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PLANT MEDIATED SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING *Acalypha Fruticosa Forssk* COMBINED WITH SPECIAL REFERENCE TO ANTIBACTERIAL

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ABSTRACT

The use of medicinal plant extract in the manufacture of metal oxide nanoparticles is a viable alternative to the traditional chemical technique. This study used a green technique to synthesis zinc oxide nanoparticles from *Acalypha fruticosa forssk* leaf extract, which is an endemic medicinal plant growing in home gardens across India. UV-DRS, FTIR, SEM with EDAX, and IR are some of the techniques used. The antibacterial efficacy of the produced ZnO nanoparticles was further tested against clinical and standard strains of *E. coli, S. aureus, and P. aeruginosa*.

Keywords: *Acalypha fruticosa forssk*, Zno, Nano particle, Antibacterial, Plant

INTRODUCTION

Biosynthesis of metal nanoparticles is one of the most modern and dynamically active in the field of pharmaceutical science which is simplest and ecofriendly method due to have safer and cost-effective alternative to other chemical and physical methods **[1, 2]**.

Natural plant extracts are promising for the production of metal nanoparticles due to possessing secondary metabolites as reducing agents as well as stabilizing agents **[3]**. The natural plants are easily available and offer simplicity. Generally, plant extract contains number of antioxidants which includes polyphenols, proteins, carbohydrates, amino acids etc which can reduce the metal ions to metal salt solution **[4]** which can leads to the formation of nanoparticles. Biosynthesis of different metal oxide nanoparticles were carried out by using many plant extract which includes *Hordeum vulgare, Rumex acetosa, Caesalpinia bonducella, Nerium oleander* etc, **[5-7]**. Metal oxide nanoparticles like CuO, MgO, NiO, TiO_2 etc were prepare by using natural origin to explore the superior biological activities **[8-10]**. ZnO is one of the essential semiconducting materials which has unique advantageous such ultraviolet resistant property, remarkable catalytic activity, high surface area and biocompatible **[11-12]**. In addition greener synthesis of ZnO nanoparticles has attracted in the field of biomedical application which is simplest and ecofriendly method **[13]**. The synthesized ZnO nanoparticles are found to have various therapeutic uses like antioxidant, anti-infalmmatory, antifungal, antidiabetic etc. **[14]** which were synthesized by using only single plant origins. However, they are not effective due to have lower biological activities. The combination of plant extract for the production of nanoparticles has attracted distinct advantages offering improve or synergetic biological activities **[15]**. This work is mooted out to synthesis the metal oxide nanoparticles using two natural sources such as *Acalypha fruticosa Forssk* and curcumin. Both plants are freely available and contain numerous bioactive ingredients such as vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids **[16, 17]**. The plants have traditionally hemotherapeutic agents due to produce the lower size particles. Green synthesis of metal oxides nanoparticles using *Acalypha fruticosa Forssk* plant extract and its antibacterial activity was reported **[18-20]**. Some of the works have been reported on the synthesis of metal oxide nanoparticles using curcumin which was tested for antibacterial activity against different pathogens **[21, 22]**.

In this study, ZnO nanoparticles were synthesized by using two plant extracts such as *Acalypha fruticosa Forssk* and curcumin. The synthesized nanoparticles were evaluated by using different analytical techniques such as UV-DRS, FTIR, SEM

with EDAX and TEM. Antibacterial activities was also investigated on *E. coli, S. aureus, and P. aeruginosa*.

MATERIALS AND METHODS

Synthesis of ZnO Nps

$ZnSO_4 \cdot 7H_2O$ (0.4 M) and NaOH (0.8 M) were dis- solved in distilled water. Co-precipitation of ZnO nanoparticles was achieved by adding NaOH dropwise to $ZnSO_4$ $7H_2O$ with steady stirring at 80 °C until a white precipitate appeared. The precipitate was washed multiple times with deionized water, then ethanol, before being dried in a hot air oven for 5 hours at 80°C. To obtain nano-sized ZnO, the dried samples were calcined at 350 °C for 3 hours.

Characterization Methods

A JASCO V-550 twin beam spectrophotometer with PMT detector was used to measure Ultra Violet-Visible-Diffuse reflectance spectroscopy (UV-Vis-DRS). The samples were placed in a quart's cuvette with a 1 cm light-path length, and the light absorption spectra were calculated using deionized water as a reference. The JSM-6701F-6701 device was used to take SEM pictures in both back scattered electron and forward scattered electron modes. The elemental analysis was identified using an energy dispersive X-ray spectroscopy (EDX) coupled to the SEM.

Antimicrobial Activity

ZnO was investigated for antibacterial activity against three bacterial strains: *E. coli, S. aureus, and P. aeruginosa*. The samples were dissolved in dimethyl sulphoxide, which was then concentrated to the appropriate levels. By employing the well diffusion method, the bacterial and fungal strains were inoculated separately in 30 mL of nutrient broth in a conical flask and incubated for 24 hours to obtain active strain. Separate petri dishes were filled with Muller Hinton agar. After solidification, 0.25 mL of test strains were inoculated separately in the media, with special attention paid to homogenization. The experiment was carried out in an aseptic environment. The test sample (40 L) was poured into the well, and the plates were incubated at 37 °C for 72 hours. Triplicates of each sample were tested. Microbial growth was measured by the diameter of the zone of inhibition.

RESULTS AND DISCUSSION

FT-IR analysis

The presence of functional groups in the synthesised samples was determined using FTIR spectroscopy. **Figure 1** shows the FTIR spectra of pure and Acalypha fruticosa Forssk aided zinc oxide samples. The absorption peak at 642 cm^{-1} is the characteristic stretching vibration of ZnO

originating from the Zn and O bond **[23]**. The peak at 1630 cm^{-1} is due to the OH group which is ascribed to water molecules **[24]**. In addition to that, Broad peaks at 693.45 cm^{-1}, 964.54 cm^{-1}, 1062.37 cm^{-1}, 1411.89 cm^{-1}, 1442.75 cm^{-1}, 1562.34 cm^{-1}, indicated the presence of the hydroxyl group, an aromatic group, amine group, saturated primary alcohol. Also the sharp peak at 2695 cm^{-1} is related to O-H stretching vibrations **[25]**. The findings show that phenolic chemicals, terpenoids, or proteins adhered to the surface of ZnO nanoparticles, which could have come from the *Acalypha fruticosa Forssk* plant extract. Moreover the proteins present in the medium might also prevent the agglomeration and aids stabilization on forming a cover on the metal nanoparticles. The metal oxygen frequencies found for each metal oxide are consistent with published values. The phenolic compounds, flavonoids, and other biological molecules included in the plant extract and curcumin can activate the decrease of zinc salt and influence the size of produced Nps, according to the results of FT IR experiments **[3]**.

Diffused reflectance spectroscopy

The absorption spectrum of pure ZnO, ZnO/*Acalypha fruticosa Forssk* and ZnO/*Acalypha fruticosa Forssk/curcumin* nanoparticles were shown in **Figure 2** which was evaluated by the UV-Vis spectroscopy to find the optical properties of zinc oxide nanoparticles. Pure ZnO and *Acalypha fruticosa Forssk* assisted ZnO nanoparticles showed a characteristic absorption bands at 359 nm and 352 nm respectively **[26]**. The ZnO/*Acalypha fruticosa Forssk*/curcumin showed a band at 350 nm. It is observed that the *Acalypha fruticosa Forssk* assisted ZnO nanoparticles which are having blue shift compared to the pure ZnO. This may be attributed to the quantum size effect and suggests that the addition of *Acalypha fruticosa Forssk* significantly affects the particle size and therefore the absorbance properties have been changed. On the other hand, sample without *Acalypha fruticosa Forssk* and curcumin shows a red shift of the UV–Visible absorption which can be attributed to the larger particle size **[27]**.

SEM with EDX analysis

SEM with EDX results was employed to explore the surface morphology and metal present in the synthesized samples. **Figure 3** shows the surface morphology of pure ZnO, ZnO/*Acalypha fruticosa Forssk* which was investigatd by SEM analysis with X55,000 magnification. Image a clearly showed flower-like morphology whereas in Fig. 4b and 4c revealed sheet-like morphology. There was no agglomeration observed due to

protein present in the *Acalypha fruticosa Forssk* as a stabilizing agent around which can prevent the particles formed from aggregating and making them more dispersible by acting as a bio template **[29]**. Meanwhile the morphology sizes of the all samples were different and confirmed the average sizes about 100 nm.

The surface of the ZnO/*Acalypha fruticosa Forssk* has slightly rougher than that of the pure ZnO, which might be due to the void between the particles. In addition to that, it seen that sheet-like structures were made up of many tiny ZnO nanoparticles in **Figure 3**. The SEM results clearly indicated that the *Acalypha fruticosa Forssk* and have an impact on the morphology and particle size of zinc oxide. Fig.4d shown the energy dispersive X-ray analysis (EDAX) spectrum of *Acalypha fruticosa Forssk* and assisted ZnO sample. The expected stoichiometric mass percent of zinc and oxygen is 72.96% and 27.04% respectively. The EDX results showed that the samples were mostly made up of Zn and O, with no other impurities, and they agreed with the XRD results **[30]**. The weak signals in EDX spectra may be due to the presence of macromolecules such as alkaloids, flavonoids and phenolic compounds in *Acalypha fruticosa Forssk* extract **[31]**.

Antibacterial Activity

The antibacterial activities of pure ZnO, ZnO/*Acalypha fruticosa Forssk* and ZnO/*Acalypha fruticosa Forssk*/curcumin nanoparticles were tested against five bacterial strains such as, *S. aeureus*, *M. luteus*, *P. vulgaris*, *E. coli* and *P.aeruginosa* at various concentrations such as 10 µg/mL, 20 µg/mL, 30 µg/mL, and 40 µg/mL as shown in **Figure 6(A-C)**. The results for all the samples are represented in **Tables (1-3)**. The analysis was carried out using positive control Chloramphenicol as standard. The maximum zone of inhibition (13 ± 0.31 mm) of ZnO is absorbed against *E. coli* and minimum zone of inhibition (9 ± 0.15 mm) is observed against *S. Aureus*. The size of the inhibitory zones varies with type of pathogens and the concentration of the samples. The maximum antibacterial activity (15 ± 0.85 mm) of ZnO/*Acalypha fruticosa Forssk* is absorbed against *E. coli* and minimum activity (13 ± 0.15mm) is observed against S. *aureus*. The antibacterial activity has increased for increasing sample concentration of 40 µl. The maximum zone of inhibition (17 ± 0.18 mm) is obtained for ZnO/*Acalypha fruticosa Forssk*/cur nanoparticles against *E.coli* and minimum activity is obtained (15 ± 0.07 mm) against S. *aureus* **(Figure 4)**. The observed value of

inhibition is in good agreement with earlier studies **[33]**.

From the observations it is seen that *E.coli* is highly sensitive to ZnO nanoparticles due to its high lipopolysachride and thick peptide glycane. The antibacterial activity of ZnO/*Acalypha fruticosa Forssk* nanoparticles has been originate to be due to a reaction of the ZnO surface with water and production of elevated levels of reactive oxygen species, namely hydroxyl radicals and in turn induces oxidative pressure. In turn, bacteria exposed to tiny ZnO nanoparticles experienced enhanced cellular internalisation of the nanoparticles as well as bacterial cell damage **[34]**. The enhanced activity is attained for ZnO/*Acalypha fruticosa Forssk* nanoparticles due to the strong attack on the surface of the cell wall and slowly penetrated into the inside cell consequently kill the bacteria. Since the ZnO/*Acalypha fruticosa Forssk* nanoparticles has smaller particles size (22.4 nm) thus made it easy to penetrate the cell wall. The less effect against of *S. aureus* due to the presence of thick layers of peptidoglycans present on the cell membrane **[35]**.

The zinc oxide nanoparticles apprehended antibacterial activity that the surface of nanoparticle could easily form a layer of water, thus zinc oxide could be released into the water. The main composition of bacteria cell-membrane is phospholipid bilayers and the protein molecules and the phosphate in phospholipid molecules owned negative electricity, important the entire cell-membrane to be negatively charged. Furthermore, the antibacterial activity of the zinc oxide nanoparticles mostly appeared on the surface bind with the thiol (-SH) groups of protein present in the cell wall. This interaction decreases the cell permeability which leads to cell lyses **[36-38]**.

In the case of Gram (−ve) bacteria, the cell membrane is easily broken as the peptidoglycon layer is thinner, but the Gram (+ve) bacteria have thick peptidoglycon layer and hence, toxicity towards Gram (+ve) cell is significantly less **[39]**. The presence of flavonoids, phenolic compounds, and tannins in biosynthesized ZnO nanoparticles compared to those synthesised by chemical methods, cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes, and microbial adhesions are all responsible for the enhanced antibacterial activity of biosynthesized ZnO nanoparticles **[40]**.

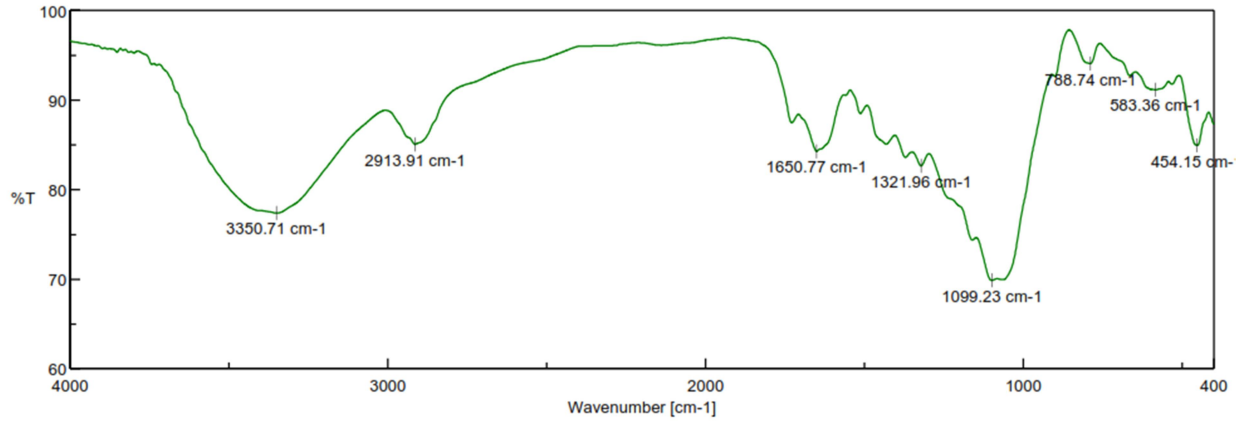

Figure 1: FT-IR spectrum of ZnO/*Acalypha fruticosa Forssk* nanoparticles

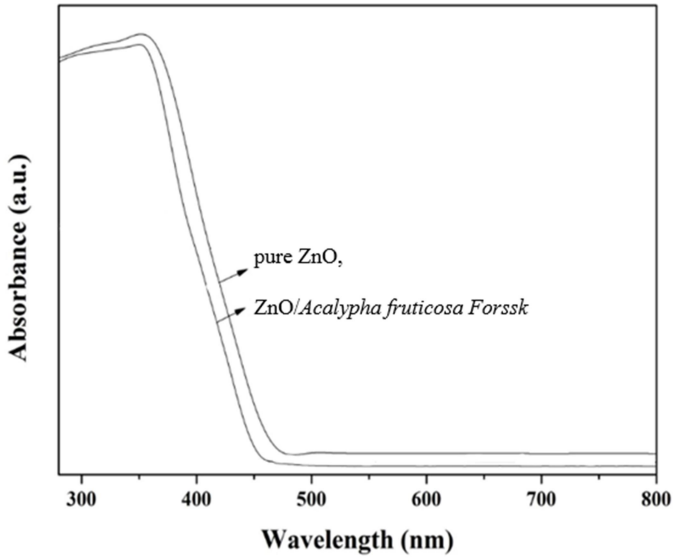

Figure 2: Diffuse reflectance spectra of pure ZnO, ZnO/*Acalypha fruticosa Forssk* and ZnO/*Acalypha fruticosa Forssk*/curcumin nanoparticles

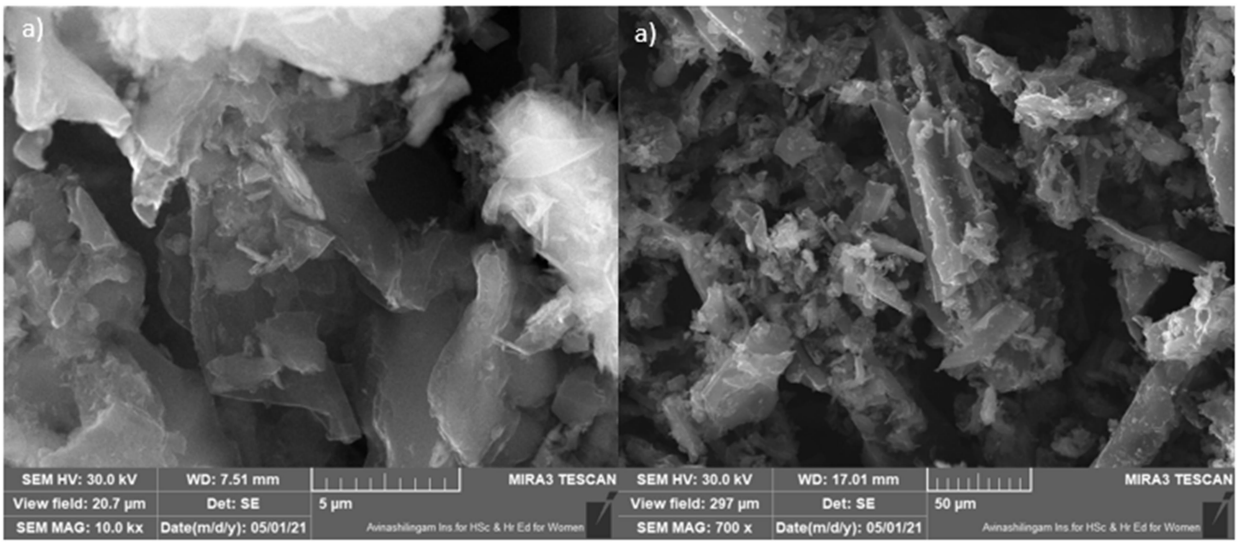

Figure 3: SEM images of Pure a) ZnO and b) ZnO/*Acalypha fruticosa Forssk* nanoparticles

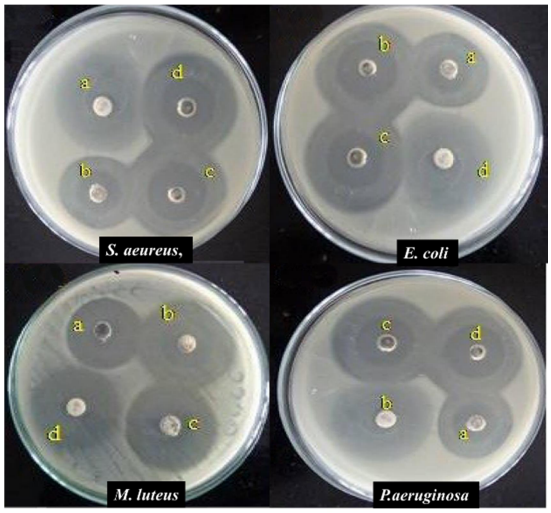

Figure 4: (B) Antibacterial activity of (a) ZnO (b) ZnO/*Acalypha fruticosa Forssk* (c) ZnO/*Acalypha fruticosa Forssk/curcumin* and (d) control against Gram (–ve) bacterial strains

CONCLUSION

Zinc oxide nanoparticles were successfully synthesized by greener method using *Acalypha fruticosa Forssk* and curcumin plant extracts and the formation of zinc oxide nanoparticles are confirmed by XRD, FTIR, UV-vis, SEM with EDX. The 30 µg/mL of curcumin loaded *Acalypha fruticosa Forssk* assisted ZnO nanoparticles showed superior antibacterial activity against two pathogens such as, *E.coli* and *P. aeruginosa*. This suggests that the ZnO/*Acalypha fruticosa Forssk*/ sample can be used as an effective commercial antibacterial agent for the detection and destruction of bacterial strains**.**

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