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Biosynthesis, characterization, and antibacterial activity of ZnO nanoaggregates using aqueous extract from *Anacardium occidentale* leaf: comparative study of different precursors

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Abstract

Background: Various parts of *Anacardium occidentale* plant possess curative qualities like antidiabetic, anti-inflammatory, antibacterial, antifungal, and antioxidant. Aqueous extract of this plant leaf was used in biosynthesizing zinc oxide (ZnO) nanoaggregates using two precursors of zinc salt (zinc acetate dihydrate [$Zn(CH_3COO)_2{\cdot}2H_2O$] and zinc chloride [$ZnCl_2$]). The synthesized ZnO samples were used in a comparative study to investigate the antibacterial activity against selected Gram-positive and Gram-negative microbes [*Staphylococcus aureus*, *Exiguobacterium aquaticum* (Gram +ve) and *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* (Gram −ve)]. The synthesized ZnO nanoaggregates from the two precursors were characterized using Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy-dispersive x-ray spectroscopy (EDX) techniques.

Results: Micrographs of SEM and TEM confirmed nanoparticles agglomerated into aggregates. While spherical nanoaggregates were identified in samples prepared from $Zn(CH_3COO)_2{\cdot}2H_2O$, flake-like structures were identified in samples synthesized from $ZnCl_2$. Particle size determined by TEM was 107.03 ± 1.54 nm and 206.58 ± 1.86 nm for zinc acetate dihydrate and zinc chloride precursors respectively. ZnO nanoaggregate synthesized using zinc acetate as precursor gave higher antibacterial activity than its counterpart, zinc chloride with *K. pneumonia* recording the highest inhibition zone of 2.08 ± 0.03 mm (67.53%) whereas *S. aureus* recorded the least inhibition zone of 1.06 ± 0.14 mm (34.75%) for ZnO nanoaggregate from zinc chloride precursor. Also, antibacterial activity increases with increasing concentration of the extract in general. However, *A. baumannii*, *E. aquaticum*, and *K. pneumoniae* did not follow the continuity trend with regards to the 250 ppm and 500 ppm concentrations.

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Conclusion: Biosynthesis of ZnO nanoaggregates using aqueous extract of *A. occidentale* leaf from zinc acetate dihydrate and zinc chloride as precursors was successful with the formation of nanospheres and nanoflakes. The study suggested that *A. occidentale* sp. could be an alternative source for the production of ZnO nanoparticles and are efficient antibacterial compounds against both Gram +ve and Gram −ve microbes with its promising effect against infectious bacteria.

Keywords: Zinc oxide nanoaggregate, Zinc acetate dihydrate, Zinc chloride, *Anacardium occidentale*, Antibacterial activity, Biosynthesis

1 Background

Nanotechnology is a growing field of study combining technology, bio-nanoscience, and material science together [1]. Serious interests arose in the last few decades among scientists into some unique properties: catalytic, magnetic, electronic, optical, antibacterial, antimicrobial, anti-inflammatory, and wound healing of nanoparticles [2–4]. When different precursors are used in the synthesis of ZnO particles at different reaction conditions (reaction time, concentration, and temperature), literature shows that different morphologies, such as flower shape and needle shape, are produced with different sizes [5–7]. The porosity of the synthesized nanoparticles enhances the surface area and chemical and photochemical stability [8, 9]. Research has also shown that ZnO nanoparticles synthesized biologically have shown strong antibacterial effect than those synthesized through chemical means [10, 11]. The type of plant extract and its concentration used in a biosynthesis reaction have an effect on the morphology of ZnO nanoparticles. For instance, extract containing functional groups such as alcohol, ketone, carboxylic acid, and amine creates spherical-shaped ZnO nanoparticles, whereas extract with –OH (hydroxyl) group gives quasi-spherical agglomerates [12, 13].

Natural products such as chitosan and extracts of fungi, bacteria, and different plant genus extracts are used as stabilizing and reducing agents which serve as alternative to chemical synthesis of nanoparticles [14]. The reason has been reported to be due to the fact that green synthesis of nanoparticles is environmentally favorable, simple, economical, and comparatively reproducible. The concentration of metabolites in the plant extracts, pH, as well as the temperature used in the plant extract preparation has an effect on the morphology of the nanoparticles to be formed [15–19]. ZnO nanoparticles are known to be one of the multipurpose inorganic nanoparticles with effective antibacterial, antimicrobial, and antifungal activities [20] even at very low concentrations.

Anacardium occidentale (cashew) belongs to the genus *Anacardium* and family Anacardiaceae, which is found worldwide. The plant is predominantly grown in Dormaa Ahenkro in the Brong Ahafo region of Ghana, West Africa, as a cash crop. Different parts of this plant species possess curative qualities like antidiabetic, anti-inflammatory, antibacterial, antifungal, and antioxidant. This was reported by different studies that investigated into these activities and has proven its viability and effectiveness [21–26].

Phytochemical constituents of this plant as investigated by Ojezele and Agunbiade [27] reported the presence of tannin = 15.38 mg/g, total polyphenolics = 2.00 mg/g, alkaloid = 39.90%, and oxalate = 8.13%. Also, Fadeyi et al. [28] identified fatty acid esters and a new androstane steroid derivative being reported for the first time. Bastos et al. [29] also in a study went further to isolate anacardic acid, cardol, and cardanol compounds from this plant to evaluate its inhibition against *Trypanosoma cruzi Sirtuins*. From their report, isolation was successful with positive inhibition against pathogens under study.

Nevertheless, literature survey reveals the synthesis of platinum nanoparticles from this plant species and its catalytic and thermal applications but with very little information on zinc oxide nanoparticle synthesis [30]. Hence, the study sort to synthesize ZnO nanocrystals from aqueous extract of *A. occidentale* from two precursors and also investigate their antibacterial activity against selected Gram-positive and Gram-negative microbes [*Staphylococcus aureus*, *Exiguobacterium aquaticum* (Gram +ve) and *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* (Gram −ve)]. Aqueous extract was preferred for this study because the extraction process is simple and free from contaminants.

2 Methods

2.1 Collection and preparation of plant extracts

The plant species (*A. occidentale*) was formally identified by the two individuals of the Centre for Plant Medicine Research, Mampong-Akuapem, Ghana, who doubles as co-authors of this paper. *A. occidentale* fresh leaves were collected from a peasant farmer in Dormaa Ahenkro in the Brong Ahafo Region of Ghana, West Africa, in September 2019 upon a request. Since the plant species was meant for study purposes and not for any commercial reasons, permission was granted to harvest the leaves