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# Nano ice based on silver nanoparticles for fish preservation

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**Abstract**
Current work deals with novel application of biosynthesized silver nanoparticles to make antimicrobial nano ice for preservation of fish. World fish production has increased by leaps and bounds. Fish products are distributed with minimal treatment before sale to the customer. In retail establishments, the fishes are regularly stored in ice to prevent their spoilage and growth of pathogens. Silver nano products are extensively used for various antimicrobial applications. Though silver based compounds are being used extensively in food storage application, there are no previous studies reporting the incorporation of metal nanoparticles in ice for enhanced storage of any food item. Silver nanoparticles biosynthesized using banana midrib extract having a size range of 20 - 40 nm has been utilized for the preparation of antimicrobial nano ice. Silver nanoparticle based ice has been found to decrease the amount of pathogens present on the surface of stored fish.

**Keywords:** Green synthesis; silver nanoparticle; antimicrobial ice; preservation

## 1. Introduction
From prehistoric period ice played a crucial role in food preservation and freshness. Eskimos survives in Polar region with ice preserved food hunted long back. Ice can preserve any beings without any aging or decay, ice aged mammoth preserved in glacier is an example [1]. Ice has been utilized extensively from time immemorial to keep food borne pathogens from infecting perishable food especially fish and fish based products. Due to technological advancement new methods have been adopted for better and long lasting preservation of sea foods. Antibiotic ice has been used for fish preservation which is nothing but usage of tetracycline based antibiotics during ice formation. Ozonated ice has also been used for antimicrobial activity utilizing the antimicrobial power of ozone. Antimicrobial ice has been developed recently [2] for control of food borne surface pathogens on fish skin. The antimicrobial agent used by them is chlorine dioxide. Also Per Acetic Acid (PAA) is the only compound which has been approved by the USFDA for usage in the formation of antimicrobial ice for fish preservation [3]. Similarly Wild Thyme hydrosol (*Thymus serpyllum*) has been used in the formation of antimicrobial ice [4]. Nanocluster Ice on hydrophobic metal surfaces has been analysed [5] and also reported on the smallest particle of ice – a water hexamer.
Chemical synthesized silver nanoparticles exhibits cytotoxicity and genotoxicity in rat and mammalian cell [6]. By green chemistry and green nanotechnology researchers [7-13] started to focus more on products and application of nanoparticles, also by green synthesis of nanoparticles toxicity and harmful environmental effects may be reduced. In biosynthesis, extracts of parts of plant or the whole plant are being utilized for the synthesis of nanoparticle. It is possible to synthesize nanoparticle at room temperature using aqueous extract of parts of plants and animals. Silver nanoparticles and its compounds possess deleterious activity against 750 or more known pathogens comparing to other metallic nanoparticles [14]. Even though nanoparticle such as iron and copper exhibits microbicidal activity, it will not be as proactive like silver nanoparticle [15]. It is true that chemically synthesized nanoparticle achieve better size and shape control comparing to biosynthesized nanoparticle, but antimicrobial activity against different pathogens with cyto-compatible nature were reported mostly from biosynthesized nanoparticle. Products or materials applications using biosynthesized nanoparticles are of few in number when compared to chemically synthesized nanoparticles and other physical methods. Biosynthesized metal nanoparticles may be utilized for development of hybrid products using the inherent properties of the leaf extract or animal

extract. Biosynthesised silver nanoparticle possesses wide range of applications such as antimicrobial cream [16], antimicrobial tissue paper [17] antimicrobial membrane [18] etc. Silver nanoparticle synthesized rapidly [19] using leaf extract exhibits stability and size controlled property. Owing to its antimicrobial property [20-22] many applications came into role play.

Recently silver nanoparticles have been allowed for direct addition in drinking water at the concentration of 100 mcg per liter by US FDA for neutralizing water borne pathogens after elaborate trials. Hence it will not be of much toxicity to allow silver nanoparticles for the formation of antimicrobial ice for preventing fish surface borne pathogens. Here we report a novel product such as antimicrobial ice using biosynthesized silver nanoparticle. In this study, antimicrobial ice formed using biosynthesized silver nanoparticles were used for antimicrobial fish [21] surface studies. The silver nanoparticle concentration used in all the antimicrobial ice experiments is 1 µg/ml which is far below the limit set by USFDA for direct addition of nanoparticles in drinking water.

## 2. Materials and method

### 2.1 Synthesis of silver nanoparticles

Banana midrib (*Musa paradisiaca*) were collected and utilized for extract preparation for the synthesis of nanoparticles. The plant leaf broth extract is prepared by taking 20 grams of thoroughly washed and finely cut leaves/ banana midrib in a round bottom flask having 100 ml sterile distilled water to the extract. The mixture is boiled for 2 hr. The broth extract is filtered using Whatman paper III and the filtered broth extract is kept at 4º C for further work. Aqueous solution (1mM) of silver nitrate ($AgNO_3$) was prepared and 2 ml of leaf extract of *Musa paradisiaca* was added to 25 ml of 1mM $AgNO_3$ aqueous solution at room temperature.

### 2.2 Characterization of biosynthesized silver nanoparticles

The biosynthesized silver nanoparticles were characterized by UV Vis spectroscopy, FTIR, Atomic Force Microscopy and HR-TEM. Synthesized silver nanoparticles by reducing respective metal ion solution with leaves extract are initially characterized by UV visible absorption spectroscopy. The samples are taken in a 1cm quartz cuvette and measured in a JASCO V 650 spectrophotometer containing double beam in identical compartments each for reference and test solution from 200 nm to 900 nm. FTIR spectral studies were performed to identify the possible biomolecules responsible for the reduction and stability of the metal nanoparticles. The FT-IR measurements were taken for the samples in the liquid state, using Perkin-Elmer Spectrometer in the range of 400-4000 $cm^1$ for transmittance values.

Atomic Force Microscopy image was taken using Park system AFM XE 100. The aqueous silver nanoparticles are deposited into a freshly cleaved mica substrate. The sample aliquot was left for 1 min and then washed with deionized water and left to dry for 15 min. The images were obtained by scanning the mica in non – contact mode. The 3D representation of nanoparticle is shown in 1x1 micron scale. High Resolution Transmission Electron Microscopy image was taken using FEI-Technai. The sample was placed on a copper grid and left to dry for 60 min under vacuum. The sample was then subjected to transmission electron microscopy studies.

### 2.3 Preparation of Antimicrobial Nano Ice

As biosynthesized silver nanoparticles were freezed at 0 ºC to form antimicrobial nano ice. This antimicrobial ice is used to preserve the fish. Antimicrobial activity of antimicrobial ice was tested against normal control ice. The control ice was made using distilled water.

### 2.4 Antimicrobial activity of Nano Ice over the fish surface

Freshly caught fresh water fish mullet (*Mugil cephalus*) was obtained from nearby pond in Tiruchirappalli. The average weight of the fishes was 5 grams. The fishes were packed with antimicrobial ice and control ice in a UV disinfected thermocol box. Antimicrobial activity of silver nanoparticle incorporated ice was evaluated at regular time intervals of 60, 90 and 120 min respectively. After each time interval, the fish were taken and put in sterile Milli Q water. Swabbing was done on Mueller Hinton agar from each test tube and no of colony forming units were counted. The scheme below depicts antimicrobial action of ice for preservation of fish inside the box and evaluated the sample after regular interval of time.

## 3. Results and discussion

Reddish brown colour was formed when banana midrib extract has been added to silver nitrate solution which is due to the formation of silver nanoparticle. Reducing property of phytochemicals in the midrib extracts plays key role in the formation of silver nanoparticle. Formation of nanoparticle has been confirmed using UV visible absorption spectrum. The graph shows the peak absorption wavelength for silver nanoparticle by addition of different concentration of leaf extract (Fig 1). The increase in intensity of absorption peak at 433 nm reveals that 2 ml extract as the optimized concentration. The functional group analysis was done using FTIR spectroscopy, were the band at 3394 $cm^{-1}$ represent OH bending due to water molecules (Fig 2). 2076.49 $cm^{-1}$ represent C=N stretch. At 1637.09 $cm^{-1}$ amide band is highlighted. Aromatic amines (C-N stretch) expressed at 1373.58 $cm^{-1}$ Polyols (C-O-) and aromatic compounds are represented at 1239.64 and 665.43 $cm^{-1}$[19].

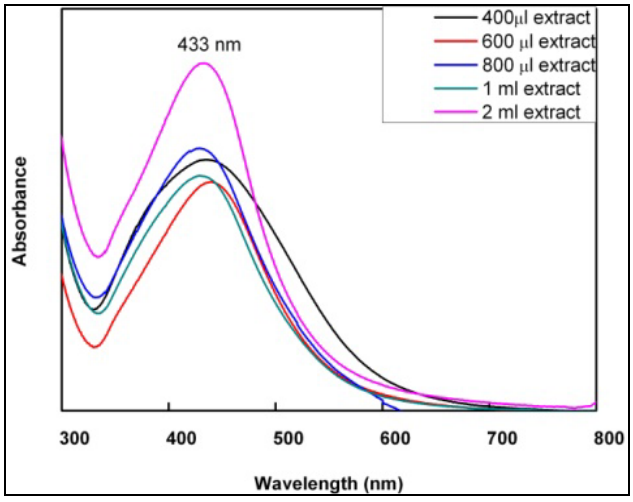


**Fig 1:** UV visible absorption spectroscopy of biosynthesized silver nanoparticles using different volume ratios of extract to 25 ml of 1 mM silver nitrate banana midrib exhibiting a SPR peak at 433 nm.

The average size confirmation and topological image analysis were done using atomic force microscopy (AFM). AFM at non contact mode gives raster scan of the nanoparticle coated on mica substrate, where x, y and z scan is possible. Fig. 3 shows the topology and three dimensional morphology of the biosynthesized silver nanoparticle which are spherical in shape and the average size of the silver nanoparticle is estimated to

be 34 nm. High resolution Transmission electron microscope (HRTEM) image shows further confirmation of the size and morphology of the biosynthesized silver nanoparticle. HRTEM image of silver nanoparticle (Fig. 4) shows spherical morphology when taken at different scales of 5nm, 10nm, 20nm and 50nm scales. The fringe pattern of silver nanoparticle can be observed in the figure at 5 nm scale with d spacing of 0.23 nm corresponds to 111 plane of silver nanoparticle, according to the JCPDS No.87-0720 [19].

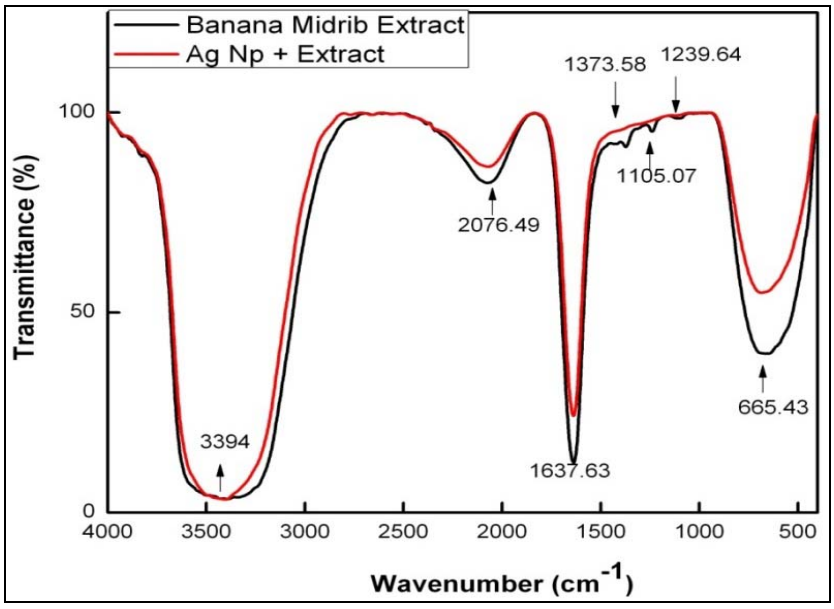


**Fig 2:** FTIR spectroscopy of biosynthesized silver nanoparticles and banana midrib extract exhibiting significant bands at wave numbers 3394, 2076.49, 1373.58, 1105.07, 1239.64 and 665.43.

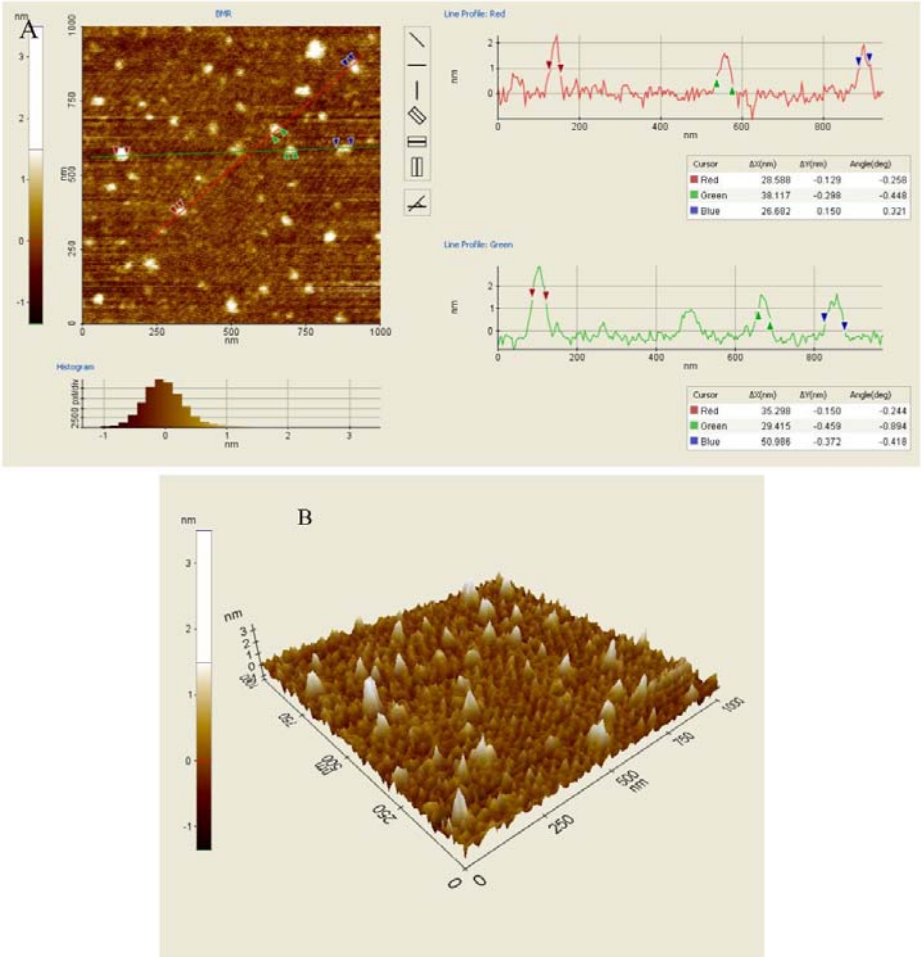


**Fig 3:** 2D and 3D **A**FM analysis (A and B) of the biosynthesized silver nanoparticle exhibiting uniform size and spherical shape.

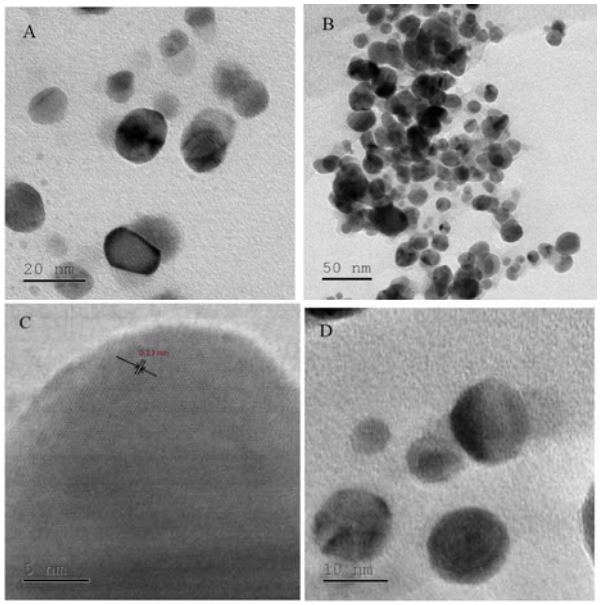


**Fig 4:** HRTEM imaging of biosynthesized silver nanoparticles for morphological analysis and structural property of the particle where the d spacing (0.23nm) for silver lattice fringes can be observed in 5 nm scale image (C).

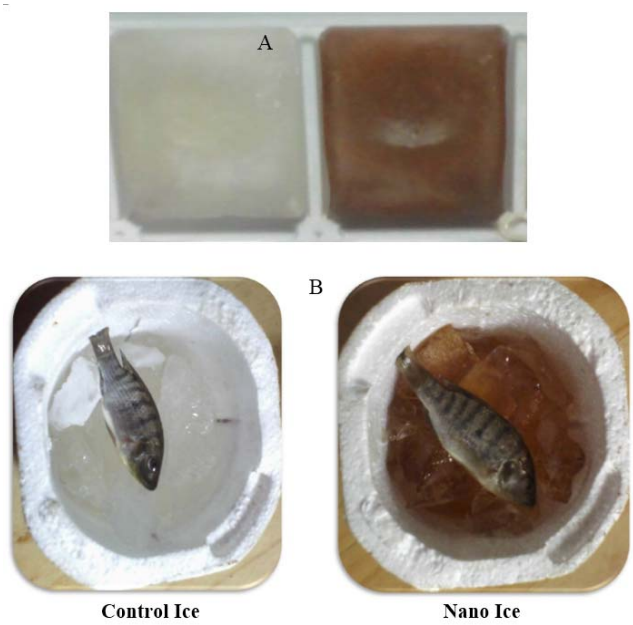


**Fig 5:** Colour variation between A - Control Ice and Nano Ice. Fish *Mugil cephalus* kept in Control Ice (without silver nanoparticle) and Nano Ice (with silver nanoparticle) in a sterile thermacol box.

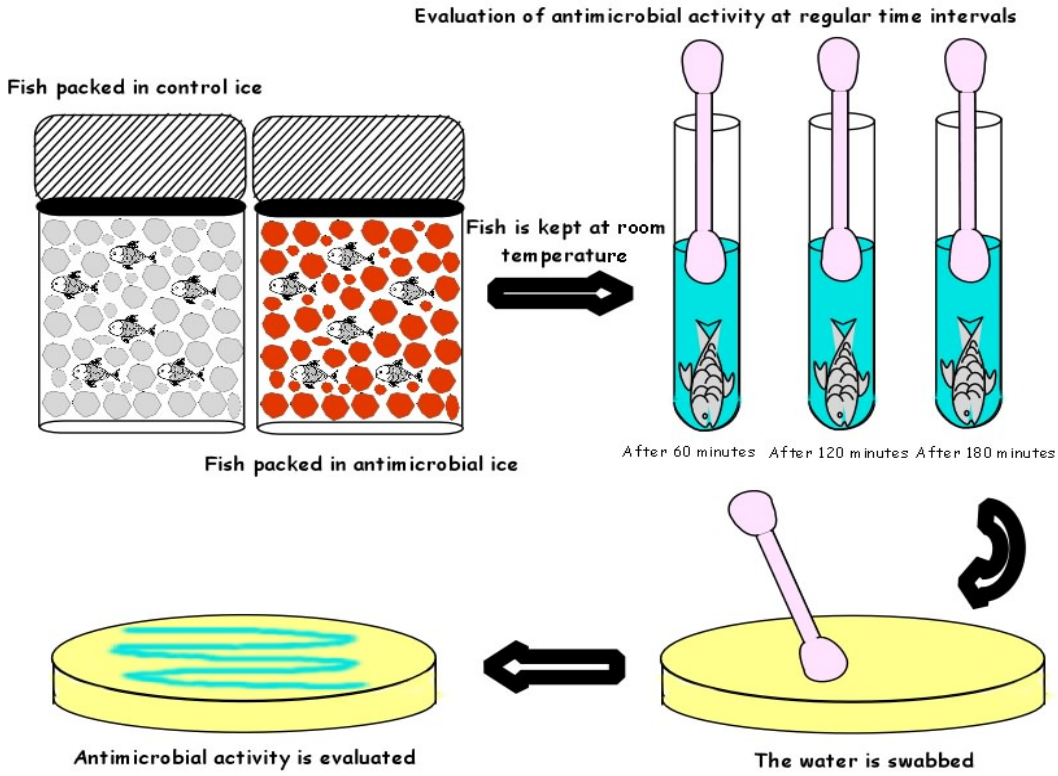


**Fig 6:** Schematic representation of the steps carried out for the evaluation of antimicrobial activity of the antimicrobial ice.

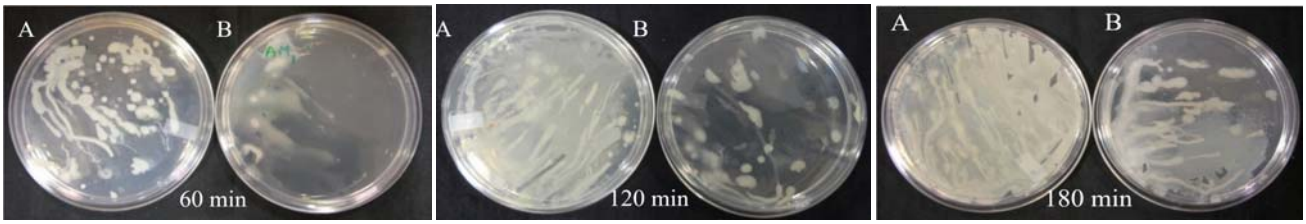


**Fig 7:** Photographic images of the CFU formed on Mueller Hinton agar plate of (A) control ice and (B) antimicrobial ice on the fish surface of *Mugil cephalus* after incubation at time intervals of 60 min, 120 min and 180 min.

Antimicrobial ice and control ice were having reddish brown and white colour respectively (Fig 5) which can be due to the presence of silver nanoparticles in antimicrobial ice. The activity of antimicrobial ice was tested on the surface of fresh water as described in the scheme (Fig 6). Commonly available *Mugil cephalus* (Mullet fish) was selected as experimental model for fresh water fish. The antimicrobial activity was carried out by swabbing the surface of fish at intervals of 60, 120 and 180 minutes on Mueller Hinton agar plates. In all the cases, the fish preserved in normal control ice exhibited increased CFUs (colony forming unit) and growth of pathogens, while the fish preserved in antimicrobial nano ice has less CFUs and growth. The antimicrobial activity of the antimicrobial ice in fresh water fish was evaluated at regular time intervals such as 60, 120, and 180 minutes. The activity for antimicrobial ice in comparison with the control ice is shown in Fig. 7. The isolate was identified as *Acinetobacter*. *Acinetobacter* is a food borne pathogen and it is widely responsible for the spoilage of fish. The antimicrobial ice inhibited the growth of *Acinetobacter* to a greater extent due the antimicrobial activity of silver nanoparticles, while the control ice exhibits the presence of *Acinetobacter*.

## 4. Conclusion

Silver nanoparticles biosynthesized using *Musa paradisiaca* (Banana) midrib extract present in the Nano Ice has thus been able to decrease the microorganism load on the *Mugil cephalus* fish surface. Antimicrobial Nano Ice incorporated with biosynthesized silver nanoparticles can be used as a nano-preservative for fish. Biosynthesized silver nanoparticles have been utilized rather than chemical synthesized silver nanoparticles to be more biocompatible. Nanoparticle impregnated ice can be further explored for various other food packaging applications.

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