*

Concentration (µg/mL)	% DPPH Inhibition observed for		
	Ascorbic acid	ZnO NPs	Leaf Extract
5	3.14	2.45	0.89
10	5.68	4.61	2.36
20	12.87	8.44	5.96
40	29.68	25.19	14.26
60	48.01	43.28	27.19
80	69.57	60.25	46.51
100	87.44	78.69	64.92
150	96.23	91.25	77.81

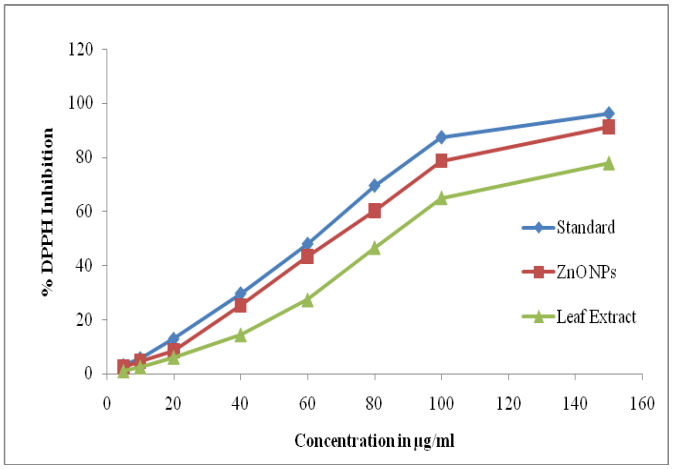

Fig. 6: Comparison of DPPH inhibition activity of standard ascorbic acid, ZnO NPs and aqueous plant leaf extract of *B. gymnorrhiza*

3.2. Anti-microbial activity

In the present study, biosynthesized Zinc Oxide nanoparticles were analyzed by twogram positive and two-gram negative bacterial strains. The zone of inhibition was found to be 2.9, 5.4 and 11.1 mm for *Bacillus subtilis*, 0, 3.6 and 9.7 mm for *Staphylococcus aureus*, 2.3, 4.9 and 10.6 mm for *Salmonella typhi* and 3.8, 6.1 and 12.5 mm for *Escherichia coli* at a concentration of 1µg/ml, 10µg/ml and 100µg/ml respectively. Penetration of ZnO nanoparticles through cell membrane in of bacterial strains are the reason for the anti-microbial activity of synthesized ZnO NPs. The plant extract is abundant with various phytochemical constituents like phenolics, alkaloids, terpenoids and flavonoids which play a major role in capturing the ZnO ions and adhering with them. The anti-bacterial comparison graph is given in figure 7.

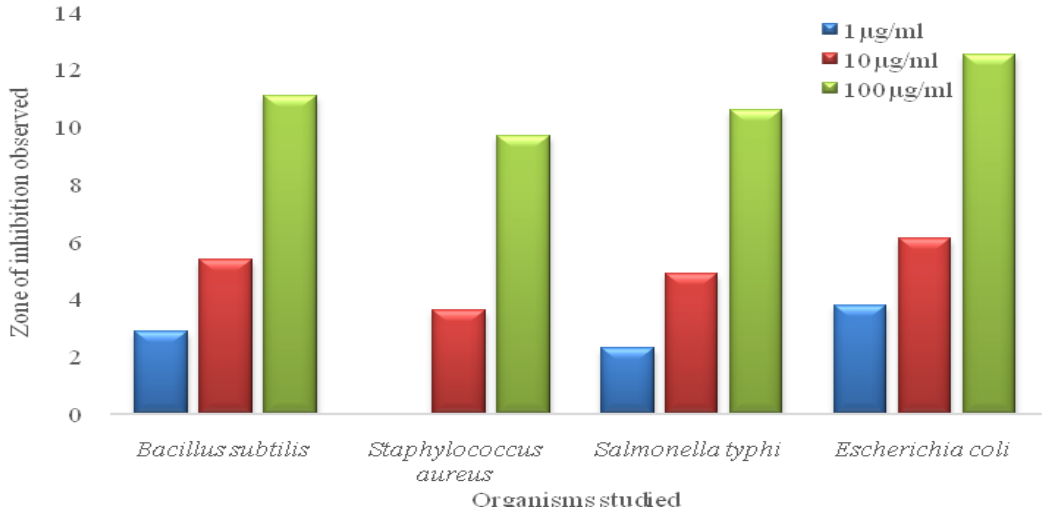

Fig.7: Comparative anti-microbial zone inhibition results of ZnO NPs

4. CONCLUSION

The investigation demonstrates a simple green synthetic approach for the synthesis of ZnONPs using aqueous *B. gymnorrhiza* leaf extract as a biological reducing agent. The leaf extract was found to comprise of significant amounts of terpenoids, polyphenols and flavonoids. These components effectively act as reducing agents and lead to the synthesis of ZnONPs. The ZnO nanoparticles were found to have the hexagonal wurtzite structure with absorption maximum at 320 assigned to the intrinsic band-gap absorption. The EDX spectra confirms the zinc content in the synthesized nano particles was found to be 58.97% whereas the SEM confirms that the average crystallite size of the synthesized nano particles was found to be 38-97 nm. The synthesized nano particles were found having potent anti-oxidant and anti-microbial activities. The study successfully demonstrates that facile synthesis of multifunctional ZnO nanoparticles was achieved using underutilized naturally occurring plant parts.

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