**Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)**
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

# Synthesis and characterization of silver nanoparticle using *Melia azedarach* for vegetable coating and antibacterial activity

**J .Uthaya Chandirika, S. Tamil Selvi, G. Annadurai***

*Division of Nanoscience, Sri Paramakalyani Centre for Excellence in Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi - 627 417, Tamilnadu, India.*

**Key words:** AgNPs, Green synthesis, *Melia azedarach*, Anitimicrobial, vegetables, coating.

***Corresponding Author: G. Annadurai,** Environmental Nano-technology Division, Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi – 627412, Tamilnadu, India.

**Abstract**

The present study was concluded that the vegetable coating affects positively on the physiochemical parameters of tomatoes. The coated sample shows significant difference in almost all parameters as compared to control (uncoated) tomatoes. As far as storage period is concerned as increase the quality parameters like weight loss and antimicrobial activity of tomatoes. *Melia azedarach* mediated silver nanoparticles were Characterize by UV-vis Spectrophotometer, Fourier infrared spectroscopy (FTIR), and Scanning Electron Microscope (SEM) examine the antibacterial activity of biological medicated silver nanoparticles against the microbial strains of *E. coli, Streptococcus sp., Klebsiella sp., Bacillus sp., Salmonella sp., Aspargillus niger.*

## Introduction

Silver nanoparticles is the most recent advances in the application and development of edible coatings for fresh and minimally processed fruits, discussing wide range of compounds that can be used in the formulation of edible coatings depending on target application, the available and potential techniques to characterize coated commodities. Have they focusing on the development of new technologies that allow for a more efficient control of coating properties and functionality, and incorporation of nutritional and anti-microbial ingredients to the coatings. Food promotional material have been traditionally conceived as simple containers to transport food from the place where they have been produced to the retail outlet and then to the consumer with no alteration of the food nutritional and organoleptic characteristics [1].
As the important of packaging is saving of vegetables from extraneous pollution, other significant characteristics such as deceleration of impairment, extension of period of time, protective cover from carry affects and sustainment of the food product. Packaging materials should protect food from environmental influences such as heat, moisture, oxygen, enzymes, loss of aromas and unpleasant odor component are contamination from macro-organisms. Moreover, the world commercialize is becoming more demanding and is continuously in need of novel and stable products which, at the same time, could retain the natural properties of food [2].
The term edible coatings in food applications correspond to thin layers of edible materials applied onto surfaces of highly perishable foodstuff, such as fresh-cut fruits and vegetables. The most recent scientific and technological developments in this field will be highlighted and our goal would be to permit the reader getting a complete survey of the increasing use of this biopolymer in food industries [3]. Fruits and vegetables were coated in low molecular of silver nanoparticles, by using sterile cotton swab thrice. Overall encapsulation has been done after drying of vegetables in time period, and stored at room temperature. Samples were stored in aseptic condition for 28 days for further analysis [4].
The major post harvest losses of tomatoes, fresh vegetables are due to fungal infection. Physical disorder many tech have been studied in order to extend the shelf life of fresh products. However, they have advantages and disadvantages. Quality maintenance of fresh products is still a major challenge for the food industry. Nanotechnology will facilitate the development of light and more precise food manufacturing equipment, non-polluting as well as cheaper packaging techniques [5].
AgNPs were synthesized, characterized, and used for the edible vegetable coating on tomato. The weight loss, firmness and antimicrobial activity of coated vegetables were also determined.

## Materials and methods

Tomatoes (*Lycopersicume sculentum)* were purchased from local farm. Fruits with uniform size and shapes, without damage and fungal activity, washed twice water and used for further analysis.

### Chemicals and reagent

All the chemicals were purchased from the HiMedia, Mumbai, India

### Collection of plant materials

*Melia azedarach* plant leaf samples were collected from Sri Paramakalyani Centre for Environmental Science campus, MS University, Alwarkurichi.

### Preparation of leaf extract

Aqueous extract of plants were prepared from freshly collected leaves. 10 g of fresh leaves were surface sterilized using tween 20 was dissolved in for 30 min at 60°C and double distilled water. Then the leaves were cut into fine pieces and dispersed in 100 ml distilled water and boiled for 15 min at 60°C. After that, the solution was filtered through Whatmann No1 filter paper and stored at 4°C in refrigerator for 2 weeks for further experimental studies.

### Synthesis of silver nanoparticles

For silver nanoparticles synthesis, 1 mM silver nitrate solution was prepared in 90 ml of distilled water and the solution was taken in 250 ml Erlenmeyer flask. About 10 ml of plant extract added into silver nitrate solution and kept the flask at room temperature. A control was also maintained without addition of leaf extract. The colour changes observed visually and the synthesis of silver nanoparticles at different time intervals were monitored by UV-vis spectrophotometer of the solution.

### Instruments used for the characterization of nanoparticles

Uv- Visible Spectrophotometer – Used for the absorption of biological mediated silver nanoparticles and plant extract.

Fourier Transform Infrared Spectroscopy (FTIR) - Fourier transform spectroscopy was used to identify the functional groups of the biological mediated silver nanoparticles and extract of *Melia azedarach* were analyzed.

Scanning Electron Microscope and (SEM) - The Scanning Electron microscope was used to analyse the morphology of silver nanoparticles.

### Vegetable coating

Vegetables were coated in biological mediated silver nanoparticle, by using sterile cotton swab thrice. Each coating has been done after drying of vegetables in equal interval of time period, and stored at room temperature. Samples were stored in aseptic condition for 28 days for further analysis [6].

### Weight loss

Two replicates of fruits were used for each treatment. (Tomato - four week), a sample of fruits was weighed regularly to determine weight loss.

### Tomatoes and chilli firmness

The colour changes of clean and stored tomatoes were weighted and firmness tester checking the insight depth by concerning an reserve penetrometer tip into the vegetables [7].

### Antimicrobial activity by well diffusion method

To determine the antimicrobial activity of Ag-NPs, nutrient agar medium was sterilized and sterile media was poured aseptically and the plates were seeded with appropriate micro organisms *E. coli, Streptococcus sp., Klebsiella sp., Bacillus sp., Salmonella sp., Aspargillus niger* by evenly on to the surface of the medium with a sterile cotton swab. The biological mediated synthesized silver nanoparticles solution in respective wells. Then the plates were incubated at $37^0$C for 24 hours. Zones of inhibition were assessed with a measuring scale.

### Result and discussion

The maximum absorption band occurs at the wavelength of 360 nm due to the excitations of surface plasmon resonance in the nanoparticles and the broadening peak indicates particles are polydispersed. While adding the *Melia azedarach* leaf extract into the silver nitrate solution the reduction starts with in 10 min and steadily increases in absorbance as function of reaction time indicates that the continuous formation of silver nanoparticles (Figure 1). After 3 hours of reaction time the absorbance was decreased indicates the completion of reaction [8]. The optimum time required for completion of reaction was recorded as 3 hours. Silver nanoparticle formation was started at 10 min is similar to the report of [9] where they used *Solanum xanthocarpum* berry extract.

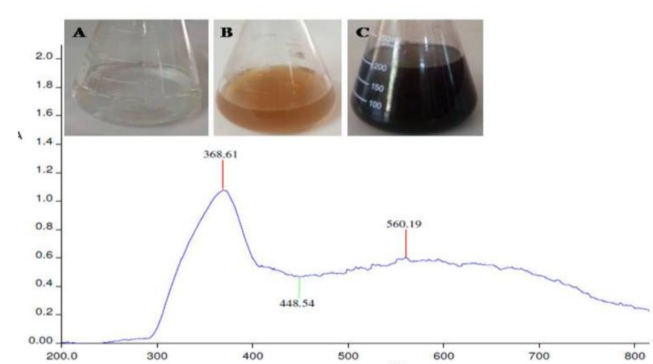


**Figure 1. Visual observation (A) Silver solution (B) plant extract (C) synthesis of silver nanoparticles and UV -vis spectra shows effect of synthesis of *Melia azedarach* leaf extract mediated silver nanoparticle.**

**Fourier transform infra-red spectroscopy (FTIR)**

Fourier transform Infra-Red spectroscopy is a technique for obtaining high quality infrared spectra by mathematical conversion of an interference pattern into a spectrum. Infrared radiations consisting of all wavelengths (eg. 5000-400 $cm^{-1}$) split into two beams, which recombine after a path difference has been introduced. Entire IR region is divided into group frequency region and fingerprint region. In group frequency region, the peaks corresponding to different functional groups can be observed. According to corresponding peaks, functional group can be determined. Each atom of the molecule is connected by bond and each bond requires different IR region so characteristic peaks are observed.

FTIR measurements were carried out to identify the potential functional groups of the biomolecules in the leaf extracts of Melia azedarach responsible for the reduction of the silver ions into silver nanoparticles (Figure 2). The band at 3306 $cm^{-1}$ corresponds to O-H Stretch of hydrogen bonded alcohols, phenols. The absorbance peak at 2918 $cm^{-1}$ was indicates to C-H stretch, alkenes. The band at 1605 $cm^{-1}$ shows characteristics of C=O stretch of alkynes. The absorbance peak at 1413 $cm^{-1}$ was indicates C-C stretch (in ring), aromatics. The band at 1162 $cm^{-1}$ corresponds to C-H, bend of alkynes. The absorbance peak at 1030 cm-1 was indicates to C-N. The presence of aliphatic amine, might act as reducing agents for the synthesis of silver nanoparticles.

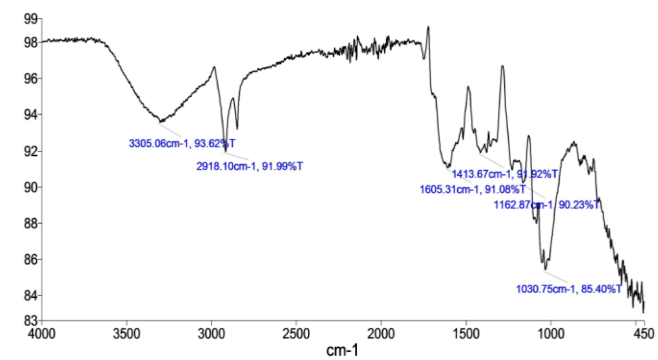


**Figure 2. FTIR spectrum of silver nanoparticles synthesized by leaf extract of (A) *Melia azedarach* (B) Silver nanoparticles.**

**Scanning electron microscope (SEM)**

The surface morphology and size of the silver nanoparticles was identified by Scanning Electron Microscope. SEM image had shown shape and size of the silver nanoparticles synthesized by using leaf extract of *Melia azedarach.* Figure 3 shows individual silver nanoparticles as well as number of aggregates synthesized using leaf extract *Melia azedarach.* It illustrates the particles are preponderantly spherical in form and a few particles aggregates into larger particles with no well-defined morphology and also the size of the silver nanoparticles starting from 75-130 nm.

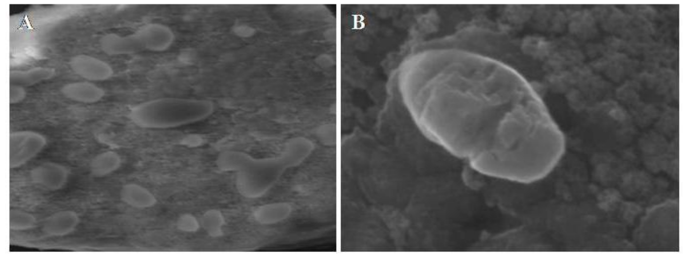


**Figure 3. SEM images of surface modified silver nanoparticles at different (A-B) 300-200nm of surface modified silver nanoparticle.**

**Weight loss**

Three replicate of fruit were used for each treatment, every week a sample of vegetable was removed from each treatment. The vegetable were weighed regularly to determine the weight loss. Weight loss depends upon transpiration of the vegetable. Permeability of coating material is another factor that could reduce the weight loss of tomatoes (Table 1). The results showed that significant weight loss was observed in 20.8% in silver coated vegetable followed by green synthesised silver nanoparticle. The transpiration rate of tomatoes and grapes depended on the thickness of film.

**Tomatoes colour changes**

The tomatoes loss of colour changes during ageing which contribute greatly to its short postgrad life and susceptibleness to microbial pollution. Change in firmness between control coated and Ag NPs coated vegetable samples during four week of storage at room temperature have been studied. Starting colour change evaluate were standardized for control and coated samples. On the second week of storage, uncoated tomatoes began to show a loss of firmness.

**Table 1. Weight loss of vegetables coating**

| Vegetable | Coating type | Storage period (week) Weight in grams | | | | | | % of weight loss |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | |
| Tomatoes | Control | 42 | 39 | 36 | 27 | 25 | 24 | 32.06 |
| | Silver nanoparticle | 47 | 45 | 43 | 42 | 41 | 39 | 20.08 |
| | *Synthesised Melia azedarach mediated silver nanoparticle* | 46 | 45 | 44 | 43 | 42 | 40 | 7.09 |

## Application of edible vegetable coating

Ag nanoparticle was coated in the outer layer of Tomato done under the sterile condition at room temperature. The results conclude that when compared with non-coating, the coated vegetables showed the good shelf life period from 16 to 21 days at room temperature (Figure 4). Ag nanoparticle coated in the outer layer of vegetables (Tomato) was done under the sterile condition at room temperature. It is concluded that when compared with non-coating, figure 4 the coated fruits and vegetables showed the good shelf life period from 20 to25 days at room temperature Moreover, since many of the research, there is still a lot of room for variation and maturation in the development of edible coatings for application in food packaging [10].

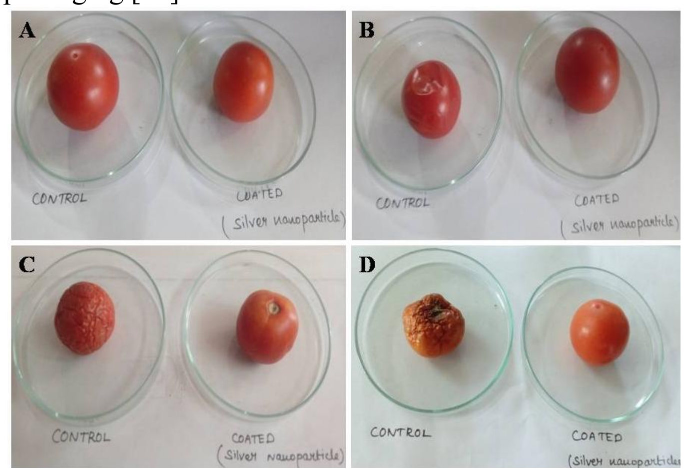


**Figure 4. Vegetables Tomato coated with biological mediated silver nanoparticle (A) day 1 (B) day 6 (C) Day 12 (D) day 16.**

## Mechanism of antimicrobial activity

Further the nanoparticles syntheses by green route are found non-toxic against *Bacillus subtilis, Klebsiella pneumonia, E. coli, Streptococcus. sp* and *Aspergillus niger* were investigated and compared their Antimicrobial activity (Table 2). Gram positive bacteria differ from gram negative bacteria in the structure of their cell walls. The cell wall of gram negative bacteria was made up of lipopolysaccharide layer at the exterior, followed underneath by a thin layer (7-8 nm) of peptidoglycan [11] and it was not as rigid as peptidoglycon because of the covalent linkage between the lipid and polysaccharide. Lipopolysaccharides contain negative charge, and attract the weak, positively charged Ag nanoparticle [12]. The rigid and extended cross-linking not only endows the cell wall with fewer anchoring sites for the Ag nanoparticle, but also makes it difficult to penetrate. However the highest inhibition rate of silver nanoparticles was observed for 1mM concentration [13]. So that nanoparticles penetration was much more in gram negative bacteria than the gram positive bacteria. Thus Ag nanoparticles are more toxic to gram negative bacteria than gram positive bacteria [13].

**Table 2. Antibacterial activity of Silver nanoparticle using bacteria.**

| Organism name | Zone of inhibition (diameter in mm) | | |
|---|---|---|---|
| | 10 µl | 20 µl | 30 µl |
| *E.coli* | 5 | 5 | 7 |
| *Streptococcus* sp. | 7 | 5 | 8 |
| *Klebsiella* sp. | 4 | 6 | 6 |
| *Bacillus* sp. | 6 | 7 | 8 |
| *Salmonella* sp. | 3 | 3 | 5 |
| *Aspargillus niger* | 7 | 8 | 11 |

## Conclusion

In conclusion typically, the biological mediated synthesis of silver nanoparticle can be used as a surface coating on perishable vegetables to enhance microbial safety and extend food shelf life. It is a green, high yield, fast and low cost approach. Therefore the presence study clearly provides novel antimicrobial material which is potentially useful in food packing.

## Acknowledgement

Authors gratefully acknowledge the DST-FIST sponsored programme, Department of science technology, New Delhi, India for funding the research Development (Ref no: S/FST/ESI-101/2010) to carry out this work.

## Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

### References

1. Arantzazu Valdés, Nuria Burgos, Alfonso Jiménez and María Carmen Garrigós: Natural Pectin Polysaccharides as Edible Coatings, Coatings 2015; 5: 865-886.
2. Krochta JM, De Mulder-johnston C: Edible and biodegradable polymer films: challenges and opportunities. Food Technology 1997; 512: 61-64.
3. Ciolacu L, Nicolau AI, Hoorfar J: Edible coatings for fresh and minimally processed fruits and vegetables. In Global Safety of Fresh Produce Ed.; Wood head publishing: Sawston 2014; 233-244.
4. Baldwin EA, Nisperos MO, Chen X and Hagenmaier RD: Postharvest Biol Technol 1996; 9: 151-163
5. Jesteena J, Ragunathan R, and Arul Mozhi K: Development of Low Molecular Chitosan and Its Silver Nanocomposites for the Edible Fruits Coating to Improve the Shelf Life Period. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2014; 5:6, 34-40.
6. EI-Beltagy A, Ayman H, Amer Eissa, E-Sayed S: Agric Eng Int 2013; 15(3): 208-216.
7. Pochanavanich P, and Suntornsuk W: Fungal Chitosan Production and Its Characterization. Letters in Applied Microbiology 2002; 35: 17-21.
8. Solomon SD, Bahadory M, Jeyarajasingam AV, Rutkowsky SA and Boritz C: Synthesis and study of silver nanoparticles, journal of chemical Education 2007; 84(2): 322-325.
9. Amin M, Anwar F, Janjua MRSA and Rashid U: Green synthesis of silver nanoparticles through reduction with Solanum xanthocarpum L. Berry extract: characterization, antimicrobial and Urease Inhibitory Activities against helicobacter. pylori. Int J Mol Sci 2012; 13:9923-9941.
10. Zhang Y, Yang D, Wang X, pandoli O and Gao G: Synergetic Antibacterial Effect of Silver Nanoparticles and Aloe Vera prepared via a Green Method. Nano Biomed Eng 2010; 24: 252-257.
11. Madigan MT and Martinko JM: Brock biology of microorganisms. 11th edition. Pearson Prentice Hall Inc, 2006.

12. Sui J, Tleugabulova D, Brennan JD: Direct and indirect monitoring of peptide-silica interactions using time-resolved fluorescence anisotropy. Langmuir 2005; 21: 4996-5001.
13. Sasirekha R, Mangayarkarasi R, Ganesan S, Santhanam, P: Biosynthesis, characterization antibacterial effects of silver nanoparticle by using *Carica papaya* fruit extract and it's interaction with an anticancer drug (5-fluorouracil). Journal of Innovations in Pharmaceutical and Biological Sciences 2018; 5(1): 01-07.
14. Tiwari, D.K. *et al*: Application of nanoparticles in waste water treatment. World Appl. Sci. J 2008; 3(3): 417-433.