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Aloe vera extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates

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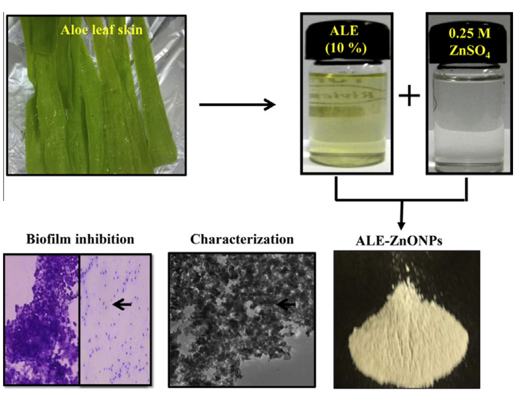
GRAPHICAL ABSTRACT



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ABSTRACT

ZnO nanoparticles (ZnONPs) were synthesised through a simple and efficient biogenic synthesis approach, exploiting the reducing and capping potential of *Aloe barbadensis* Miller (*A. vera*) leaf extract (ALE). ALE-capped ZnO nanoparticles (ALE-ZnONPs) were characterized using UV–Vis spectroscopy, X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and transmission electron microscopy (TEM) analyses. XRD analysis provided the average size of ZnONPs as 15 nm. FTIR spectral analysis suggested the role of phenolic compounds, terpenoids and proteins present in ALE, in nucleation and stability of ZnONPs. Flow cytometry and atomic absorption spectrophotometry (AAS) data analyses revealed the surface binding and internalization of ZnONPs in Gram +ve (*Staphylococcus aureus*) and Gram −ve (*Escherichia coli*) cells, respectively. Significant antibacterial activity of ALE-ZnONPs was observed against extended spectrum beta lactamases (ESBL) positive *E. coli*, *Pseudomonas aeruginosa*, and methicillin resistant *S. aureus* (MRSA) clinical isolates exhibiting the MIC and MBC values of 2200, 2400 μg/ml and 2300, 2700 μg/ml, respectively. Substantial inhibitory effects of ALE-ZnONPs on bacterial growth kinetics,

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exopolysaccharides and biofilm formation, unequivocally suggested the antibiotic and anti-biofilm potential. Overall, the results elucidated a rapid, environmentally benign, cost-effective, and convenient method for ALE-ZnONPs synthesis, for possible applications as nanoantibiotics or drug carriers.

1. Introduction

A rapid stride in synthesis and applications of nanomaterials, in recent years, has been perceived in almost every domain of life including health care, cosmetics, biomedical, food and feed, drug-gene delivery, environment, electronics, mechanics, catalysis, energy science, optics, chemical and space industries [1]. Nanoparticles (NPs) of noble metals, such as gold, silver, platinum, and zinc oxide are widely used in medical and pharmaceutical applications, and in an array of consumer products [2]. Synthesis of NPs has been reported using various chemical and physical methods, such as sol-gel process, chemical precipitation, chemical vapour deposition, hydrothermal and microwave methods [3]. Lately, single-pot biomimetic and/or biological methods of synthesis are preferred over chemical and physical methods because of their rapidity, eco-friendliness, non-pathogenic, and economical attributes. Besides, these methods exclude the use of high temperature, pressure, energy and toxic chemicals [4]. Furthermore, the metallic NPs are recently being comprehended as promising nanoantibiotics, due to their remarkable antimicrobial properties. This has elicited enormous research interest owing to growing incidence of multiple antibiotic resistance in microorganisms [5]. Lately, environmentally friendly single-step protocols using plants extracts without involving any extrinsic surfactants, capping agents, and/or templates have been explored for metal NPs synthesis [6]. This intrinsically green approach involves the biomolecules such as proteins, amino acids, enzymes, vitamins, alkaloids, phenolics, saponins, tannins, and terpenoids, present in plant extracts, for reduction and stabilization of metal ions [7]. Some of these biomolecules act as electron shuttles in metal reduction, while other constituents are responsible for capping of resulting NPs, which not only controls the aggregation of NPs but also results in post-surface modification of NPs [8].

This has prompted us to use *Aloe barbadensis Miller* (*A. vera*) *plant* extract for synthesis of zinc oxide nanoparticles (ZnONPs). Zinc oxide has been chosen because of its wide spectrum applications in sunscreens, paints, plastic and rubber manufacturing, pharmaceutical products, diagnostics and micro-electronics [9]. The ZnONPs have also been used to remove arsenic and sulphur from water [10], and in dental applications [11]. They have also

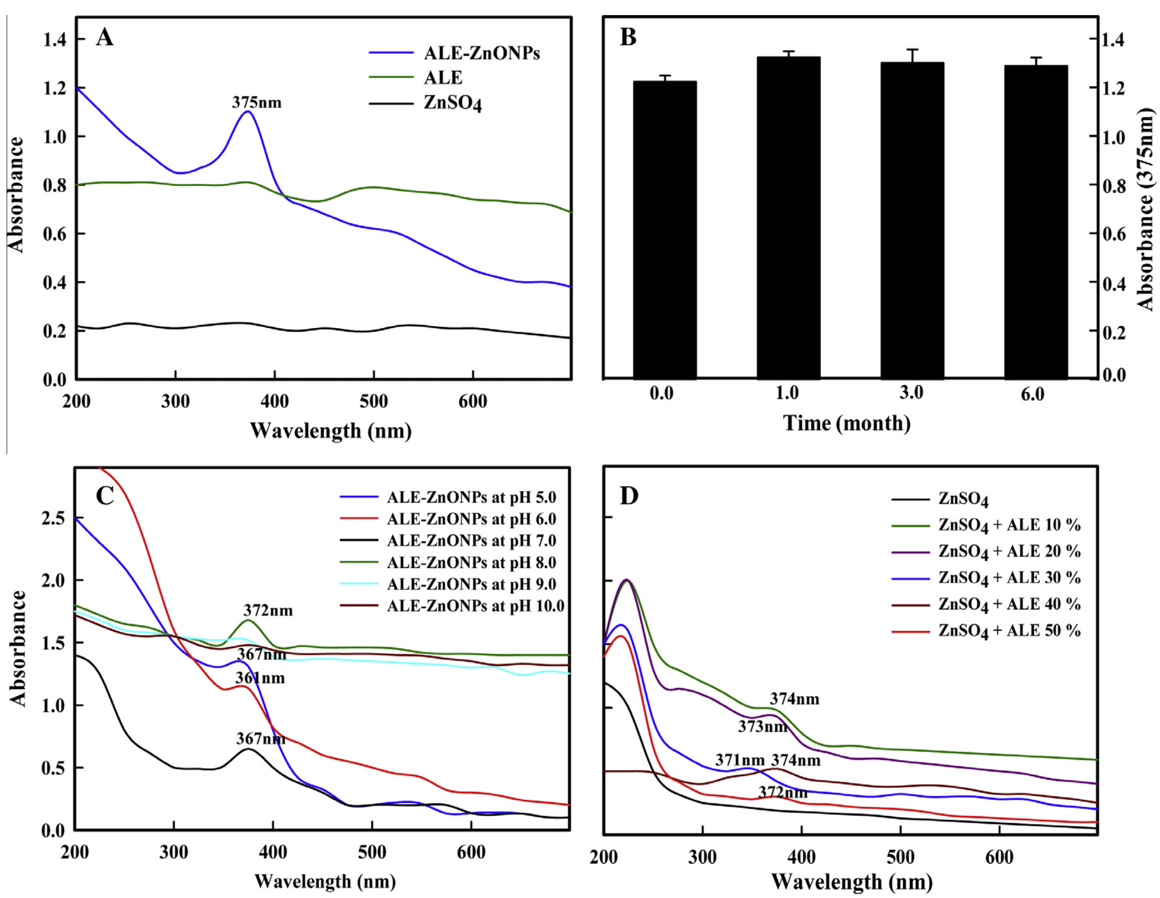

Fig. 1. Synthesis and surface plasmon resonance (SPR) properties of ALE-ZnONPs under varying physico-chemical conditions. The panels represent: (A) UV–Vis spectra of ALE-ZnONPs, ALE and $ZnSO_4$. (B) stability of ALE-ZnONPs based on SPR measurements up to six months (error bars represent the mean ± SE of three replicates). (C) UV–vis absorption spectra of ALE-ZnONPs synthesized under different pH (5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) conditions. (D) effect of ALE concentration (10%, 20%, 30%, 40% and 50%) on ALE-ZnONPs synthesis.