Biological Activity Of Crab Shell Mediated Silver Nanocomposites Against Bacteria Via Minimum Inhibition Concentration And DNA Fragmenatation In MCF 7

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ABSTRACT
Crab shells represent an abundant source of chitosan, a biodegradable biopolymer with limited bioactivity in bulk form. To overcome this limitation, chitosan was converted into silver Nano composites. The objective of this study was to evaluate the minimum inhibitory concentration and DNA fragmentation analysis of potential chitosan Nano composites derived from mud crab and sea crab shells. The minimum inhibitory activity was assessed against *E. coli,* a Gram-negative bacterium, using various concentrations to determine the microbial inhibition concentration. Further DNA fragmentation assay was carried out against MCF-7 breast cancer cells. This study aims to develop eco-friendly chitosan-based Nano composites from marine waste for biomedical applications.

Keywords: chitosan, Nano composites, Crab shell, Minimum inhibitory concentration, DNA fragmentation assay, MCF 7

INTRODUCTION

Nanoparticles made from various polymers, typically within the 1–100 nm size range, are commonly used in nanomedicine and biomedical applications. Nanoparticle production can be achieved through top-down techniques, including high-pressure processing, homogenization, sonication, and bottom-up procedures such as reactive precipitation and solvent displacement [1]. Owing to their nanoscale dimensions, nanoparticles possess distinct properties compared to their bulk counterparts, enabling their precise engineering with unique characteristics [2]. Synthesis of AgNPs using plant extracts have popped up as a novel approach but their stability and dispersion are very critical. Nowadays, stabilizers like natural polymers are gaining much interest among the scientific community than the synthetic polymers derived from petroleum oils as they are non-toxic and biodegradable materials [3,4].

Chitosan, a chemically degradable biocompatible polymer, is obtained through the deacetylation of chitin and is structurally similar to cellulose, consisting of alternating units of N-acetyl glucosamine and glucosamine linked by 1-4 glycosidic bonds [5,6]. It is a pale, inelastic carbohydrate used for various purposes, such as agriculture, biomedicine, the food sector, water purification, preventing pollution, photography, and paper production [7]. The nanoparticles derived from chitosan, known as chitosan nanoparticles (CNPs), combine the advantageous properties of both chitosan and nanomaterials [8]. The economic feasibility and widespread availability of chitosan make it particularly valuable for medicinal applications, such as enhancing wound healing [9] and the development of drug delivery systems [10]. Moreover, chitosan possesses mucoadhesive characteristics that enable the administration of CNPs through several transmucosal pathways, including the intratracheal, intranasal, intravaginal, intrapulmonary, and intraocular pathways [11].There are 3 forms namely α,β,γ chitin [12]. Applications of chitin has been restricted due to its compact structure it is insoluble in most of the solvents like water, acetone, dilute acids or dilute alkalines [13]. In order to make it applicable, chitin is partial deacetylated with 40% NaoH which yields chitosan. Chitosan is a straight chain polymer consists of both D-glcosamine and N-acetyl D- glcosamine [14]. It consists of more amine group and readily soluble in acetic acid or formic acid. Thus it became highly biocompatible with unique physical, chemical and biological properties [15]. Molecular weight, degree of deacetylation and source of raw material used are the main factors in determining the physicochemical properties of chitosan [16]. Being a polymer derived from natural resource, it possesses antimicrobial, antioxidant, antidiabetic, antitumor, hypocholesterolemic, haemostatic and wound healing properties. The molecular weight and viscosity of chitosan in aqueous solutions critically affect its potential use in biochemistry, nanomedicine, and pharmacology. Other essential factors include moisture content, crystallinity, ash content, crystal size, and levels of heavy metals. Additionally, chitosan extraction is an environmentally friendly process that mitigates the ecological impact of discarded crustacean shells from the seafood industry. The waste produced

annually from crab shells poses a challenge for decomposition, contributing to environmental pollution. Transforming these shells into chitin and chitosan effectively reduces contamination, imparting both compounds with many valuable applications [17]. In the present study, silver nanoparticles (AgNPs) were evaluated against human pathogenic bacterial strains namely *E.coli*, *and DNA fragmention of MCF-7 breast cancer cells*.

MATERIALS AND METHODS

Crab Shell Collection and Recovery of Chitosan from Crab Shells, Chitosan extracted from previously collected crab shells served as the starting material for this work [18].

MINIMUM INHIBITORY CONCENTRATION (MIC) DETERMINATION

This method was initiated by preparing nutrient broth using sterile 96 well plates [19]. The wells of each row were filled with 200 mL sterilized nutrient broth. Crab nano-compisites were added into the well at various concenrations (10, 20,30,40,50 µL) and 50ul of culture *Escherichia coli* respectively. The plate was incubated for 24 hours at 37℃. The resulting turbidity was observed ,after 24 hours MIC was determined to be where growth was no longer visible by assessment of turbidity by optical density readings at 570 nm with a 96 well ELISA plate reader (Robonik).Percentage of cell death was calculated using the formula : % of cell death= Control OD-Sample OD Control ODX 100 After the calculation the higher percentage of cell death occurred concentration was selected to identify the viability.

DNA FRAGMENTATION ASSAY

DNA About 500ul of 106 cells were treated and lysed with RNase A and incubated at room temperature for 2.30 hrs. Followed by addition of proteinase K (0.2 mg ml–1) and incubated at 50^{O}C overnight. After incubation 10m Ammonium acetate solution was added during the incubation period, and DNA precipitation is done by adding 100% ice-cold ethanol. Ethidium bromide was added in 1.5 % agarose gel and electrophoresed for 45 min 75 mV. The DNA imaged was taken using gel documentation system [20].

RESULTS AND DISCUSSION

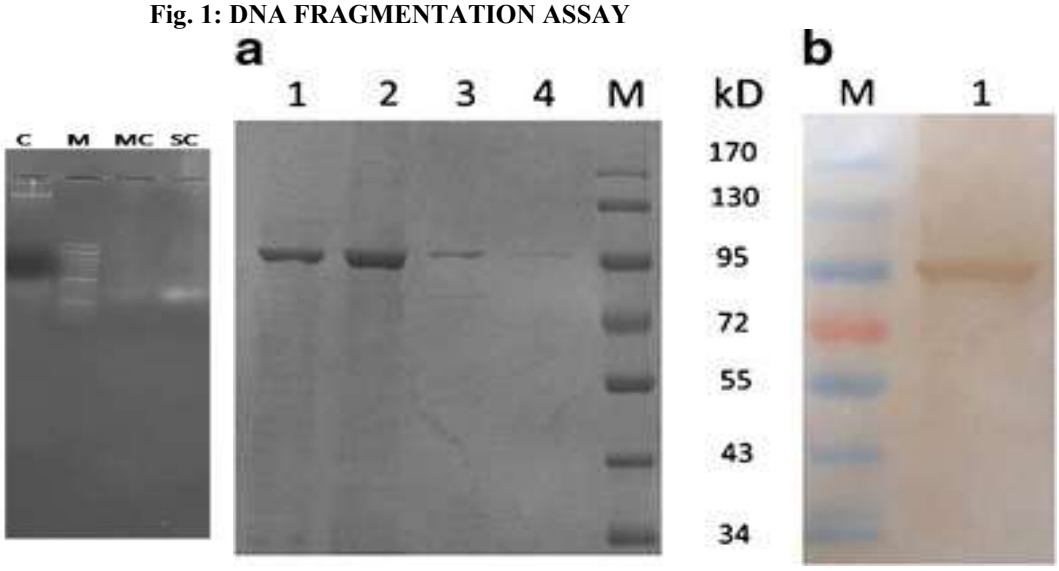
Determination of Minimum Inhibitory Concentration (MIC) The minimum Inhibitory Concentration (MIC) assay was conducted to assess the concentration-dependent antibacterial activity of chitosan and nanoparticle against the isolated gram negative bacteria *E.coli*

All composite solutions of other ratios also cause decrease in the antibacterial activity but to more or less to constant value compared to the 1:3 ratio. This comes in agreement with literature and can be attributed to the great surface area offered by the much small particles' size, reaching the nuclear content of bacteria easily stated that chitosan-based Ag nanoparticles have a dual mechanism of action for antibacterial activity, namely, the bactericidal effect of Ag nanoparticles and cationic effect of chitosan[21,22.23]. Three most common mechanisms proposed are: (i) gradual release of free Ag-ions followed by disruption of ATP production and DNA replication, (ii) Ag nanoparticles direct damage to cell membranes, and (iii) Ag nanoparticles and Ag-ions generation of ROS. Several studies insist that the mode of antimicrobial action of Ag nanoparticles is similar to that of Ag-ions [24], which contradict to our results. Many authors demonstrate that the small particles mainly in the range of 1–10 nm could enter the cell based on indirect microscopic evidences [25]. The presence of hexagonal shaped nanoparticles at 12 h (of size range 103–193 nm with average 148 nm) and spherical ones (p.s. 14–162 nm with average 88 nm) in amount relatively 30% less than that of the spherical shape only (at 16 h) produced higher antibacterial activity against E. coli (23.68%). Series of five concentrations (10 folds dilution) of a selected composites prepared from 1:3 AgNO3 : Chitosan ratio at 80°C for 16 h, 1:2 AgNO3 : chitosan ratio at 80°C for 16 h and 1:2 AgNO3 :chitosan ratio at 90°C for 16 h were examined for their inhibition percentage to G+ve and G−ve bacteria. Results showed that all composites under investigation at the highest concentration 220 µg/ml produced maximum inhibition % in the range 89–96% for all bacteria. The minimum inhibition % range (56–74%) was achieved by the smallest three concentrations 2.2-0.02 µg/ml for all bacteria.

Table 1: Minimum inhibitory concentration of chitosan and nanoparticles against *E.coli*

Volume of sample (mg/ml)	% of cell death in chitosan	% of cell death in nanoparticles
10	25	42.10
20	30.52	57.63
30	41.57	66.05
40	48.15	68.83
50	51.57	78.68

Fig. 1: DNA FRAGMENTATION ASSAY



DNA extraction from MCF-7 cells treated with AgNPs and detection of it in an agarose gel were used to confirm DNA fragmentation. Control DNA was not fragmented. Mud crab and Sea crab treated cell's DNA was fragmented and degraded. Mudcrab shell nanoparticles induced more extensive DNA fragmentation than sea crab shell nanoparticles, suggesting stronger growth-inhibitory potential against breast cancer cells.

CONCLUSION

Crab shells are a rich source of chitosan, and to enhance its bioactivity, chitosan was converted into nanocomposites. The antimicrobial potential of both chitosan and its nanocomposites was evaluated against E. coli, a Gram-negative bacterium. Various concentrations were tested to determine the microbial inhibition concentration at which maximum bacterial killing occurred, and the results showed improved inhibition by nanocomposites compared to bulk chitosan. The study further extended to anticancer evaluation through DNA fragmentation assay. Nanoparticles derived from mudcrab and sea crab shells were tested against MCF-7 breast cancer cells to analyze DNA degradation patterns using a specific marker. The assay revealed that mudcrab shell-derived nanoparticles induced greater DNA fragmentation, indicating higher potential to control the growth of breast cancer cells compared to sea crab shell nanoparticles.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

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