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RESEARCH ARTICLE

ISOLATED POLYSACCHARIDE FROM THE PULP OF *SPONDIAS MOMBIN* USED FOR THE SYNTHESIS OF CAPPED ANTIBACTERIAL AG-NANOPARTICLES AND CATALYTIC ACTIVITY OF SYNTHESIZED AG-NANOPARTICLES

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ABSTRACT

Nanotechnology is the understanding and control of matter at dimension of roughly 1-100 nanometers, where unique phenomena enable novel applications. Recent innovation in nanotechnology has transformed a number of scientific and industrial areas including the microbiology and other biological field. In this experiment Ag-nanoparticles were prepared by using polysaccharide isolated from *Spondias mombin* and characterized by UV-VIS spectrum band at 420 nm. We found those nanoparticles were very effective against some human pathogenic bacteria like *E.coli, K.pneumoniae*, *Pseudomonus*. We found that Ag-nanoparticles of *Spondias mombin* are very good effective against *E.coli*, *K.pneumoniae* and *Pseudomonus* sp. compared with the antibiotic ciprofloxacin. It has strong photocatalytic activity against congo-red. Those particles showed nuclease activity on plasmid DNA.

Key words: Ag-nanoparticles, *Spondias Mombin*, Polysaccharide, Antimicrobial Activity.

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INTRODUCTION

The Yellow mombin (*Spondias mombin* L.) belongs to the *Anacardiaceae* family and it is found in the tropical areas of America, Asia and Africa and in Brazil mainly in the regions North and Northeast. It is known as cajá or taperebá in Brazil; ciruela amarilla in Mexico and Ecuador; jobo in Central America and hogplum or Yellow mombin in North America. The fruit is a small ovoid drupe (3 to 5 cm long) with thin yellow skin and a sour–sweet taste (Bosco, Soares, Aguiar Filho, and Barros, 2000). However, the knowledge of the nutritional value of these fruits has also a great importance and potential contribution for the consumption enhancement, considering the great concern of the consumers worldwide about healthy eating habits (Sloan, 2003). The decoction of the leaves is commonly used to treat laryngitis, sour throat, and tooth decay; this product has also been very popular in traditional birth practices for its periand postpartum obstetrics uses (Akubue *et al*., 1983; Nworu *et al*., 2007). The leaves of Spondias mombin have been reported to contain antiviral ellagitannins and caffeoyl esters, as well as antibacterial and molluscicidal phenolic acids (Corthout *et al*., 1994).

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The leaves have also been demonstrated to have antihelminthic (Ademola *et al*., 2005) and abortifacient (Offiah and Anyanwu, 1989; Nworu *et al*., 2007) properties. In traditional medical practice of southern Nigeria, the freshly boiled aqueous leaf extract of Spondias mombin is used to treat dizziness, especially after childbirth, while the bark is used to treat mothers after delivery (Onwuka, 1992). The more general wound-healing properties of extracts from the leaves of this plant have also been investigated (Villegas *et al*., 1997). The yellow mombin frozen pulp is one of the most prized in Brazilian markets due to its exotic and appreciated flavor besides its excellent nutritional quality which is more and more valued by consumers. It is used for the preparation of juices, popsicles, ice creams, yogurts and jams (Janick and Paull, 2008; Soares *et al*., 2006).

MATERIALS AND METHODS

Isolation and purification of Polysaccharide (PS) from fruit fibre of *Spondias mombin:* Green Hog plum was collected from the local market of Panskura, West Bengal, India. At first the fruit fibre of *Spondias mombin* (250gm) was cutted into small pieces and boiled for 4 hours in water and freeze cool. Then the water extract was filtered from the solution and then centrifuged at 8000 rpm, 4°C for 30 mins.

Then the filtrate solution was precipitate in ethanol drop by drop. Then precipitate was centrifuged in 8000 rpm for 30 minutes. The precipitate was collected and was dialyzed in dialysis bag and dried through lyophilization and get 86 mg Polysaccharide (P.S.)

Synthesis of Ag-nanoparticles: The silver nanoparticles were prepared by using polysaccharide as reducing agent. The process performed by adding 20 mL of 0.5 mg/ml of polysaccharide into 20 ml of aqueous solution of 1 mM $AgNO_3$ for reduction of Ag^+ ions into Ag^0. The total mixture was stirred with magnetic bar for 14 hours at 100ºC. The reddish brown color of the solution indicated the presence of Ag nanoparticles which was confirmed by UV-Visible spectrophotometer.

Determination of optimum temperature of synthesis of ag-nanoparticles: 20ml of 1mg/ml polysaccharide was divided into 5 test tube with equal volume. 4ml of 0.001M $AgNO_3$ solution was added in each test tube. Then all test tubes were kept in respective temperature such as 16ºC, 30ºC, 40ºC, 50ºC, 100ºC for 2 hours.

The antimicrobial activities of Ag-nanoparticles: The antimicrobial activity of polysaccharide based SNPs were done on human pathogenic *Salmonella typhi*, *E. coli*, *K. pneumoniae*, *Pseudomonas sp.* , *S. typhimurium* by disc method using 0.5 mM and 1mM concentration of prepared nano solution and standard nano as control.

Nuclease activity of synthesized Ag-nanoparticles on plasmid DNA: Total DNA was extracted from bacterial (E. coli) by the phenol/chloroform method. DNA concentration was measured with the Bio-Photometer (Eppendorf). 2 mL of diluted DNA sample (200 µg) was prepared and 20 µL of polysaccharide based Ag-nanoparticles (500 µg/mL) was added to it. Then the sample was left undisturbed at 37^0C for 10 min and analyzed the effect using UV-VIS spectroscopy taking same quality and quantity of DNA as control in sterilized distilled water. Again the degradation effect on DNA of polysaccharide based silver nanoparticles confirmed by taking 5 µL of DNA solution (400 µg/mL) were mixed with 200 µL of a suspension containing the nanoparticles (500 µg/mL) and incubated at 37^0C for 10 minutes. After incubation, an aliquot was sampled and analyzed by electrophoresis run at 100 V for 2 h in 1% agarose gel. The gels were stained with ethidium bromide and images were acquired.

Compare the effectiveness of Ag-nanoparticles and Ciprofloxacin against different microorganisms: By using disc method 0.5mM ciprofloxacin antibiotic was compared with 0.5mM nanoparticles.

Photocatalytic activity of synthesized Ag-nanoparticles on congo red (dye): In this experiment Ag-nanoparticles, synthesized from hog plum was used to decolourize. 0.0001mg of congo-red was added in 50 ml distilled water and equally divided into two beakers. Then, 2 ml of 1mg/ml synthesized nanoparticles were added in 1 beaker. So to maintain the concentration 2ml water was added in another beaker. After that the reaction was carried in presence of light and 2ml aliquot was taken from each beaker after 30 mins. This reaction was continued for 4 hours. All of the aliquot was scanned by an UV-VIS spectrophotometer.

RESULTS AND DISCUSSION

Isolation and purification of Polysaccharide (PS) from fruit fiber of *Spondias mombin*: The *Spondias mombin* was boiled in 250 ml of distilled water for 5 hours. The mixture was filtered. The aqueous extract was precipitated in ethanol and then centrifuged at 8000 rpm for 30 min. The precipited material was dissolved in distilled water and dialyzed through DEAE cellulose bag for 2 hours. The material was lyophilized. The lyophilized material was 86 mg.

Figure 1. *Spondias mombin* plant

Synthesis of Ag-nanoparticles: The synthesis of Silver nanoparticles by reduction of the aqueous metal ions during exposure of 20 mL of 0.5mg/mL of polysaccharide extract from *Spondias mombin* 20 mL of aqueous solution of 1 mM $AgNO_3$ detected by color change from light brown to brownish. In case of negative control (silver nitrate solution alone), no change in color change was observed. The silver nanoparticles synthesis further confirmed by UV-VIS spectra. UV-VIS absorption spectrum of silver nanoparticles in the presence of polysaccharide extract was shown in figure 2. The Surface Plasmon band in the silver nanoparticles solution remains close to 436.46 nm throughout the reaction period, suggesting that the nanoparticles were dispersed in the aqueous solution with no evidence for aggregation in UV-VIS absorption spectrum.

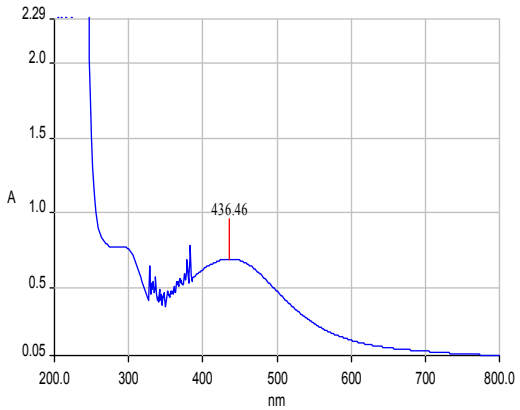

Figure 2. UV-VIS absorption spectrum

Determination of optimum temperature of synthesis of ag-nanoparticles: 20 ml of 1mg/ml polysaccharide was divided into 5 test tube with equal volume. 4ml of 0.001M $AgNO_3$ solution was added in each test tube. Then all test tubes were kept in respective temperature such as 16ºC, 30ºC, 40ºC, 50ºC, 100ºC for 2 hours. So 100ºC is the optimum temperature for the synthesis of nanoparticles.

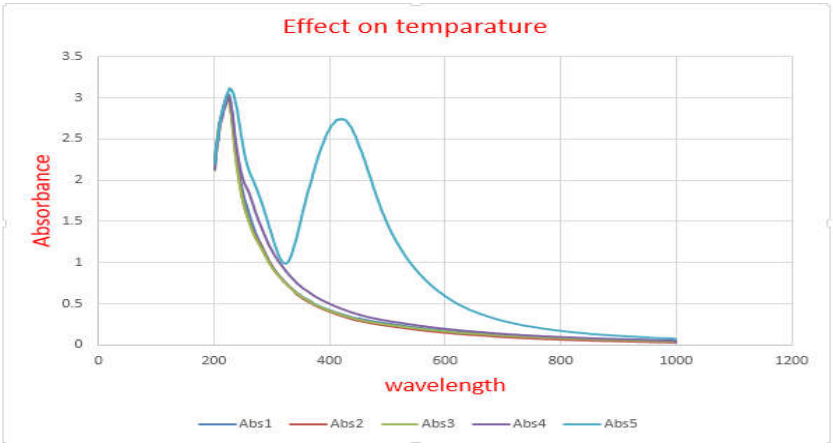

Figure 3. Determination of optimum temperature of synthesis of ag-nanoparticles

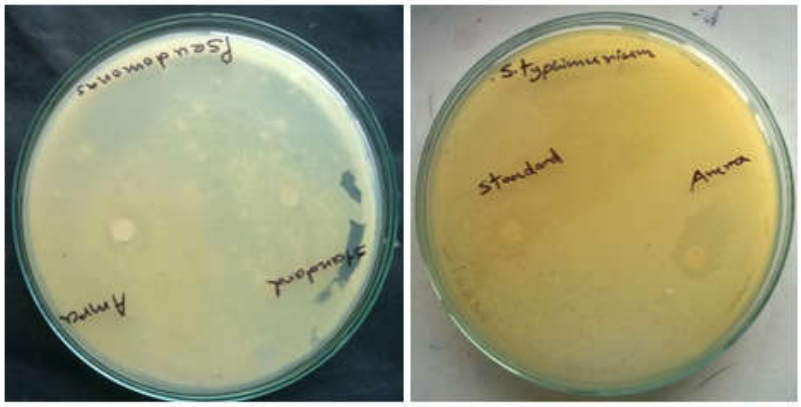

Figure 4. The antimicrobial activities of Ag

Figure 5. The effectiveness of Ag-nanoparticles and Ciprofloxacin against different microorganisms

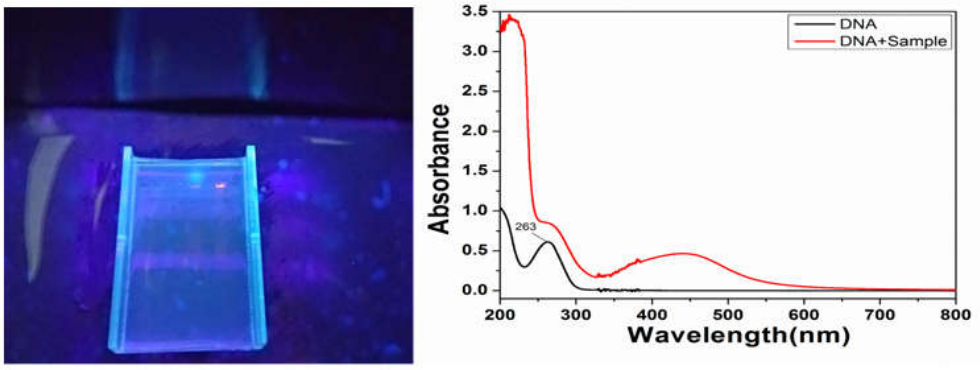

Figure 6. Nuclease activity of synthesized Ag-nanoparticles on plasmid DNA

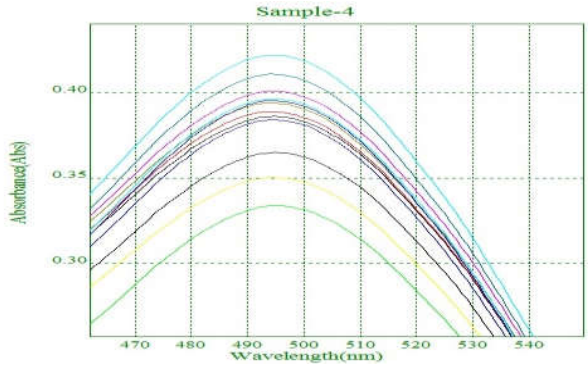

Figure 7. Photocatalytic activity of synthesized Ag-nanoparticles on congo red (dye)

The antimicrobial activities of Ag-nanoparticles: The antimicrobial activity of polysaccharide based SNPs were done on human pathogenic *Pseudomonas sp.*, *S. typhimurium* by disc method using 0.5 mM and 1mM concentration of prepared nanoparticles solution and standard nano as control. Respective nanoparticles showed antibacterial activity against *Pseudomonas* sp.*, S.typhimurium* by creating clear zones on agar plates.

Compare the effectiveness of Ag-nanoparticles and Ciprofloxacin against different microorganisms: By using disc method 0.5mM ciprofloxacin antibiotic was compared with 0.5mM nanoparticle. Ciprofloxacin gave larger clear zone than nanoparticles.

Nuclease activity of synthesized Ag-nanoparticles on plasmid DNA: The effect of polysaccharide based Ag-nanoparticles on plasmid DNA was given. The nanoparticles was responsible for degradation of the plasmid DNA into nucleotides. This result predicted that the bactericidal effect of polysaccharide based SNPs due to degradation of the genomic DNA.

Photocatalytic activity of synthesized Ag-nanoparticles on congo red (dye): After 30 mins interval samples were withdrawn and analyzed spectrophotometrically using UV-Visible spectrophotometer at 420 nm. A significant decolourization rate was observed for the dye Congo red. The % of reduction of the dyes was calculated using the formula,
%Red = (*O D. of control - O.D.of test) x 100)/ OD of control*
A significant decolourization rate 66.67% of congo red dye by synthesized silver nanoparticles was observed within 4 hr of incubation.

Conclusion

Synthesis of Ag- nanoparticles by using polysaccharide extracted from fruit fiber of *Spondias mombin*, an eco-friendly and simple process and also economically cheap. The polysaccharide based SNPs responsible for destruction of different multi drug resistant (MDR) human pathogenic bacteria.

In conclusion it has been demonstrated that bactericidal activity of polysaccharide based SNPs were due to degradation of bacterial DNA. These nanoparticles showed a significant catalytic activity (decolonization rate) ona few times (specifically Congo red). The effectiveness of these nanoparticles was highly comparable with respect to famous antibiotic Ciprofloxacin.

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Conflict-of-Interest Statement: The author declares that he has no conflict of interest.

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