Biosynthesis of Silver Nanoparticles from Orange Peel Extract and its Antibacterial Activity against Fruit and Vegetable Pathogens

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Abstract :- The biological syntheses of silver nanoparticles emerge as an eco-friendly and exciting approach in the field of nanotechnology. Silver nanoparticles (AgNPs) have been known to have inhibitory and bactericidal effects. Resistance to antibacterial agents by fruits and vegetable pathogenic bacteria has emerged in recent years. Here, we report on the synthesis of metallic nanoparticles of silver using a reduction of aqueous Ag+ ion with the extract of Orange peel (*Citrus sinensis*). Water-soluble organics present in the Orange peel materials were mainly responsible for the reduction of silver ions to nano-sized Ag particles. Synthesised nanoparticles were characterised using UV-visible spectroscopy, Transmission Electron Microscope (TEM), Energy-Dispersive X-ray (EDAX). XRD result confirmed the presence of nano-crystalline Ag particles. The antibacterial effect of silver nanoparticles produced in this study was studied against four fruits pathogenic bacteria. This work was to use biological method to deposit AgNPs as a protection layer fruit and vegetable. AgNPs were coated on surface of orange and tomato by layer coating method at room temperature. Production of AgNPs and time of these protections were investigated, time length production of the AgNPs has on increasing trend for orange and tomato.

Keywords: Silver nanoparticles, Biosynthesis, *Citrus sinensis*, Antibacterial

I. INTRODUCTION

Nanotechnology is now creating a growing sense of excitement in the life sciences especially in biomedical devices and biotechnology [1]. Nanoparticles exhibit completely new or improved properties based on specific features such as size, distribution and morphology [2]. Nanoparticles of noble metals such as Gold, Silver, Titanium, Iron, Zinc, Carbon, Copper, Palladium and Platinum are widely applied in products that directly come in contact with the human body, such as shampoos, soaps, detergents, shoes, cosmetic products, and tooth paste, besides medical and pharmaceutical applications[3], [4]. Although chemical and physical methods may successfully make pure, well defined nanoparticles, these methods are rather expensive and potentially hazardous to the environment. Use of biological organisms, plant extract or plant biomass could be alternative to chemical and physical methods for production of nanoparticles in an eco-friendly manner [4].

The silver nanoparticles have various and important applications and they have been known to have a disinfecting effect and has applications ranging from traditional medicines. It's been reported that silver nanoparticles (AgNps) are non-toxic to humans and are effective against microbes at low concentrations and have no side effects. Moreover, several salts of silver and its derivatives are commercially manufactured as antimicrobial agents [5]. In small concentrations, silver is safe for human cells, but toxic for microorganisms [6]. Various fruit peels extracts have been shown to have some antimicrobial activity. In this study orange, pomegranate, lemon, custard apple fruit peels extracts are used for synthesis of silver nanoparticles. Their antibacterial activity is evaluated.

II.MATERIALS AND METHODS

In this study orange, guava, pomegranate, lemon fruit peel were collected from local fruit juice shop at Adyar, Chennai in the month of December 2013. The Samples were bought to the laboratory in Polythene covers and cleaned thoroughly using distilled water to remove dust particles adhering the surface of these fruits. The washed fruits were then kept under aeration for complete drying, for a period of 3 to 4 days. The dried fruit peels were cut into small pieces using scissors and were powdered into fine particles. 2 g of the powdered fruit peel was added to 50 ml of distilled water and boiled for 20 min at 50°C .The Crude extracts were filtered through Whatman's filter paper and the filtrates were stored in conical flasks at 4°C for further use.

In a typical biosynthesis production scheme of silver nanoparticles, 1 mL of the extract was added to 9 mL of double distilled water making the solution to 10 mL in a test tube. The synthesis of silver nanoparticles involves addition of AgNO3 1mM solution to 10 mL of filtrate in test tubes and kept under dark conditions for 48 hrs without adding silver nitrate were kept as control.

The reduction of silver ions from AgNO3 to Silver Nanoparticles was monitored by measuring the absorbance as a UV-vis spectrum of the reaction mixture on a HITACHI U-2900 UV-Visible Spectrophotometer. The absorbance was recorded from 300 to 700 nm for detection of biologically synthesized Silver Nanoparticles. TEM observations were performed on a HITACHI, H-7650 (Japan) instrument operated at an accelerating voltage of 100kV. The size distribution of the resulting nanoparticles was estimated on the basis of TEM micrographs. Energy-dispersive x-ray (EDX) spectroscopy analysis for the confirmation of elemental silver was carried out for the detection of elemental silver. After freeze drying the purified Silver Nanoparticles were analysed by XRD using SEIFERT JSO- DEBYEFLEX 2002 model.

The antibacterial activity was done on bacterial organisms like Escherichia coli (E.coli), Pseudomonas aeriginosa, Salmomella typhi and Staphylococcus aureus. The well diffusion method was carried out by using Muller Hinton Agar. The inoculums was prepared in sterile nutrient Broth and then incubated at 37°C until the turbidity was reached up to 0.5 McFarland standards [NCCLS (National Committee for Clinical Laboratory Standards), M100-S12 Performance Standards for Antimicrobial Testing: Twelfth Information Supplement, 2014]. The Muller-Hinton agar plate was inoculated with 2 ml of inoculums by spreading the swab of culture on the plate. Agar was punched using of sterile cork borer to create 6 mm wells. 40 µl of fruit extract, 1mM of silver nitrate 40 µl, 40µl of synthesized silver Nanoparticles and 40 µl of antibiotics were added in respective wells. Streptomycin was used as a control. All plates were incubated at 37°C for 18 hrs. Zone of inhibition was measured after incubation using a scale.

The antimicrobial activity of silver nanoparticles was checked by determination of Minimum Inhibitory Concentration. Suspension of bacterial culture was prepared and swabbed on the agar plates then wells were made on the plates. Different concentrations of the Silver Nanoparticles i.e. (10, 20, 30, 40 µl) were added to the wells. All the plates were incubated at 37°C for 24 hours in order to determine the inhibitory growth of the silver nanoparticles on specific pathogen. The MIC was defined as the lowest concentration at which the nanoparticles inhibited the growth of test organisms.

Coating Silver Nanoparticles on Fruits: The tomato and orange fruits were coated with the silver Nanoparticles 20 ml of synthesized silver nanoparticles was taken in a beaker, the tomato and orange of same size was washed with running tap water. Then the fruits were dipped in the 20 ml of synthesized silver Nanoparticles. Tomato and orange were marked as control and test respectively. The control and test samples were kept at normal room temperature for observation of results.

III. RESULTS AND DISCUSSION

On screening among the four Fruit peel extract the *Citrus sinensis* fruit peel extract showed good stability **Fig. 1** and the remaining three fruit peels showed aggregation and no stability.

Fig.1 Synthesised AgNPs from *Citrus sinensis*

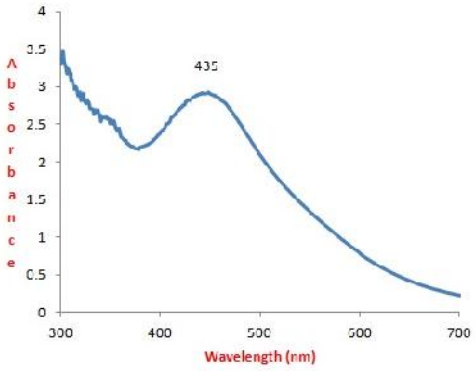

Fig .2 UV-Visible Spectrum of Orange Fruit peel Extract

Silver nitrate has distinctive properties such as good conductivity, catalytic and chemical stability. The formation of AgNPs was found to be successful as suggested by initial changes in colour. It is well known that AgNPs exhibit brown colour in aqueous solution due to excitation of surface plasmon vibrations in AgNPs. In the present study the Silver Nanoparticles were characterized by UV-Vis spectroscopy, a mainly used technique for structural characterization. The absorption spectrum of the silver nanoparticle solution showed a surface Plasmon absorption band of 425 nm, signifying the existence of spherical shaped silver Nanoparticles. The peak shows that the synthesized nanoparticles had a plasmon absorption band of 435 nm after 48 hrs.

Fig. 3 The TEM image of the Silver Nanoparticles produced at different concentrations were roughly circular, spherical in shape and polydispersed shows a representative TEM image recorded from the drop-coated

film of the Ag-NPs. The particle size histograms of silver particle range in size from 25 to 30 nm and possess an average size of 30 nm.

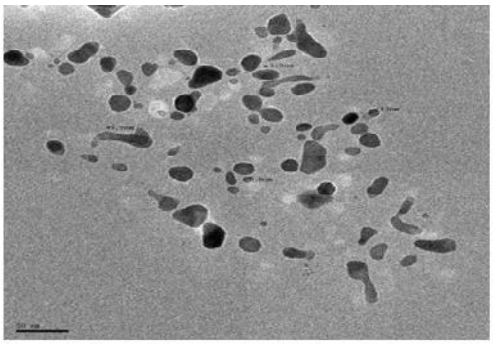

Fig. 3 Transmission Electron Microscopy

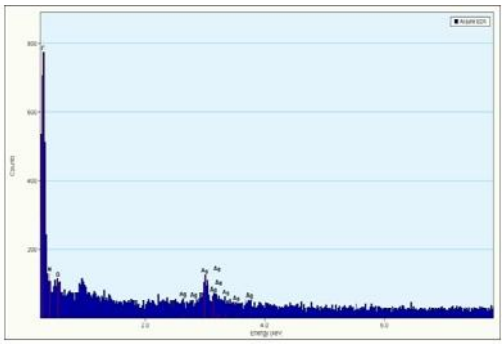

Fig. 4 Energy dispersive X-ray Analysis (EDAX)

The energy dispersive X-ray analysis (EDX) reveals strong signal in the silver region and confirms the formation of Silver Nanoparticles. The typical optical absorption peak at 3 KeV confirms the metallic nanoparticles due to surface plasmon resonance. The EDX on the SEM analysis of the Silver Nanoparticles was performed for elemental study. The freeze-dried Silver Nanoparticles were coated with gold in a sputter coater and mounted on specimen stubs with double-sided taps**. Fig. 4** shows the EDX spectrum of spherical nanoparticles prepared with this bioreduction method. The peaks around 3.5 keV correspond to the binding energies of AgL. During the scanning range of binding energies, no peak of impurity was detected. The results indicated that the product was composed of high purity Ag nanoparticles. A similar EDX spectrum was obtained for each sample analyzed.

The dry powders of the Silver Nanoparticles were used for XRD analysis. The diffraction model in **Fig. 5** corresponds to pure silver metal powder. Later analysis of Silver Nanoparticles precipitate by powder-XRD revealed four peaks at 47° (111), 40° (200) and 25° (220).The peaks were compared with X-ray diffraction database. The XRD database result of silver powders collected from test filtrate strongly supported the presence Silver Nanoparticles. Comparison of test database with online database JCPDS (card No. 04-0783) indicated that test solution consists of Silver Nanoparticles which have face centered cubic (FCC) structure.

The particle size histogram obtained Silver Nanoparticles shows broad distribution of particle size. The average particle size comes out to be 30.29 ± 0.22 nm and the size of particles. The X-ray diffraction pattern obtained for Silver Nanoparticles synthesized by *Citrus sinensis* shows that the silver nanopaticles are crystalline in nature.

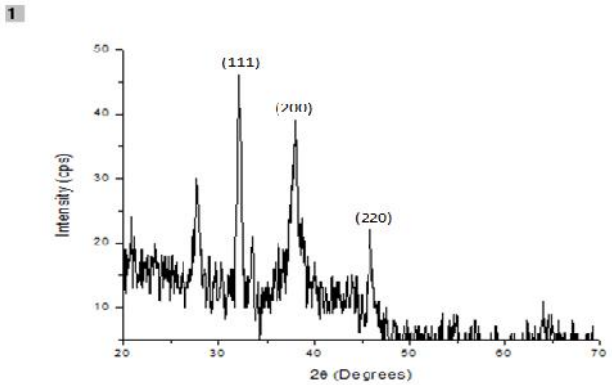

Fig 5 X-ray diffraction analysis

The antibacterial effects of biologically synthesized Silver Nanoparticles have been investigated against, *Staphylococcus aureus, Pseudomonas aeriginosa., Escherichia coli* and *Salmomella typhi* . The test was performed by loading the biologically synthesized nanoparticles into the well A followed by silver nitrate in well B, then Antibiotic (Streptomycin) in well C and fungal extract in well D. It was observed that clear zone of growth inhibition was produced in *Staphylococcus aureus, Pseudomonas aeriginosa, Escherichia coli,* and *Salmomella typhi* . which confirms the antibacterial property of biologically synthesized nanoparticles and higher zones was observed in the well loaded with Streptomycin and no zones was observed in the well loaded with Fruit peel extract.

In this susceptibility test *Pseudomonas* showed more sensitivity in biologically synthesized nanoparticles, *Salmomella typhi* 13 mm, *Pseudomonas aeriginosa* 15mm, *Staphylococcus aureus* 13mm, *Escherichia coli* 15 mm. Among the four pathogenic bacteria experimented bacteria showed resistance to biologically synthesized nanoparticles and all the experimented bacteria showed resistance to Streptomycin throughout the experiment. There is no activity for the fruit peel extract but $AgNO_3$ shows least zone of clearance.

This study has showed that, Streptomycin has highest antibacterial activity than synthesized nanoparticles. The second mean zone of inhibition was found in *Pseudomonas aeriginosa* in Silver Nanoparticles and Streptomycin. The least zone of inhibition was found in S*almonella typhi*.

Table 1: ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES

S. No	**Name of the organism**	**Zone of inhibition (mm in diameter)**			
		Silver Nanoparticles	**Silver Nitrate**	**Fruit peel Extract**	**Streptomycin**
1.	*Staphylococcus aureus*	13	-	-	24
2.	*Salmomella typhi*	10	-	-	19
3.	*Escherichia coli*	15	-	-	24
4.	*Pseudomonas aeriginosa*	19	13	-	20

Table.2 The synthesized Silver Nanoparticles were very effective in controlling the bacterial human pathogens. The Minimum Inhibitory Concentration was checked with human pathogens like *Staphylococcus aureus Salmonella Typhi, E.coli,* and *Pseudomonas aeriginosa*. The silver nanoparticle was experimented in diverse concentrations such as 10, 20, 30, 40. The Silver Nanoparticles had MIC of 20 µl on *Pseudomonas aeruginosa, Staphylococcus aureus and E.coli,* had MIC of 10 µl whereas *Escherichia coli* had MIC of 40 µl of Minimum Inhibitory Concentration.

Table 2: MINIMUM INHIBITORY CONCENTRATION OF SILVER NANOPARTICLES

S.No	**Name of the organism**	**Synthesized Silver Nanoparticles**			
		Zone of inhibition (mm in diameter)			
		10 µl	**20 µl**	**30 µl**	**40 µl**
1.	*Staphylococcus aureus*	**10**	7	10	12
2.	*Salmonella Typhi*	6	11	10	13
3.	*Escherichia coli*	**10**	11	**13**	**15**
4.	*Pseudomonas aeriginosa*	8	**29**	11	13

In the present study we coated Silver Nanoparticles on tomato and orange. Previous studies were carried out with gold nanoparticle coated on apple, cucumber, lettuce and tomato which have the ability to retard oxygen diffusion and it is highly resistant to oxidation [8]. It was observed that all samples were found to be fresher with less wrinkles without any mold thereby it takes long time to decompose.

Fig .6 Coating of silver nanoparticle on orange Fruit

Fig .7 Coating of Silver Nanoparticle on Tomato

It's obvious that duration of production by silver nanoparticles depends upon the kind of experimented samples. The results have shown that it took 10 days for tomato and 15 days for orange to ripen as shown in **Fig.6**. As researchers reported silver nanoparticles are used as biomedicals [10]. Eating the Fruits that was coated with Silver nanoparticles have benefits for human health by having Antibacterial properties [2] [9].

IV. CONCLUSION

The physicochemical properties of nanoparticles with the inhibitory capacity against microbes have led to the increase in the research on nanoparticles and their potential application as antimicrobes.The direct coating of silver nanoparticle is reported for the first time in research. Silver nanoparticles can be used as a protection by delayed ripening of fruit and vegetables. In this study , silver nanoparticle has the ability to retard oxygen diffusion and it is highly resistant to oxidation and we investigate the relation of physical and biological Factors.

V. ACKNOWLEDGEMENT

We would like to thank Prof. R. Rengasamy, Director, CAS in Botany, Univesity of Madras , Guindy campus for providing laboratory facilities. We also thank NCNSNT, University of Madras, Guindy campus for providing characterization studies and UGC herbal science scheme for financial support.

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