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Aegle marmelos phytochemical stabilized synthesis and characterization of ZnO nanoparticles and their role against agriculture and food pathogen

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Abstract: Nature and nanotechnology have not yet achieved a lucid correlation in the field of science but together they have exhibited immense potential towards the advancement and modification in future science and technology. Due to the unique properties of nanomaterials like nanoparticles gained considerable importance. Among all the nanoparticles, zinc oxide (ZnO NPs) are greatly utilized in sensors, catalysis, bioscience, etc. Antimicrobial activity by ZnO NPs had more attention by the implementation of nanotechnology for the preparation of ZnO NPs. At present, antimicrobial activity of ZnO NPs using *Aegle marmelos* (*A. marmelos*) leaves extract was done. The ZnO NPs subjected to UV-Vis, FT-IR, XRD, SEM and TEM analysis. The presence of phenolic group in extract has the capacity to form ZnO NPs and act as stabilizing agent. Every 5 min interval ZnO NPs was formed which recorded by UV-Vis spectrophotometer. The SEM analysis displayed a fine spherical ZnO NPs and EDAX report showed that the existence of zinc and oxygen in the ratio of 30.51% and 69.49%. The antimicrobial activity of ZnO NPs has high percentage inhibition against *A. niger* at 1000 ppm. Till now, no research carryout on *A. marmelos* mediated ZnO NPs and applications towards antimicrobial activity.

Keywords: *Aegle marmelos*; zinc oxide nanoparticles; antimicrobial activity; food pathogens

List of abbreviations

ZnO NPs	Zinc oxide nanoparticles
UV-Vis	Ultra violet visible spectroscopy
FT-IR	Fourier-transform infrared spectroscopy
XRD	X-ray powder diffraction
SEM	Scanning electron microscope
TEM	Transmission electron microscopy
EDAX	EDAX offers Energy Dispersive Spectroscopy
ppm	parts per million
°C	Degree Celsius
mM	Millimolar
min	Minutes
mg	Milligram
mL	Millilitre
h	Hours
µL	Microliter
mm	Millimeter
nm	Nanometer
$FeCl_3$	Iron III chloride
JCPDS	Joint Committee on Powder Diffraction Standards
θ	Theta
cm	Centimeter
SAED	Selected area (electron) diffraction
%	Percentage

1 Introduction

The science behind the nano-sized materials is dealt with tiny particles which were used across all the fields of sciences including synthetic chemistry, biological sciences, electric and electronic engineering, etc. [1-3]. Today's researchers are finding a wide assortment of approaches to purposely synthesis materials in nano-size to increase their properties in various aspects such as biological activities, optical property, mechanical strength, magnetic property, etc. [4]. The nano-sized particles can be effectively integrated using various techniques with

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different methodologies accessible for the synthesis of nanoparticles which include chemical, electrochemical, radiation, Langmuir-Blodgett, and biological techniques. The synthetic methods for the preparation of nanoparticles include the use of toxic, hazardous chemicals which are harmful and lead to increase the risks of bioaccumulation followed by biomagnifications. This enhances the need for an eco-friendly method to synthesis nanoparticles. The extract mediated nanoparticles are one of the easy, safer and non-toxic methods which have gained more interest towards environmentally conscious products, synthetic chemistry, natural products, etc. [5-16].

The metal and metal oxide nanoparticles have been considered as promising materials that possess remarkable antimicrobial properties caused by their high surface area [17-24]. The ZnO NPs have a long history of usage for antimicrobial activity and still have gained interest in antimicrobial studies. The microbes such as bacteria and fungi must be controlled because it causes infection to human and also contaminates in the water source. The microbes can also contribute to several non-infectious chronic diseases including cancer and heart diseases (Figure 1). To address the problem caused by microbes, ZnO NPs is one the key which can leads to cell death of microbe in a non-toxic way to the environment. Hence, this study we have taken *Aegle marmelos* (*A. marmelos*) leaves to synthesis ZnO NPs and further applied for antimicrobial activity.

The *A. marmelos* is one of the traditional plants belongs to Rutaceae family and have been used to cure ophthalmia, deafness, aggravations, catarrh, diabetes, and asthmatic dissensions. The natural products are utilized as a part of treating loose bowels, stomach hurt, and cardiovascular afflictions. Logical studies have approved huge numbers of the potential application of ZnO NPs such as antimicrobial impacts, hypoglycemic, astringent, antidiarrheal, antidysenteric, demulcent, pain relieving, mitigating, antipyretic, injury mending, insecticidal, photocatalytic and gastroprotective activities [25-33]. Still, there is no report found that synthesis of ZnO nanoparticles using *A. marmelos* and we have utilized to study the synthesis and antimicrobial activity of *A. marmelos* extract mediated ZnO NPs.

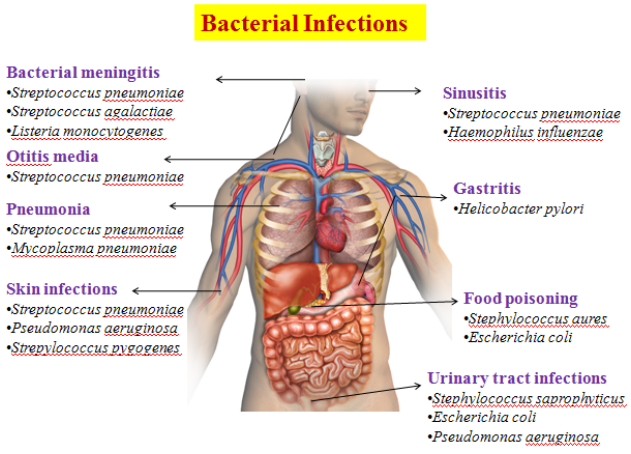

Figure 1: Bacterial infection on Human.

2 Materials and methods

2.1 Materials

The commercially available leaf powder of *A. marmelos* was procured from Vellore local market, Vellore, Tamil Nadu, India. The zinc acetate was purchased from AVRA chemicals, Hyderabad, India.

2.2 Conventional extraction process of *A. marmelos*

About 5 g of *A. marmelos* powder was used to extract the phytochemicals using distilled water (100 mL) further heated at 60°C on water-bath. The aqueous solution was taken for filtration using Whatman filter paper further evaporated on the water-bath. The extract was stored in the refrigerator for further use to synthesis ZnO NPs.

2.3 Conventional green method to synthesis *A. marmelos* mediated ZnO NPs

The green method of *A. marmelos* mediated ZnO NPs were synthesized as mentioned in our earlier report [33] with slight modifications. In brief, 10 mg of extract was mixed with 1 mM of zinc acetate and heated over the water-bath for 30 min. The UV-Vis spectrophotometer utilized to screen the preparation of ZnO NPs at 5 min interval.

2.4 Analytical technique adopted for ZnO NPs

The progress of ZnO NPs formations was recorded at a wavelength of 200-800 nm by using Shimadzu UV-1800 PC, Japan. The crystalline nature was analyzed using Brucker, Germany D8 X-ray diffractometer. The functional analysis was carried out using Alpha T, Brucker, Germany instrument. The Hitachi H-7100 SEM voltage of 120 kV with the parameters to be set as EHT 19.79 kV, extractor V4.4.0 kV

was utilized to identify the shape of ZnO NPs. The carbon coated grid and JEM-1230, JEOL, USA TEM instrument was used to identify the nanosize of the ZnO NPs [33].

2.5 Antimicrobial efficacy

2.5.1 In vitro antibacterial activity

The green synthesized ZnO NPs was applied for antibacterial activity against *Escherichia coli (E. coli)*, *Bacillus cereus (B. cereus)*, and *Proteus vulgaris (P. vulgaris)*. The antibacterial activity was done using disc diffusion method [34-39] with slight modification. About 25 µL of ZnO NPs was poured in 7 mm well and ampicillin used as a standard. The Petri plates were incubated at 37°C for 24 h. Each experiment was done in triplicates and measured zone of inhibition.

2.5.2 In vitro antifungal efficacy

The antifungal efficacy of ZnO NPs was carried out on *Aspergillus flavus (A. flavus)* and *Aspergillus niger (A. niger)* as reported by Sompalle et al. (2016) [40,41] with slight modification. About 500 ppm and 1000 ppm of ZnO NPs was used to determine the antifungal activity. The potato dextrose broth (10 mL) mixed with 1 mL of fungal strain and place in an incubator at 37°C for 3 days at constant stirring (180 rpm) on the shaker for complete aeration. The percentage inhibition was calculated using the following Eq. 1.

$$[(\text{Control weight} - \text{Sample weight})/\text{Control weight})] \times 100 \quad (1)$$

3 Results and discussion

3.1 Observation of ZnO NPs formation

The absorption spectra were monitored against water to observe formation and stability of ZnO NPs. The turbid formation change in the mixture of plant extract and Zinc acetate is recorded by visual observation. The surface plasmon absorbance bands were monitored every 5 min interval and the optimum time interval was 30 min to get a broad peak at 250-350 nm [42,43]. The white color turbid was formed 5 min due to plasmons at the colloid surface that confirmed the development of ZnO NPs

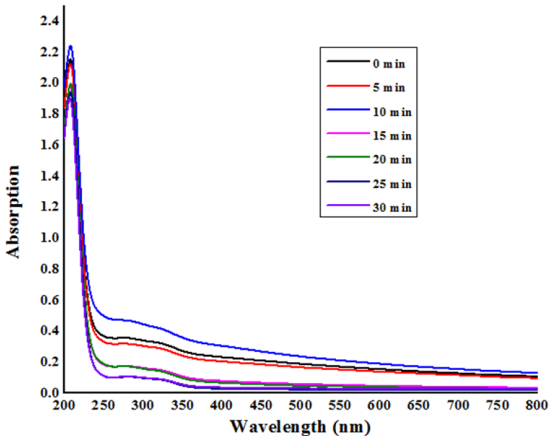

Figure 2: Observation of ZnO NPs formation by UV-Vis spectroscopy.

(Figure 2). The same peak was obtained at another each 5 min interval and found to be 30 min is an optimum time to get a broad spectrum. The phytochemical including alkaloids, phenolic, and flavonoids of *A. marmelos* might be a capping agent for ZnO NPs synthesis. The qualitative analysis reported by Mujeeb and co-workers was found that phenolic content was high in aqueous extract of *A. marmelos* [44]. Here we confirmed the presence phenolic compounds in *A. marmelos* using preliminary test for phenols. Minimum amount of the aqueous extract of *A. marmelos* added with the 1 to 2 drops of Iron III chloride ($FeCl_3$). An intense green colour was obtained. This confirms the presence of phenolic compounds.

3.2 Determination of crystalline nature

The crystallinity of ZnO NPs had an intense peak were obtained in XRD at 2θ values of 31.65°, 34.21°, 36.30°, 47.67°, 56.50°, 62.90°, 67.55° and 69.10° which are corresponding to the (100), (002), (101), (102), (110), (103), (200) and (201) planes respectively (Figure 3). This was agreed with the hexagonal structure and agreed with Joint Committee Powder Diffraction Standard data (JCPDS: 36-1451). The extra peaks in XRD may due to the capping and stabilizing agent present in the extract [45,46].

3.3 Functional group analysis

The functional group of capping and a stabilizing agent was analyzed by Fourier transmission infrared (FTIR) spectroscopy. The FTIR result of extract showed more peaks at 1082 cm 1which is corresponding to C-O stretching

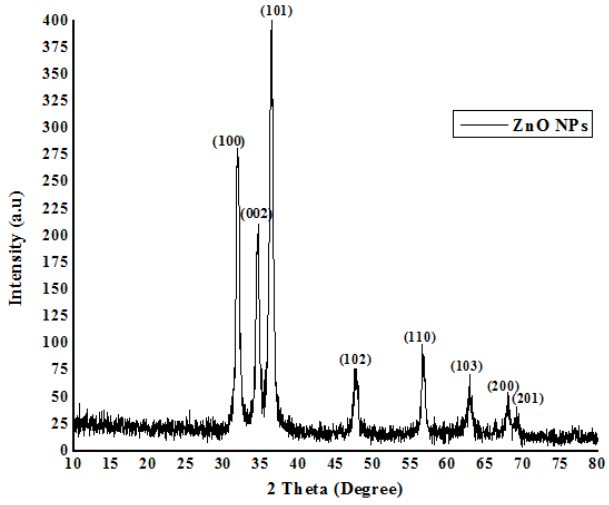

Figure 3: XRD pattern for *A. marmelos* capped ZnO NPs.

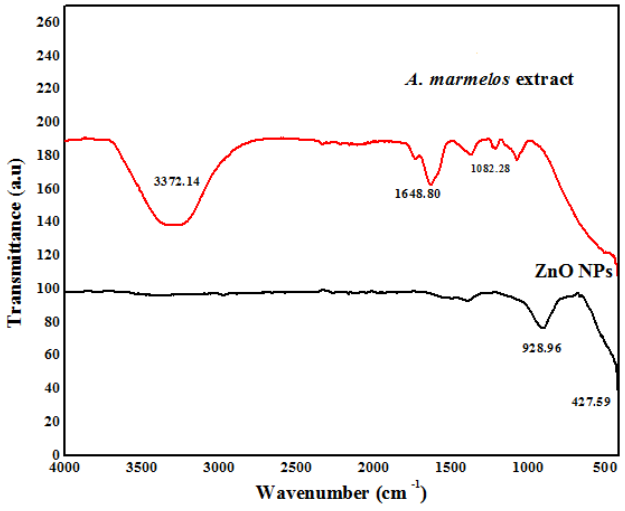

Figure 4: Functional group analysis of ZnO NPs.

of ester, 1648 cm^{-1} strong peak appeared for amide and a strong broad peak at 3372 cm^{-1} corresponding to O-H group of phenolic compounds (Figure 4). The FTIR result of ZnO NPs showed stretching and vibration peaks at 427 cm^{-1} and 928 cm^{-1} respectively. Therefore, after addition of Zinc acetate to extract the O-H peak at 3372 cm^{-1} was reduced and it can be seen in FTIR of ZnO NPs. It can be concluded that phenolic group present in the extract are responsible for the synthesis of ZnO NPs formation [47,48].

3.4 Morphology study by SEM and TEM

The structures of ZnO NPs were analyzed using scanning electron microscope (SEM) and displayed a spherical

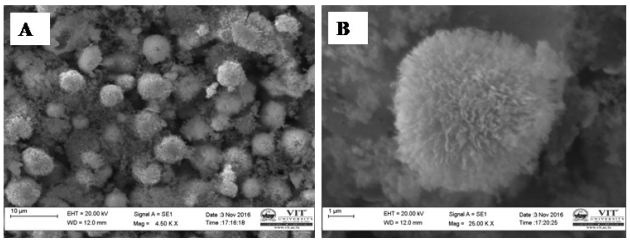

Figure 5: (a,b) Morphology study of ZnO NPs by SEM analysis.

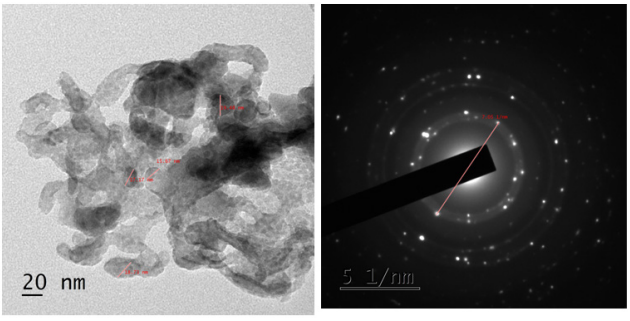

Figure 6: TEM and SAED pattern of ZnO NPs.

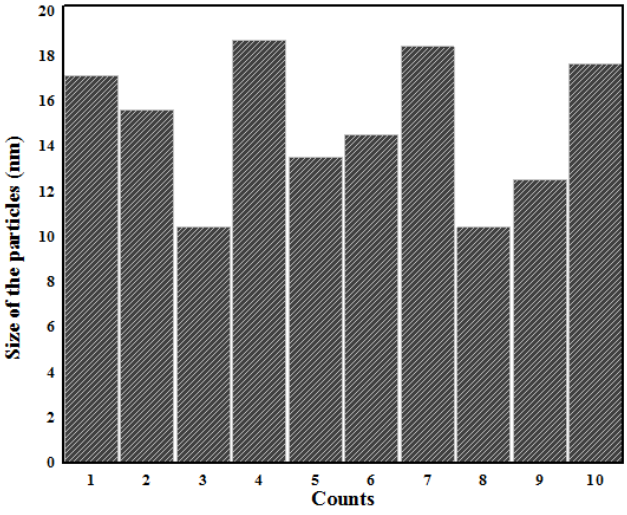

Figure 7: Particle size distribution of ZnO NPs.

shape in SEM. The spherical shaped contains an irregular shape of crystal on the surface of Spherical shape and agglomerated (Figure 5). The particles were aggregated but the distance between each spherical shape was found to be considerably high. The TEM images of synthesized ZnO NPs were the quasi-spherical shape and the average nanosize was 18 ± 2 nm (Figures 6 and 7). The SAED pattern obtained from TEM result reveals that the diffraction rings of ZnO NPs exhibited the same miller indices values assigned as (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3),

(2 0 0) and (2 0 1) respectively and the lattice planes of the face-centered cubic (fcc) indicates the nano-crystalline in nature.

3.5 Antimicrobial efficacy of ZnO NPs

The antifungal efficacy of ZnO NPs was done on *A. flavus* and *A. niger* fungus and antibacterial activity were done on *E. coli*, *B. cereus*, and *P. vulgaris*. From the obtained results, 1000 ppm concentration of ZnO NPs was very effective against *A. flavus* and *A. niger*. About 500 and 1000 ppm of ZnO NPs was showed 46.35% and 63.57% inhibition against *A. flavus* whereas 60.92% and 66.95% against *A. niger* (Figure 8). Therefore, *A. marmelos* mediated ZnO NPs was very effective against *A. niger* at 1000 ppm than standard fluconazole values 40.21%, 63.04% on *A. flavus* and 60.21%, 63.04% on *A. niger*.

The gram-positive (*B. cereus*) and gram-negative (*E. coli* and *P. vulgaris*) bacteria were tested for antibacterial activity of ZnO NPs by well diffusion method. The diameter of inhibition zones (mm) around well was measured and it was found to be 14 mm for *E. coli*, 12 mm for *B. cereus* and 16 mm for *P. vulgaris*. Therefore, the synthesized ZnO NPs has high antibacterial activity against *P. vulgaris* than other tested bacteria. The antibacterial efficacy of ZnO NPs was compared with antibiotic drug ampicillin. Still, the exact mechanism for bacteria death is not clear but researcher found that it may due to electrostatic interaction of ZnO NPs on bacteria membrane [49-55]. Further, the nanotoxicity of ZnO NPs can form oxidative stress which leads to cell death (Figures 9 and 10). Generally, the gram-negative microorganisms are susceptible to cell damage than gram-positive bacteria. Therefore, the ZnO NPs were showed high cell death on gram-negative bacteria *P. vulgaris* and *E. coli* than gram-positive bacteria *B. cereus*. On the basis of this research, it can be concluded that the inhibition formed by ZnO NPs could be attributed to cell damage resulting in the death of bacterium.

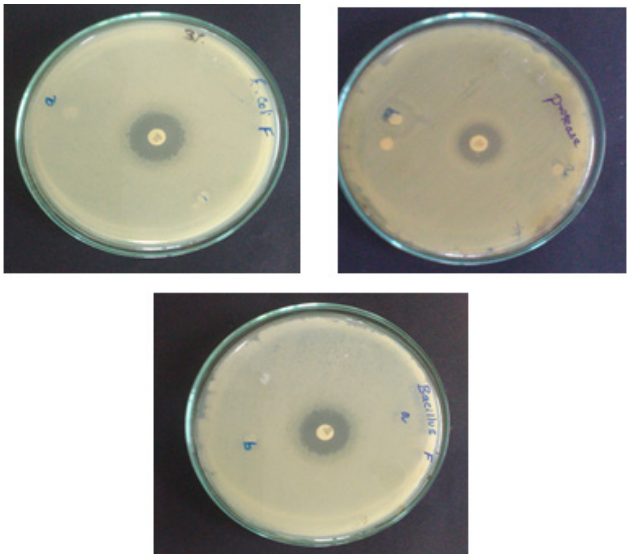

Figure 9: Antibacterial activity of ZnO NPs.

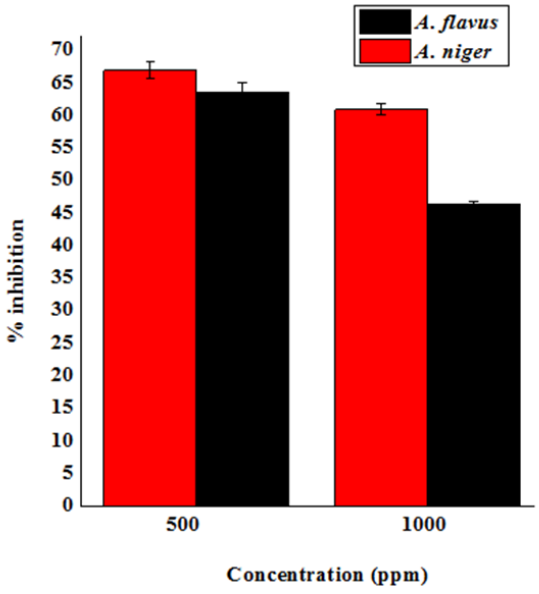

Figure 8: Antifungal potential of ZnO NPs against *A. flavus* and *A. niger*.

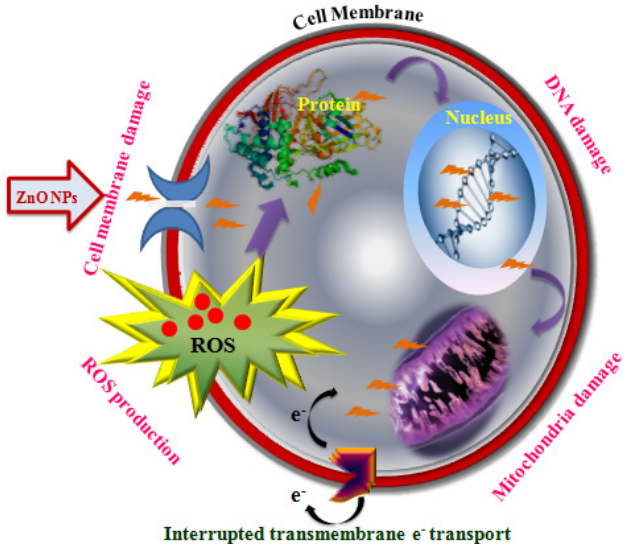

Figure 10: Possible mechanism for antibacterial activity.

4 Conclusion

The *A. marmelos* extract mediated ZnO NPs was synthesized in green method and has been described by UV-Vis spectrophotometer, XRD, FTIR and SEM analysis. The ZnO NPs showed a good spherical shape in SEM and FTIR analysis confirmed that phenolic compounds have the capacity as capping and stabilizing agent in ZnO NPs synthesis. The antifungal potential of ZnO NPs showed high inhibition against *A. niger* at 1000 ppm. Therefore, this green method is one of the eco-friendly, economical and effective process to synthesis ZnO NPs and it may lead to the further study on *A. marmelos* in the area of biomedical and nanotechnology.

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