

Green Synthesis, Characterization, Cytotoxicity Studies of Alumina Nanoparticles Using *Carica Papaya* and Evaluation for Their Potential Antibacterial Properties

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Abstract

The environmentally friendly synthesis, characterization, evaluation of potential toxicity and antibacterial development of alumina nanoparticles (Al₂O₃NPs) has become one of the best approaches to address the problem of antimicrobial drug resistance. The recent advances in the field of nanotechnology have thus made a great contribution to medical, biomedical, pharmaceutical and drug discovery research. Moreover, the tiny Al₂O₃NPs have a great success in inhibiting various types of microbes that cause diseases in humans and animals. In this study, we use the simplest, cheapest, less time-consuming and most effective green synthesis approach to produce environmentally friendly Al₂O₃NPs. In this study, the Al₂O₃NPs were synthesized from *Carica papaya* leaf extract (CPL) and examined microscopically for their size and shape. Fourier transform infrared spectroscopy (FTIR) was performed to determine the concentration of metal ions and the shape of the molecules. In vitro cytotoxicity was performed on different mammalian cell lines to determine the safety of the Al₂O₃NPs. Finally, bactericidal activity was evaluated using the agar plug diffusion method. The result showed 50–60 nm sized spherical nanoparticles. Turbidity analysis revealed MIC and MBC of >25 µg/mL against both Gram-positive and Gram-negative bacteria on Mueller-Hilton agar cultures. In conclusion, the Al₂O₃NPs synthesized from CPL exhibited acceptable nanoparticle size and shape as well as effective bactericidal activity.

Keywords: *alumina nanoparticles, carica papaya, antibacterial activity, agar diffusion method.*

INTRODUCTION

Recent advances in the field of nanotechnology have thus made a major contribution to medical, biomedical, pharmaceutical and drug research. Alumina nanoparticles (Al₂O₃NPs) have dimensions between 1 nm and 100 nm and can be synthesized green in either a bottom-up or top-down approach. Large surface area, durability, increased stability and recyclability are properties that nanoparticles possess.

Recent advances in the field of nanotechnology have therefore contributed greatly to medical, biomedical, pharmaceutical and drug discovery. Al₂O₃NPs are continuously produced due to their application in medicine, agriculture and industry by adding aluminum nitrate to the leaf extracts to obtain Al₂O₃NPs. Existing literature confirms favorable and unique properties such as porous morphology and highly stable nature that qualify Al₂O₃NPs as nanoparticles of interest for scientific research.

Among the existing methods for the synthesis of Al₂O₃NPs, which include chemical and physical methods, the novel green synthesis approach has been shown to be an effective, affordable, safe, natural, non-toxic and simple method for the preparation of nanoparticles. As a bottom-up approach, it can serve as either a reducing agent, capping agent or solvent due to the secondary plant compounds contained in the plant extract. The novel approach to synthesize nanoparticles using green plants aims to minimize the use of toxic chemicals, which in turn cause serious problems to the vital organs of humans and animals. In addition, the green synthesis approach is relatively inexpensive and sustainable compared to the conventional chemical approach.

Papaya, botanically known as *Carica papaya* L., belongs to the Caricaceae family. It is often referred to as 'papaya' in Australia and as 'tree melon' in some other countries in Africa and Asia. Papaya is a very important biomaterial with a special therapeutic effect and efficient antibacterial activity. Several studies have reported that the excellent antibacterial epoxy coatings of the extract have been shown to have a self-healing effect. Recent studies suggest that *C. papaya* fruit and leaf extracts are used in the treatment of fever to increase both whole blood and platelet counts. In another study, *C. papaya* leaf extract was found to have antioxidant and immunostimulatory properties against acrylamide toxicity in rats. The extract from the leaves and fruits of *C. papaya* is rich in vitamins, phenols and proteolytic enzymes and acts as a good antioxidant and excellent antimicrobial agent.

In the recent years antimicrobial resistance has become a serious problem in the treatment of infectious diseases a significant worldwide concern. Microbes can courteously be fought through the application some antimicrobial substances derived from plant extract coupled with compound derived from chemical sources. The good news is that the effectiveness of green synthesized nanomaterials in the treatment of an infectious disease has been reported in several studies.

RESEARCH METHODS

The leaves of *C. papaya* were collected from the Biological Garden of the Science laboratory Technology of Ramat Polytechnic, Maiduguri, Borno State, Nigeria. The reagents such as aluminum nitrate (Cat. No.: 209139, Sigma Aldrich, India), alumina, ethanol, distilled water (Cat. No.: 209140, Sigma Aldrich, India), Muller-Hinton agar (Cat. No.m1245, Invitrogen, USA), Nutrient Agar (Cat. No.: M001, Invitrogen, USA), Evergreen Malaysia. The pure cultures of *Staphylococcus aureus* and *Escherichia coli* were kindly provided by Mustapha Abubakar from the Department of Medical Microbiology,

Green synthesis of Al₂O₃NPs using *C. papaya* leaf extract has been previously described with antibacterial activities against medically important bacteria [14]. In this study, *C. papaya* Al₂O₃NPs (CPL- Al₂O₃NPs) were synthesized using a one-pot method in agreement with the aforementioned study. CPL- Al₂O₃NPs were synthesized by mixing an Al₂NO₃ solution with an extract of *C. papaya* leaves. The resulting reaction

leading to the formation of the nanoparticles is the reduction of aluminum oxide ions by the aqueous extract. The obtained nanoparticles were characterized by electron microscopy (SEM and TEM) and FTIR and investigated for their cytotoxicity and antibacterial properties with bacterial strain.

RESULTS AND DISCUSSION

Determination and Synthesis of Al₂O₃NPs

The aqueous papaya leaf extract and 1 mM Al₂NO₃ were mixed in a 1:4 ratio and heated on a heating mantle at 70 °C for 30 minutes until a colour change was observed. The colour change indicated the formation of Al₂O₃NPs by C. papaya leaf extract (CPL-Al₂O₃NPs) (Fig. 1). The yellow precipitate shown in Figure 2 also indicates the successful synthesis of Al₂O₃NPs. The precipitate containing the Al₂O₃NPs was carefully collected and dried overnight at 70 °C in an oven. It was then calcined in a muffle furnace at 500 °C for 1 hour. At the end of the calcination process, a uniform powder was obtained, as shown in Figure 3.



The mixture of Alumina nitrate and C. Papaya leave was heated at 70°C for 30 min in a Heating Mantle

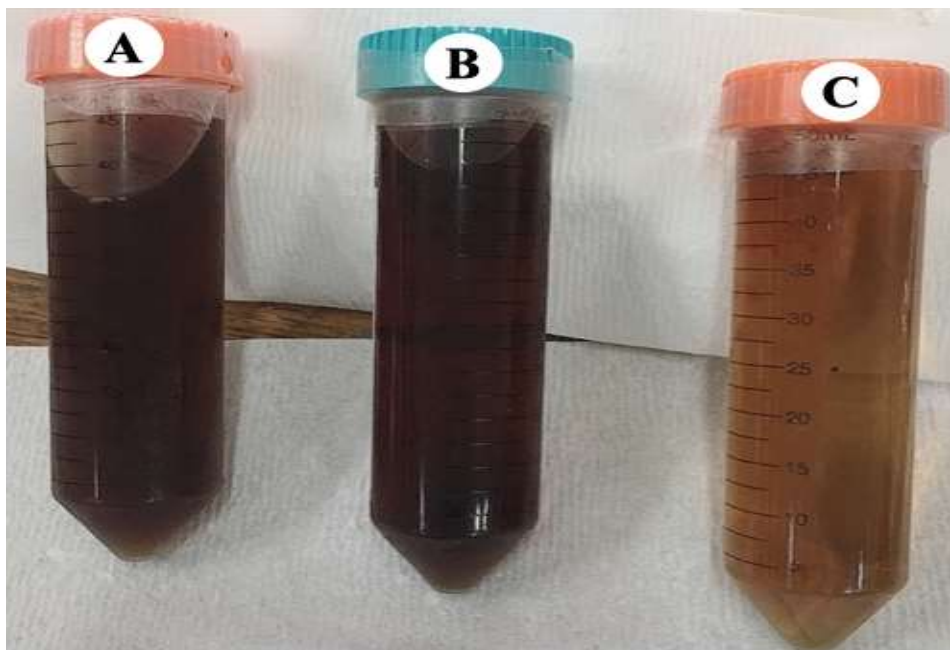


Figure 1. Colour Change Of Carica Papaya Leaf Extract From Deep Brown Colour Before Mixing With Aluminium Nitrate To Yellowish Colour After Mixing With Aluminium Nitrate Powder.

• Figure 2: Green synthesis of Alumina nanoparticle using *Carica papaya* leaves extract.



• Figure 3: Green synthesized of Alumina nanoparticle powder using *Carica papaya* leaves extract.



The results of the SEM and HRTEM studies clearly showed a particle size between 50 and 60 nm and an average size of the nanoparticles of 10 and 40 nm with a spherical morphology (Fig. 4). The FTIR spectrum also showed a very strong absorption between 1000 and 4000 cm^{-1} . There are further peaks in the spectrum at 1020, 1350, 1420, 1600, 2050 and 3300, which could be esters, ethers, carbonyl (polyols) or aromatic compounds. The sharp FTIR curve indicates the presence of alumina ions and confirms the green synthesis of alumina nanoparticles (Fig. 5)

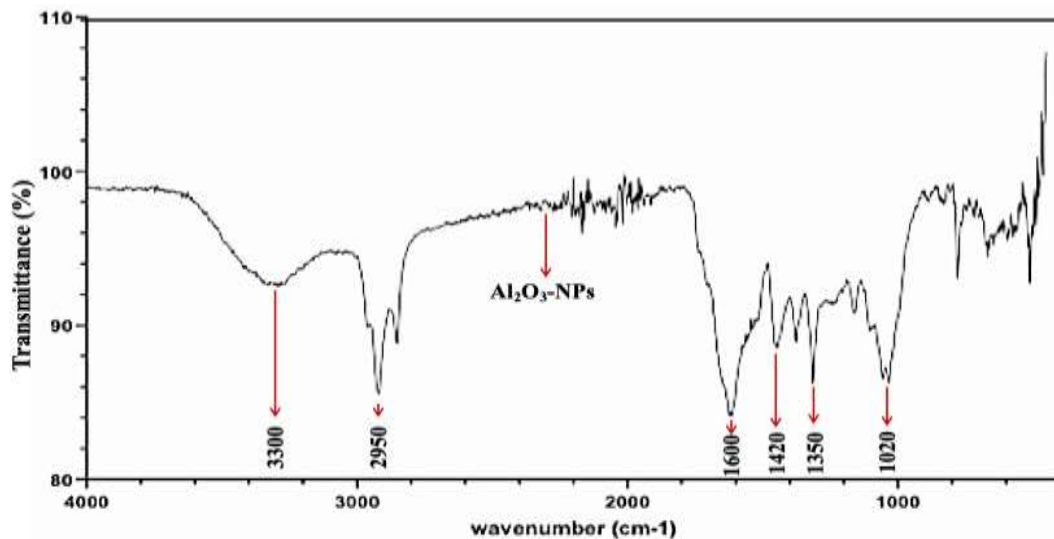


Fig. 5: Fourier transform infrared analysis (FTIR) of green synthesized alumina nanoparticles using leaves extract of *Carica papaya*

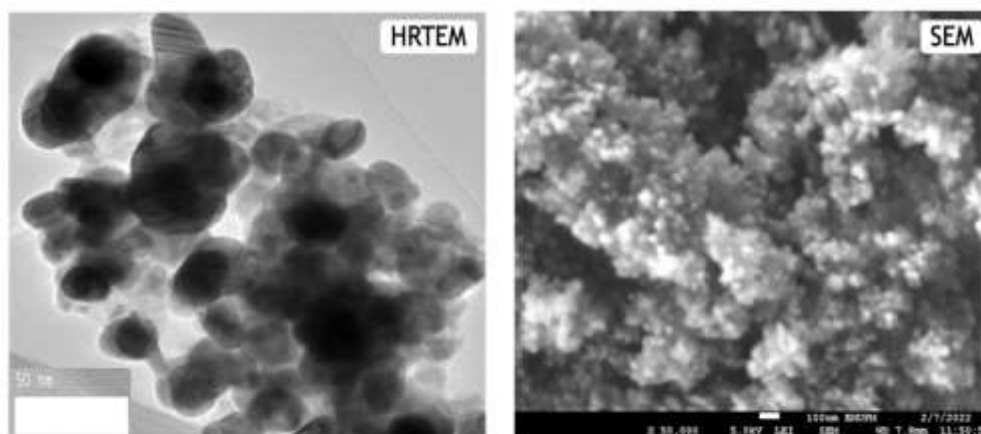


Fig. 5: Show the morphology of nanoparticles High-resolution transmission electron microscopy (HRTEM) and Scanning electron microscopy (SEM) micrographs of synthesized *C. papaya* leaf alumina nanoparticles (CPL- Al_2O_3 -NPs).

The finding from agar well diffusion method clearly indicates the effective antibacterial activity of the plant extract (negative control), ampicillin (Positive control), and four treatment groups treated with (100 $\mu\text{g}/\text{mL}$, 200 $\mu\text{g}/\text{mL}$, 300 $\mu\text{g}/\text{mL}$, and 400 $\mu\text{g}/\text{mL}$) of alumina nanoparticles (Fig. 6). The visible zones of inhibitions were recorded when the bacteria were exposed to the highest concentration of alumina nanoparticles which are recorded as follows *S. aureus* (26 mm), *K. pneumoniae* (28mm), *E. coli* (36 mm), and *Salmonella spp* (31 mm). Relatively, lower zones of inhibition were recorded when the bacteria were exposed to 100, 200, and 300 $\mu\text{g}/\text{mL}$ as shown in table 1.

Table 1: Antibacterial Activity Of Alumina Nanoparticle Against Two Pathogens

Bacteria	Inhibition zones
Positive control	Conc. of AlNPs
	Neg. control

	1000 $\mu\text{g/mL}$	100 $\mu\text{g/mL}$	200 $\mu\text{g/mL}$	300 $\mu\text{g/mL}$	400 μL
<i>S. aureus</i>	52 mm	16 mm	18 mm	21 mm	26 mm
<i>S. pneumoniae</i>	43 mm	8 mm	17 mm	24 mm	28 mm
<i>E. coli</i>	48 mm	10 mm	21 mm	32 mm	36 mm
<i>Salmonella spp</i>	39 mm	14 mm	23 mm	27 mm	29 mm

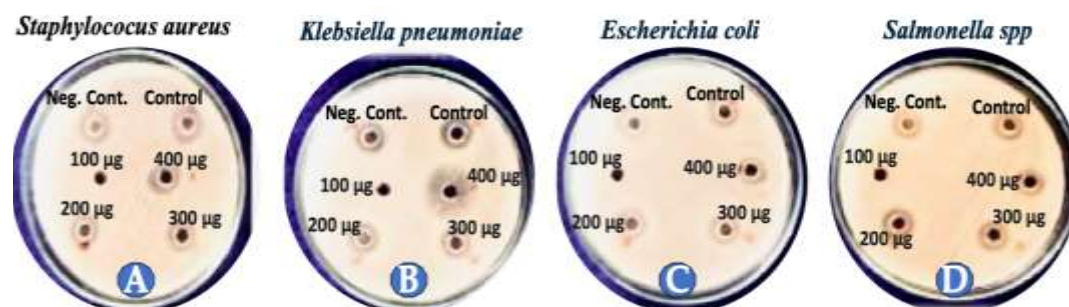


Fig. 6: The antibacterial efficiency of *C. papaya*-alumina nanoparticles (CPL- $\text{Al}_2\text{O}_3\text{NPs}$), *C. papaya* leaf (CPL) extract and ampicillin against *S. aureus* and *K. pneumoniae*, *E. coli*, and *Salmonella spp*. Note: Disc No. 1 (5 $\mu\text{g/mL}$ CPL-AgNPs, Disc No. 2 (10 $\mu\text{g/mL}$ CPL-AgNPs, Disc No. 3 (5 $\mu\text{g/mL}$ CPL extract), Disc No. 4 (10 $\mu\text{g/mL}$ ampicillin).

Discussion

The environmentally friendly synthesis, characterization, evaluation of potential toxicity and antibacterial development of alumina nanoparticles ($\text{Al}_2\text{O}_3\text{NPs}$) has become one of the best approaches to address the problem of antimicrobial drug resistance. In present days, there has been a huge demand in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, a greater attention has been paid to antimicrobial activity screening and evaluating methods. Nanomaterials had proven to be the efficient mode of drug delivery in modern science. The utilization of medicinal plant materials, microbes, enzymes for synthesis of nanoparticles has been revolutionized in recent years and could serve as alternative for antibiotics. In the present study, the CPL- $\text{Al}_2\text{O}_3\text{NPs}$ showed particle size ranging in between 5 and 200 nm, which may confer the ability to penetrate the cells/microbes and execute the bactericidal property. The mechanism of action of alumina nanoparticles is ambiguous in microorganism.

The characterization of nanoparticles was done using various techniques such as FTIR electron and microscopy. The results obtained from the techniques and antibacterial studies have coincided with the literature in the field of nanoparticle research and certainly gives proof about the nanoparticle synthesis and its efficiency as antibacterial agent. The bio-compounds in leaf extract having the carbonyl groups are believed to be reducing the alumina ions and converting to unsaturated carbonyl groups by auto-oxidizing. The FTIR analysis of CPL- $\text{Al}_2\text{O}_3\text{NPs}$ showed two sharp absorption peaks at between 1000 and 4000 cm^{-1} . There are further peaks in the spectrum at 1020, 1350, 1420, 1600, 2050 and 3300, which could be esters, ethers, carbonyl (polyols) or aromatic compounds. The absorption peak at 1640 cm^{-1} could be due to the amide bond coming from the carbonyl group of a protein and the peak at 3359 cm^{-1} may be because of OH groups present in alcohols and phenolics.

The characterization studies by scanning electron microscopy has provided more information on the synthesized Al₂O₃NPs in the presence of papaya leaf extract such as size and morphology of nanostructures at scale of 200 nm, 1 μm and 2 μm (Fig. 4). The transmission electron microscopy studies have given further inputs on the morphology and size of biosynthesized alumina nanoparticles ranging between 50 and 61 nm with a scale of 100 nm and histogram showing sizes of particles with spherical morphology (Fig. 6)

The existing findings suggests that there are several mechanisms by which Al₂O₃NPs could be killing the microorganisms: (i) destructuring the cell wall and ceasing the cell permeability (ii) formation of free radicals, (iii) inactivating important enzymes by interacting with thiols, (iv) interaction of Al₂O₃NPs with DNA and interruption of DNA replication and translation and by dephosphorylating the tyrosine residues on peptides inhibiting the signal transduction and growth in bacteria. The antibacterial studies with CPL-Al₂O₃NPs showed profound antibacterial effect against both Gram positive and Gram negative strains. The disc diffusion studies demonstrated larger inhibition zones in comparison to papaya leaf extract and similar to that of ampicillin (Positive control) which are analogous to the results reported by [1,19]. Based on the varying sizes (in mm) of zone of inhibitions, we could confirm the bactericidal efficiency against individual bacterial strains (Fig. 6, table 1). The MIC studies showed varied concentrations of CPL- Al₂O₃NPs against both Gram positive and Gram negative strains.

CONCLUSION

The green synthesis and characterization of CPL- Al₂O₃NPs was done and confirmed by FTIR, SEM, and TEM techniques. The nanoparticles appeared to be spherical in shape with smooth surface and the size of the particles varied from 50 to 60 nm, but amongst them most of the particles obtained were sized in between 5 and 15 nm. The zones of the inhibitions CPL- Al₂O₃NPs had exhibited inhibitory value >25 μg/mL against both Gram positive and negative bacterial species. In summary, the CPL extract mediated synthesis of Al₂O₃NPs was efficient and provides additional property such as bactericidal efficiency and might act as long searched alternative and could be the answer to antibiotic resistance.

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