

Aloe Vera Mediated Green Synthesis of ZnO Nanostructure under Sol-gel Method: Effect of Antimicrobial Activity

Seifunnisha O., Shanthi J.*

Avinashilingam Institute for Home Science and Higher Education for Women, 641043 Coimbatore, India

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Synthesis of nanoparticles from plant extract does not require any harmful chemicals for the preparation process. Green synthesis of nanoparticles is one of the non-toxic, cost-effective and as an alternative approach compared to physical and chemical methods. The present study reveals a simple and effective method for the preparation of nano composite by ZnO. Here, a green sol-gel method has been reported for the preparation of ZnO nano-composite using Aloe vera gel as a capping agent. The structural morphological and antimicrobial activity of as synthesized nanoparticles was studied. The infrared studies were carried out to validate the presence of phytochemicals in the extract and also the purity and nature of the nanoparticles. Finally antimicrobial activity efficacy was evaluated by agar well diffusion method against bacterial strains of E.coli, Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus and fungal strains of Aspergillus niger and Aspergillus flavus. It was observed that the green synthesized ZnO nanoparticles have better antimicrobial activity compared to pristine ZnO. These findings are gives new avenues for the synthesis of ZnO nanoparticles from plant extract as eco-friendly coating for Antimicrobial activity.

Keywords: Aloe vera, ZnO, Well diffusion method, FTIR.

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1. INTRODUCTION

In recent years nanomaterials are created good impact due to its outstanding (optical, electrical, mechanical and magnetic) properties [1]. It has variety of applications in various fields like catalyst, flat panel displays, solar energy converters, optical amplifiers, thermal barrier coatings and so on [2]. The metal and metallic oxides have created great attention in last decades due to their extraordinary properties. ZnO nanoparticles is a promising photocatalytic and antimicrobial agent [3, 4]. ZnO nanoparticles have antimicrobial activity against Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, M13 bacteriophages, and skin-specific pathogens with and without UV irradiation [5]. Recently, biosynthesis of nanoparticles via eco-friendly processes has attracted more attention due to the several advantages over the conventional chemical and physical synthesis techniques [6].

Recently biosynthesis of nanoparticles are concentrated more compared to the physical and chemical synthesis methods because of the several advantages like biocompatible, non-toxic and eco-friendly [6]. There are thousands of plants are used for the preparation of nanoparticles, but among various plants, Aloe vera is a most popular and commercially important plant in research field. Aloe vera is consists of 75 active nutrients and 200 active compounds including amino acids, enzymes, vitamins, minerals, anthraquinones, and polysaccharides. The flower and rind of the Aloe vera contains volatile compounds, ascorbic acid, polysaccharides, lignin, pectin and cellulose while organic acids, vitamins, minerals and enzymes are present in the leaves [7].

In some recent studies, there are more number of articles related to the synthesis of ZnO nanoparticles using plant extract have been reported. For instance,

antibacterial, self cleaning, UV protective and photocatalytic properties have been studied using ZnO nanoparticles [8]. The composite wound care devices are prepared by non woven cotton fabrics with the help of Aloe vera and Curcumin loaded oxidized pectin-gelatin matrices [9]. In another study, the removal different aquatic pollutants were discussed using Aloe vera waste bio-mass based sorbants (raw, treated, ash ad activated carbons) [10]. In another study, the antimicrobial activities of Aloe vera gel in acetone, ethanol and methol extract against E.coli, S.aureus, S.typhi and K.pneomonia were investigated using the agar well diffusion method. Synthesis of Silver and Iron nanoparticles with Aloe vera leaf extract and their antimicrobial activities against 9 reference bacterial strains were performed [11]. The objective of this work is to synthesize nanopartilces under sol-gel method using Aloe vera leaf extract and ZnO. And the functional properties of structural, morphological and antimicrobial activities are explored.

2. MATERIALS AND METHODS

2.1 Collection of Plants

The fresh and matured plant leaves of Aloe vera were collected from local area of Coimbatore District. The collected plants were washed several times with running tap water and double distilled water to remove the dust particles. The gel of the Aloe vera leaf was separated in a container and dried at 40 degree Celsius for two days by Hot air oven.

2.2 Preparation of Aloe Vera Extract

The dried Aloe vera gels were ground as fine powder using Agate Mortar and pestle. 5g of Aloe vera gel

* shanthinelson@gmail.com

powder was dissolved in 100 ml of DI water and stirred for 4 hours at room temperature followed by filtration process using centrifuge at 300 rpm for 15 min. The prepared Aloe vera extract was used for the synthesis of ZnO nanoparticles.

2.3 Synthesis of ZnO Nanoparticles

The prepared 60 ml of Aloe vera gel powder extract was added with 0.5 g of 1M (Merck, 99.9 %) zinc acetate under stirring for 1 and half hour at 70 °C. The obtained precipitate was washed several times with DI water and the powder is heated in a hot-air oven at 120 °C for 2 hours followed by the thermal treatment at 250 °C using Muffle furnace. The schematic representation of ZnO nanoparticles synthesis procedure is shown in Fig. 1.

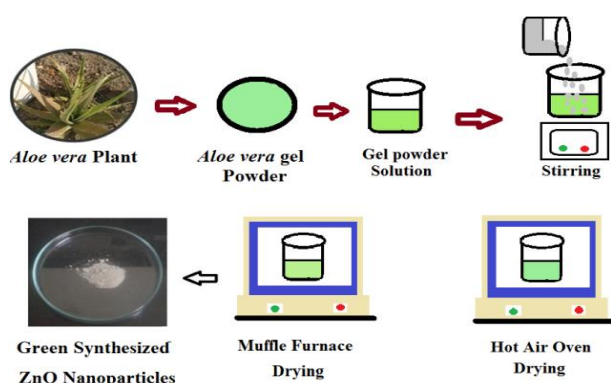


Fig. 1 – Schematic representation of ZnO nanoparticles

2.4 Selection of Standards and Culture Preparation

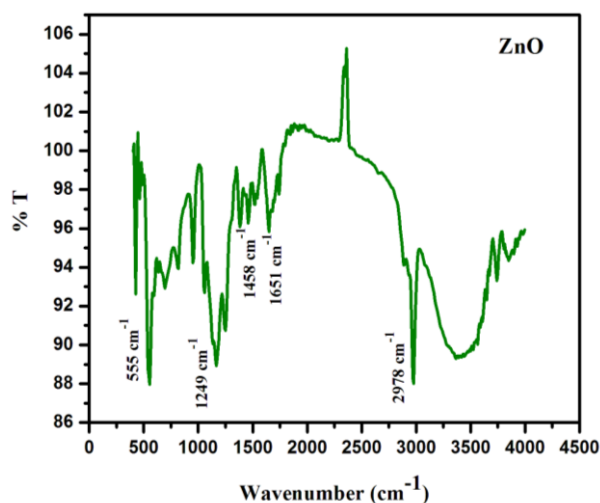
The antimicrobial activity of the ZnO and ZnO (Aloe) nanoparticles was studied by the well diffusion method. The streak plate method was used for the preparation of cultures. Culture plates were inoculated with different bacterial and fungal strains. 5mm cork borer was employed on the agar surface area of the wells. The sterile syringe was used to pour the extract. The plates were incubated at 37 ± 2 °C for 48 hours for fungal activity and 24 hours for bacterial activity. The Ciprofloxacin and Nistatin standards were used for bacterial and fungal activity measurement [12].

3. RESULTS AND DISCUSSION

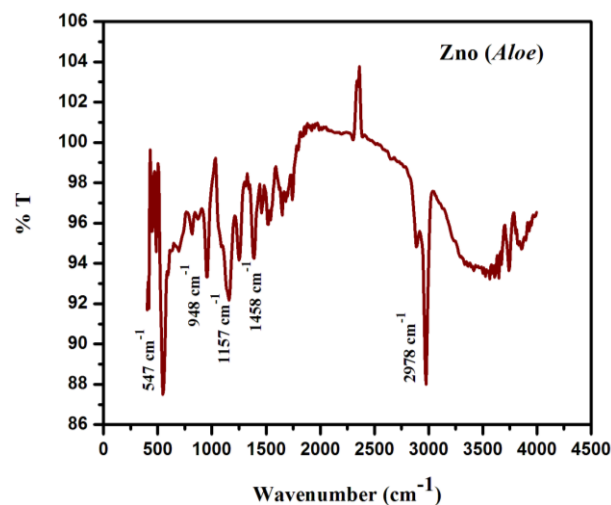
3.1 FTIR

The FTIR spectra for the ZnO and ZnO (Aloe) samples were recorded in the range of 400-4500 cm^{-1} as depicted in Fig. 2 (a, b). Infrared studies were used for the analysis of appropriated compounds in the chemicals, purity, and nature of the nanoparticles and also the presence of phytochemicals in the extract. The alcohols, phenols, amines, carboxylic acids, and other molecules can interact with the zinc surface and aid in the stabilizations of ZNPs. The peak that appeared between 3500 cm^{-1} and 3000 cm^{-1} confirms the presence of water molecules [13]. From ZnO (Aloe) Spectra, the characteristic peak at 1388 cm^{-1} corresponds to

aromatic C=C stretching vibrations. The peak at 813 cm^{-1} indicates the presence of C-H bond in the alkene group [14]. The vibration peak of 447 cm^{-1} corresponds to ZnO deformation vibration respectively. Generally, the presence of metal oxides is confirmed by the absorption peaks between 400 and 600 cm^{-1} [15]. However, the possible bio-molecules from Aloe vera might be responsible for the reduction of ZnO and capping agent of bio-reduced ZnO nanoparticles through particular bond vibrations peaks appearing at definite wave numbers was identified [16].



a



b

Fig. 2 – FTIR Spectra for ZnO nanoparticles (a) and FTIR Spectra of ZnO nanoparticles synthesized from Aloe vera (b)

3.2 Crystalline Structure

The structural characterization of the ZnO nanoparticles was confirmed by X-ray FTIR spectra for the ZnO and ZnO (Aloe) samples were recorded as a function of 2θ angle. All the diffraction patterns were performed in the range of 10°-80° with step size of 0.01° and a scanning rate of 0.02° steps/second using PANALYTICAL-PRO X-ray powder diffractometer. XRD Pattern of the ZnO and ZnO (Aloe) samples are shown in Figs. (3a)

and (3b). The following modified Scherer equation explains structure and size of the ZnO nanoparticles

$$D = K\lambda/\beta\cos\theta \quad (1)$$

where, D is the average crystallite size, λ is the full width half maximum (FWHM) of the 2 θ peak, K is the shape factor of the particles (it equals to 0.89), θ and λ are the incident angle and wavelength of X-rays respectively [16, 21]. XRD patterns of green synthesized ZnO nanoparticles using Aloe vera extract showed diffraction peaks at 2θ values 31.91°, 34.56°, 36.39°, 47.68°, 56.73°, 62.98°, 66.49°, 69.21°, 72.69° and 77.08° planes of hexagonal Wurtzite structure of ZnO nanoparticles. The plane values of XRD patterns are in good agreement with [JCPDS card no. 89-1397]. ZnO

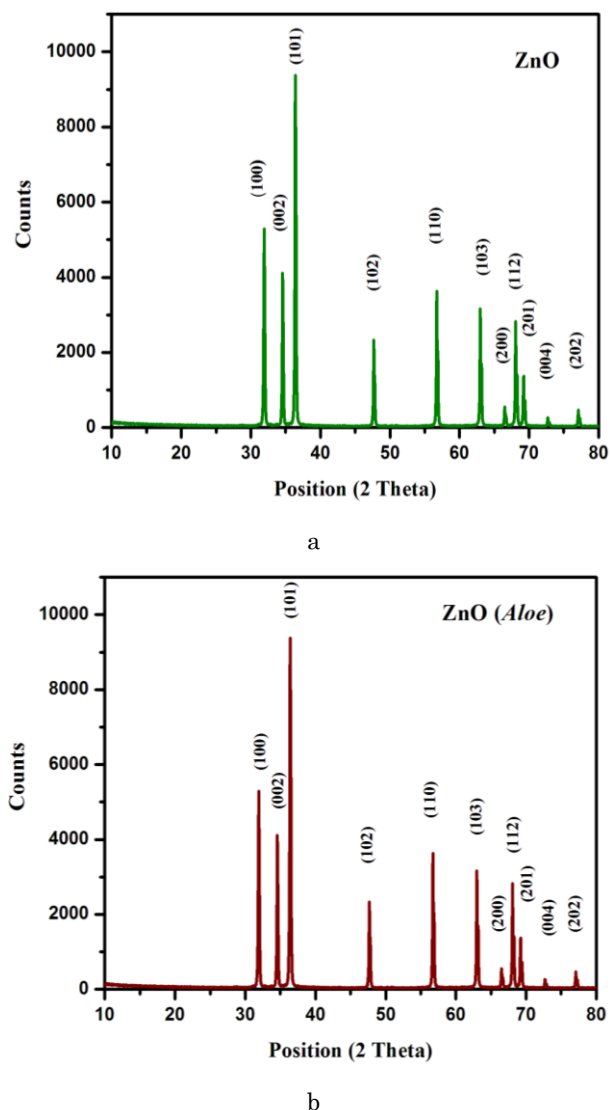


Fig. 3 – XRD Pattern for ZnO nanoparticles (a) and XRD Pattern for ZnO nanoparticles synthesized from Aloe vera (b) and ZnO (Aloe) nanoparticles shows similar peak intensity profiles without considerable shift in the peak position, characteristics of the hexagonal wurtzite structure. The stiff and narrow diffraction peaks are confirms the purity and good crystalline structure. And there is no other crystalline impurities are observed [14]. The crystallite size of the ZnO and ZnO (Aloe)

nanoparticles are found to be 35.89 nm and 25.64 nm respectively. The crystallite size of the ZnO nanoparticles between 10-20 nm may achieve by blending of other materials into the ZnO solution or by increasing temperature of drying. This will be our future goal of investigation. Thus, this study could be an effective one to generate the Green synthesized ZnO nanoparticles by sol-gel method.

3.3 Surface Roughness and thickness behavior

The surface roughness and thickness of the nanoparticles at the micrometer level are shown in Fig. (4a) and (4b), with a focus on the comparison between ZnO and ZnO (Aloe) nanoparticles. The roughness and thickness of nanoparticles using 3DLaser profilometry was found to be 26.06 μm and 76.82 μm for ZnO (Aloe) and 12.24 μm and 35.34 μm for ZnO.

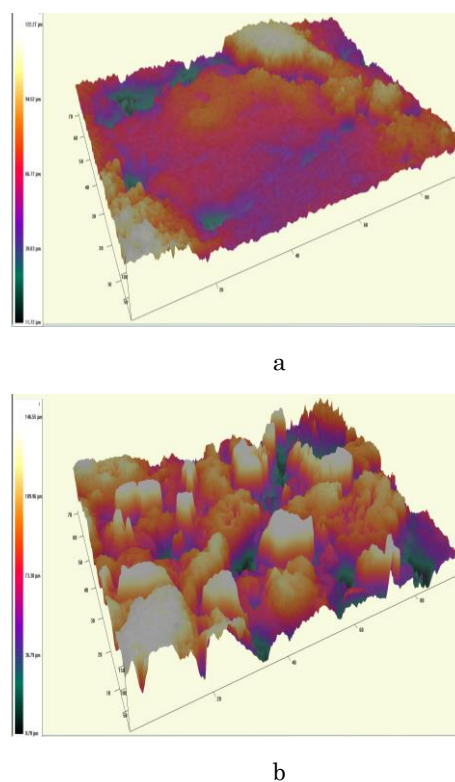


Fig. 4 – Surface Roughness of ZnO nanoparticles (a) and Surface Roughness of ZnO nanoparticles from Aloe vera

ZnO (Aloe) nanoparticles show a higher roughness and thickness compared to ZnO nanoparticles. The obtained result can be explained by the fact that by blending of Aloe vera extract in the mixture solution of ZnO [15, 18]. Crystalline size and hexagonal wurtzite structure of the ZnO and ZnO (Aloe) nanoparticles ensures the high purity of the nanoparticles and hence, lower crystalline size of the nanoparticles is observed to be improves the roughness and thickness of the surface.

3.4 Antimicrobial Study

The antimicrobial activity of ZnO and ZnO (Aloe) nanoparticles were tested against two Gram-ve (*E.coli*, *Bacillus subtilis*) and one Gram +ve (*S.aureus*) bacteria and two fungi (*A. niger* and *A. flavus*) by disc diffusion

method. The sterilization process was conducted in all discs using autoclave before the experiment. Muller Hinton Agar was used as sources for culturing bacterium at 37 °C on a rotary platform in an incubator. The colony of respective bacteria was picked by sterile wire loop and suspended in 5 mL of nutrient broth for the standardized bacterial suspension. The distilled water is used to dissolve ingredients and sterilized at 121 °C at 15 lbs for 15 min. Nutrient agar medium was prepared and plated aseptically into the sterile plates. The growing of single colony in nutrient broth at overnight is used for the preparation of bacterial inoculums and made a lawn culture using the sterile swab over the nutrient medium plates [14]. The extracts of ZnO, ZnO (Aloe) were used throughout the study. The zone of inhibition was calculated by measuring the diameter (mm) around the well. The zone of inhibition was measured by three different fixed directions in all the replicates, and average values are tabulated in Table 1.

Table 1 – Antimicrobial activity of ZnO and ZnO (Aloe)

No	Micro Organisms	Zone of Inhibition (mm)		
		ZnO	ZnO (Aloe)	Control
1	Escherichia coli	11.33 ± 0.3	14.16±0.20	27.1±0.17
2	Staphylococcus aureus	21.2 ± 0.26	28.06±0.11	32.13±0.15
3	Bacillus subtilis	17.33 ± 0.30	19.23 ± 0.25	34.03 ± 0.05
4	Aspergillus niger	12.23 ± 0.25	14.03 ± 0.05	10.02 ± 0.04
5	Aspergillus flavus	9.1 ± 0.10	11.2 ± 0.20	10.06 ± 0.11

The well loaded with ZnO (Aloe) nanoparticles show the maximum zone of inhibition against selected pathogens. In contrast, ZnO nanoparticles show lesser inhibition zone against selected pathogens. The above assessments shows the green synthesized ZnO has higher antimicrobial activity, which is ascribed to the fact that the presence of phytochemicals such as vitamins, enzymes, minerals, sugars, salicylic acids, lignins, saponins, amino acids and anthraquinones present in plant leaf extracts which is confirmed by the Fourier Transform Infrared analysis. Indeed Aloe vera leaves are reported to contains 200 active components of the major components of anthraquinones (aloin, anthranol and aloetic acid) with vitamins (B1, B2, B6, cholinefolic, acid ascorbic, b carotene and so on) [7, 9, 16]. Hence, the addition of Aloe vera into ZnO increases the degree of contact with the bacteria with high surface area

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which result in the formation of wide zone of inhibition [8]. In the current study, ZnO (Aloe) nanoparticles showed a maximum ability to inhibit the growth of the resistant against Staphylococcus aureus with inhibition zone (28.06 ± 0.11) comparing with ZnO nanoparticles (21.2 ± 0.26). This observation is in the accordance with the synthesis of ZnO nanoparticles using Aloe vera and Hibiscus sabdariffa. The antibacterial activity of both chemically and biologically synthesized ZnO nanoparticles were tested against three Gram negative and one Gram positive bacteria, in which the biologically synthesized ZnO nanoparticles showed excellent bactericidal activity over that obtained by chemical method [14, 20].

Similar antimicrobial activity was reported by (19), the multi-drug resistant was prepared by the combination of Aloe vera/Zinc Oxide nanoparticles. The antimicrobial activity of the prepared nanoparticles was tested against E.coli, S.aureus, and P.aeruginosa. The stronger cell proliferation and interaction with cellular components is the main reason for higher antimicrobial activity of E.coli bacteria. In another study, the flower like ZnO nanoparticles are prepared and their antibacterial mechanism toward Enterococcus faecalis and Micrococcus luteus was carried out by (3). Based on the literature review and antimicrobial report, the ZnO nanoparticles synthesized from plant extract exhibited exotic medicinal properties and therapeutic uses.

4. CONCLUSION

The sol-gel method was employed for the synthesis of ZnO nanoparticles from Aloe vera without adding any other chemical additives. The structural, morphological and antimicrobial activities of prepared nanoparticles were characterized by FTIR Spectroscopy, XRD, 3D laser profilometry and Well diffusion method. The obtained results from the present study revealed that Aloe vera leaf extracted several natural bioactive compounds which those could be effectively utilized in the nanoparticles synthesis. XRD pattern has revealed the hexagonal Wurtzite structure with crystallite size of 35.89 nm for ZnO and 25.64 nm for ZnO (Aloe). Roughness and thickness of the ZnO (Aloe) is high compared to ZnO which could be confirmed by 3D laser profilometry. The ZnO (Aloe) nanoparticles exhibited maximum zone of inhibition against bacterial pathogens of E.coli (14.16 ± 0.20) mm, S.aureus (28.06 ± 0.11) mm and B.subtilis (19.23 ± 0.25) mm and fungal pathogens of green synthesized ZnO nanoparticles proves the valid usage of face masks would benefit in minimizing the risk respiratory tract infections during mass gatherings.

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