

Green synthesis of silver Nano particles using *Aloe vera* extract for the extension of shelf life period in food using packaging films

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Abstract

In this present study, an eco-friendly and easy method for the synthesis of silver nanoparticles using aloe vera extract as a reducing agent has been done. The absorbance of the aloe vera silver nanoparticles is measured in UV-visible spectrophotometer and it showed a peak at 430nm. The shape and size of the synthesized particle were visualized by scanning electron microscope. The AgNPs were found to have enhanced antimicrobial properties and showed zone of inhibition against *Staphylococcus* and *Pseudomonas* species. From the results it is suggested that the silver nanoparticles synthesised from aloe vera extract showed good antimicrobial activity, thus it can be coated in food wrap films as it prevents the fruits and vegetables from microbial growth and extends the shelf life.

Keywords: Green synthesis, *Aloe vera*, silver nanoparticle, antimicrobial study, edible coating

Introduction

Aloe vera (*Aloe barbadensis*) is a succulent plant species that probably originated in northern Africa. The name *Aloe vera* derives from the Arabic word "Alloeh" is a shining bitter substance, while "Vera" in Latin means true. It is a kind of cacti grows in all seasons and temperature, especially rapid growth is found during summer season. *Aloe vera* is a miracle plant known for several health benefits. *Aloe vera* is very good in antimicrobial resistance. It is used in treating hair fall, skin disease, and weight loss. *Aloe vera* is rich in vitamins, minerals, sugars, enzymes, lignins, amino acids, anthraquinones, saponins, fatty acids, salicylic acid. It is widely used in cosmetics, pharmaceutical and food industry. *Aloe vera* coating maintained the firmness of the fruit and was the best coating to both extend the postharvest shelf life and maintain the sensory properties of the product through the storage period [1]. *Aloe vera* extract acts as a reducing agent for the production of Ag nanoparticles [4]. Green synthesis is a natural method of obtaining nanoparticle. Green synthesis of nanoparticle use biological routes such as those involving microorganisms, plants etc. This method is chosen as they are pollution free, use for renewable feedstock, designing safer chemicals, nontoxic etc. The green synthesis of AgNPs method provides a simple, efficient, environmental eco-friendly and cost effective route for the synthesis of nanomaterials with tunable optical properties directed by particle shape [5].

The biosynthesis of nanoparticles has been proposed as a cost-effective and environmentally friendly alternative to chemical and physical methods. Advantages of using plant extracts: easily scaled up for large synthesis of nanoparticles, no need of high temperature, pressure, energy and toxic chemicals and more advantageous over use of microorganisms by less elaborate process of maintaining cultures, reduces cost of microorganism isolation and their culture media. Nanoparticles are particles between 1 and 100 nanometres (nm) in size with a surrounding interfacial layer. The interfacial layer is an integral part of nanoscale matter, fundamentally affecting all of its properties. The

interfacial layer typically consists of ions, inorganic and organic molecules. Organic molecules coating inorganic nanoparticles are known as stabilizers, capping and surface ligands, or passivating agents. Silver nanoparticles (colloidal solution) have unique optical, electronic, and antibacterial properties, and are widely used in areas such as biosensing, photonics, electronics, and antimicrobial applications. Most applications in biosensing and detection exploit the optical properties of silver nanoparticles, as conferred by the localized surface plasmon resonance effect. Application and the benefits of nanotechnology in different areas of food industry that include bioactive nanoencapsulation, edible thin film, packages and nanosensors [7]. A specific wavelength (frequency) of incident light can induce collective oscillation of the surface electrons of silver nanoparticles. The particular wavelength of the localized surface plasmon resonance is dependent on the silver nanoparticle size, shape, and agglomeration state. Silver nanoparticles incorporated sodium alginate thin film shows good antibacterial activity against test strains [9]. Silver nanoparticles of various concentrations were supplemented in liquid systems. As results, Yeast and *E. coli* were inhibited at the low concentration of Ag nanoparticles, whereas the growth-inhibitory effects on *S. aureus* were mild. The free-radical generation effect of silver nanoparticle on microbial growth inhibition was investigated by electron spin resonance spectroscopy. The formation of nanoparticle is analysed through visible UV spectroscopy. Food wrap is a thin plastic films typically used for sealing food items in containers to keep them fresh over a longer period of time. Plastic wrap, typically sold on rolls in boxes with a cutting edge, clings too many smooth surfaces and can thus remain tight over the opening of a container without adhesive. Common plastic wrap is roughly 0.5 thousandth of an inch (12.5 μm) thick. The trend has been to produce thinner plastic wrap particularly for household use. *Aloe vera* gel treatments may be a useful non-chemical way of maintaining raspberry fruit quality and extending their postharvest life [15]. In this work the prepared

Aloe vera silver nanoparticle sample is coated in the food wrap films to increase shelf life period and to prevent from antimicrobial growth in packed material.

Materials and Methods

1. *Aloe vera*, Silver nitrate with molecular weight - 169.87, Fresh fruits and vegetables, Food wrap films / use & throw cups, Glass wares.

2. Preparation of *Aloe vera* extract

The matured *Aloe vera* leaves collected from the residential terrace garden has been washed thoroughly until the dirt is removed. The washed leaves divided into two parts as gel and the outer leaf. To make it as an extract the gel was grounded in a blender and the mixture was filtered to remove the fibres. In the same way the outer leaf along with 50ml of distilled water was grounded in a blender and the mixture was filtered. The filtered extracts have been refrigerated for the further studies.

3. Preparation of Silver Nitrate

3mM silver nitrate (molecular weight-169.87) crystals dissolved in 100ml of distilled water.

4. Antimicrobial activity

4.1. Revival of the Isolate from Glycerol stock/ Lyophilized vials

The Laminar Air Flow was prepared aseptically, by switching on the UV- light for 5min and swiping the surface of the workspace with 70% isopropyl-alcohol. The freeze dried vials (lyophilized) or Glycerol stock was kept on crushed ice and allowed to be thawed. The UV light was put off and the light and airflow were put on. All procedure was handled aseptically hereforth. To take 3ml of autoclavable Tryptic Soya Broth in a test tube, 100 µl of glycerol stock was inoculated and incubated at 37° C at 150rpm/min for 16-18 hours overnight. The turbid culture was re-inoculated into 3ml of Nutrient broth or (TSB) and incubated at 37° C with 150 rpm/min for 16-18 hours.

5. Making a Smear from the bacterial culture

The culture revived from Glycerol stock has to be checked for its purity with Gram's staining. For making a smear, 1ml of the overnight culture was aliquoted in a microfuge and centrifuged at 5000rpm for 5minutes. The supernatant was discarded and the bacterial pellet mixed in the remnant moisture. A thin smear of the bacterial pellet was made in the middle of a grease free glass slide and labelled appropriately. The smear was passed across the Bunsen flame, twice or thrice and heat fixed which prevents the smear from getting washed off during rinsing.

6. Gram's Staining Method

The heat fixed bacterial smear on the glass slide was placed on a staining rack near a sink and tap. First, Gram's crystal violet was poured over the smear to completely cover it. Allow it to stand for 3 minutes. Now, pour off the crystal violet and gently rinse the glass slide under running tap water. Then, Gram's Iodine was poured over the smear, which is the mordant and allowed to stand for 1 min, and washed off gently as above. Next, Gram's decolouriser was poured over the smear for brief 30 seconds duration and immediately rinsed off again. Finally, Saffranin, the counter stain was poured over the smear and allowed to stand for 2min. The stain was rinsed off under running tap water, until no traces of any dye is present. Gently tap off the extra

water and blot the slide on a blotting paper. Place it upright and allow to air dry.

7. Observation of the Smear under microscope:

When the smear is fully dry, place it on the stage of the microscope and focus it under low power (30X). Then put a drop of immersion oil on the smear and view it under oil immersion objective (100X). Fine tune is made to get the best resolution and observe the morphology of the smear, like the colour and shape of the bacteria (purple/red colour/bacilli or rods or cocci). The entire smear was uniform with a purple colour (Gram positive) and spherical cocci as bunches of grapes and evenly distributed in the field. There was no contaminating other bacteria. The bacteria were confirmed as *Staphylococcus* spp. Similarly, the other smear showed uniformly pinkish red colored (Gram negative) slender rods or bacilli. There were no contaminating other bacteria. The bacteria were confirmed as *Pseudomonas* spp.

8. Propagation of the Bacterial isolate and Inoculum culture

The two respective bacteria being pure, were sub-cultured from the remaining broth onto Trypticase Soy agar plates and respective selective plates. The *Staphylococcal* spp culture was streaked onto Mannitol Salt Agar and *Pseudomonas* spp onto Nutrient or Cefrimide agar plates. The plates were incubated at 37° C for 16-18 hours. The *Staphylococcus* spp grew as golden yellow colored colonies on the pink MSA plates and also changed the pH, hence the plates also turn yellow in colour. The *Pseudomonas* spp grew on the Nutrient agar plate as moist colonies. Also they produce a greenish pigment (Pyocyanin) which colours the surface of the agar plate. The isolates grew as pure cultures on the plates and were used for preparation of the inoculums for antimicrobial assay. A single colony from each of the pure plate was picked up using a sterile inoculation loop and inoculated into a test tube containing 3ml of Nutrient broth. The broths were incubated at 37° C with 150 rpm for 16 -18 hours. The turbid broth culture in log phase was used as inoculum for antibacterial studies. Suitable dilutions of the culture was made before put to use.

9. Dilution of Culture for Antimicrobial Studies

The turbidity of the broth culture was compared against bright light with the McFarland Standards to equate the approximate cell density. The culture was equal to 4 X10⁸ cells/ml of the broth. Tenfold serial dilutions were made from the neat culture up to the 10th dilution for each of the bacterial cultures separately. The 5th, 6th and 8th dilutions for the *Pseudomonas* culture and 7th, 8th and 9th dilutions for *Staphylococcal* culture were selected. All the three dilutions were smeared on separate Muller Hinton agar plates (90mm) diameter, by the Spread Plate Method. The Kirby Bauer procedure by the Disc Diffusion Method was followed for studying the antibacterial effect of commercial antibiotic discs. Totally 9 different antibiotic discs were used for each bacterial culture. The Zone of Inhibition of each drug was measured and recorded.

10. Antimicrobial Activity of Silver Nitrate Nano particles prepared by Green Synthesis Method

The silver nitrate nano particles from both Set I and Set II was used. For each of the chosen dilutions, 4 wells were

used for two different volumes of silver nano particles/well. The antibacterial effect of the silver nano particles used was found out by measuring the Zone of Inhibition produced. The ZOI that was maximum at a given dilution of the culture for the volume of Silver nano particles used in each set was taken as the antibacterial effect of the silver nano particles against the respective bacterial culture used.

11. UV-Visible Spectrophotometer Analysis

UV-Vis spectral analysis was done by using SHIMADZU UV-Visible absorption spectrophotometer with a resolution of 1 nm between 200 and 800 nm was used. One millilitre of the sample was pipette into a test tube and subsequently analysed at room temperature. From the spectra, the reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the *Aloe vera* extract and further it was observed that UV-Vis optical spectra of the colloidal solution of silver nanoparticles has been recorded as a function of time by using a quartz cuvette with water as reference. The colour change was noted by visual observation in the *Aloe vera* extracts when incubated with Ag solution. The colour of the extract changed to light brown within an appearance of the yellowish brown colour was an indication of formation of colloidal silver nano particles in the medium. The occurrence of brown colour may be due to the excitations of surface Plasmon resonance vibrations in Ag nano particles. The dark brown colour of the silver colloid is attributable to surface 40lasmon resonance arising from the group of free conduction electrons induced by an interacting electromagnetic field. The strong surface Plasmon resonance small band appears at the range of 410 – 430 nm and the broadening peak indicates that the particles are mono dispersed. These colour changes arise because of the excitation of surface Plasmon vibrations in the silver nanoparticle. Maximum absorbance was observed at 430 nm for Ag *Aloe vera* extract which is indicating that the formation of spherical silver nanoparticles in majority or anisotropic particles whose appearance and ratio increases with time, further the sample 10:10 Ag *Aloe vera* extract shows the shift in optical absorption spectra.

12. Characterization of silver nanoparticles

The shape of the synthesized silver nanoparticles was analysed by Scanning Electron Microscope, representative SEM micrographs of control and treated AVE magnified at 6000X to 30000X. Shape and size of the silver nanoparticles was observed.

Results and Discussion

1. Sample selection

Totally three samples were prepared with different concentration i.e., (5:5), (10:10) and (10:20). In that sample-II (10:10) were selected as the final sample and was ready for coating purpose.

2. Optiacal Analysis

Sample – II showed a good colour change from green to yellowish brown and higher peak at 430nm when observed in room temperature. The reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the *Aloe vera* extract and further it was observed that UV-Vis optical spectra of the colloidal solution of silver nanoparticles has been recorded as a function of time by using a quartz cuvette with water as reference. The colour change was noted by visual observation in the *Aloe vera* extracts when incubated with Ag solution.

3. Antimicrobial activity

The anti-bacterial effect of the silver nano particles used was found out by measuring the Zone of Inhibition produced. The ZOI that was maximum at a given dilution of the culture for the volume of Silver nano particles used in each set was taken as the antibacterial effect of the silver nano particles against the respective bacterial culture used. The samples were tested against *Staphylococcus* and *Pseudomonas* species. The result showed that *Staphylococcus* species was more resistance comparing to the *Pseudomonas* species.

4. Structural and Morphological Analysis

The shape of the synthesized silver nanoparticles was analysed by SEM, which is agglomerated in nature. More over the square shaped particles are found that indicating the particle agglomeration in the order of few hundred nanometres.

5. Edible Coating

The finalized sample is coated in cut fruits and vegetables and shelf life is observed. The experiment is performed in tomato, mango and papaya. Finally the results showed that sample coated film extended the shelf life of tomatoes for 3 days. In papaya 3 days extension was seen and in mangoes the shelf life of fruit was increased for 2 days. Extension of shelf life has observed between samples coated film and non- coated food wrap film. Weight loss, colour, texture and dryness of juice observed between non-coated film and sample coated film.



Fig 1: Separated leaf and gel, Extract preparation and Samples with different Concentration

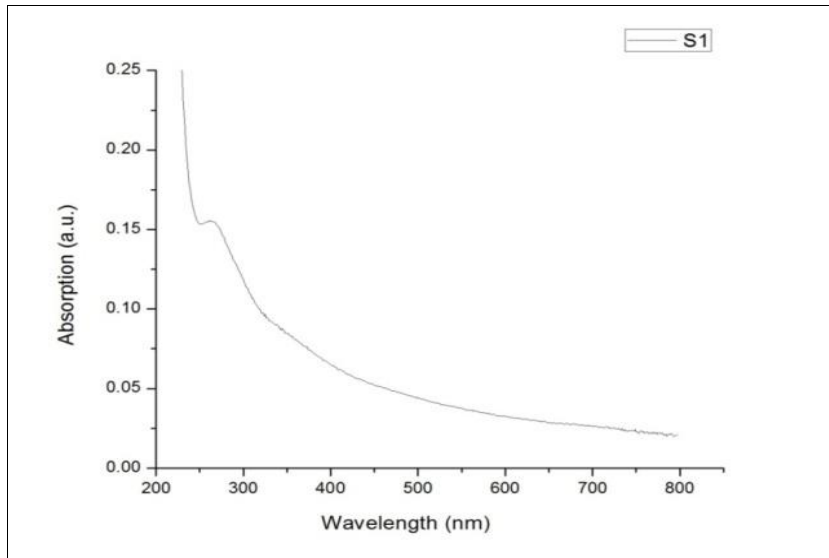


Fig 2: UV absorbance graph Sample

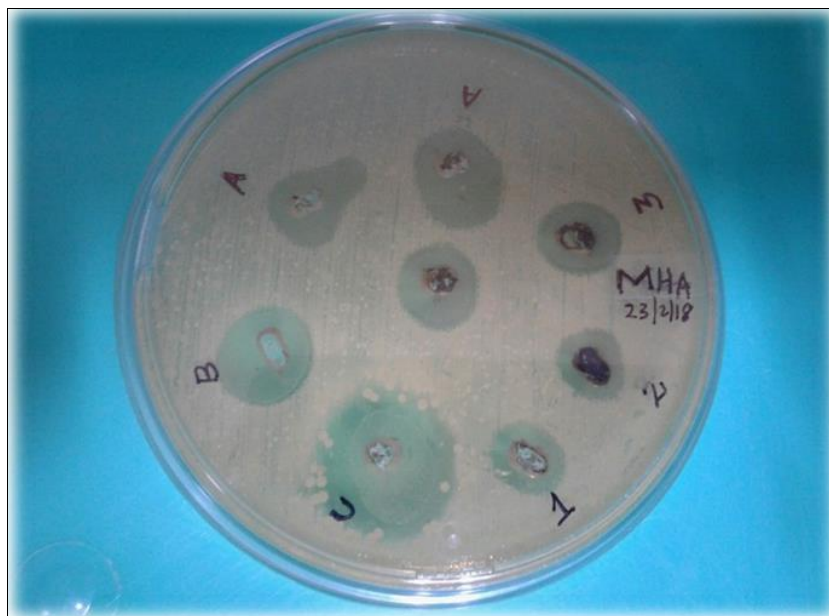


Fig 3: Zone of inhibition found in *Staphylococcus* species

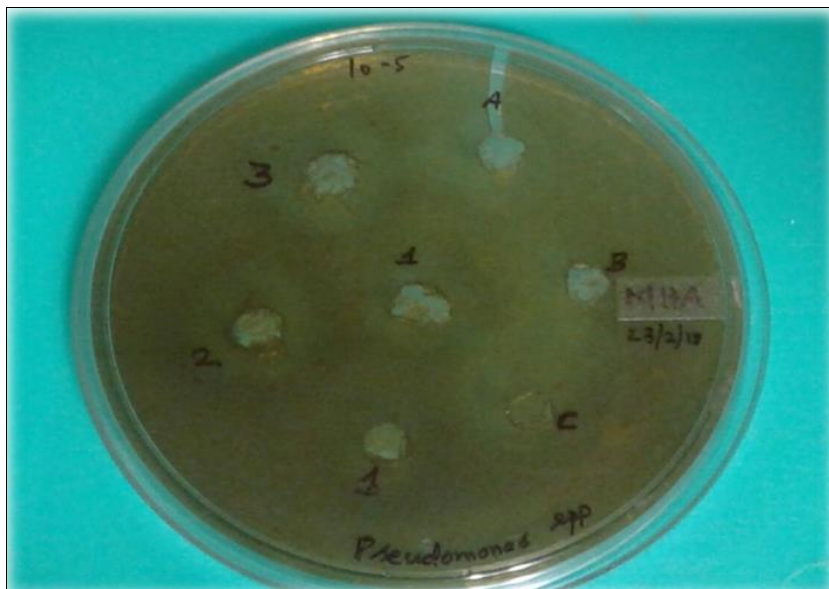


Fig 4: Zone of inhibition found in *Pseudomonas* species

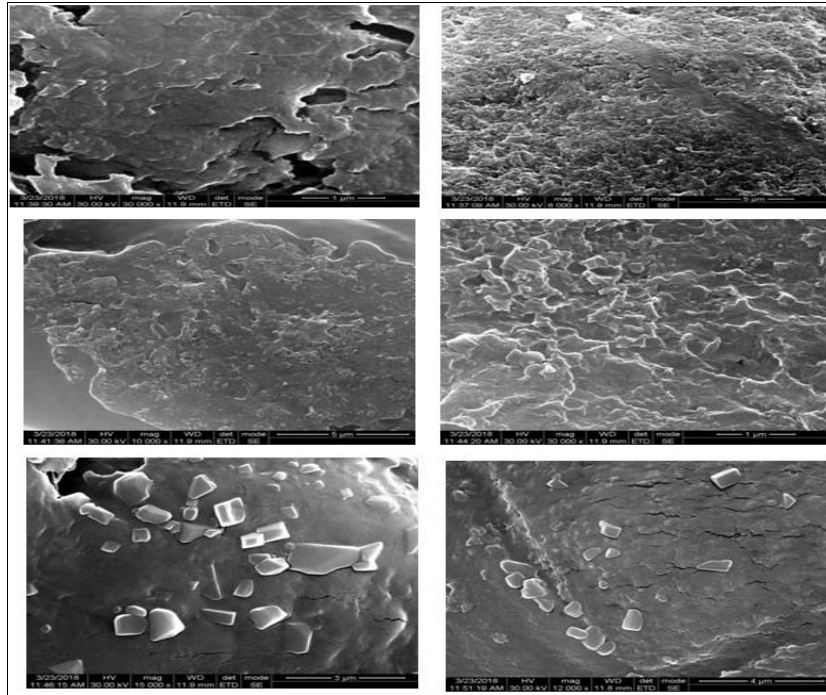


Fig 5: SEM images of the samples

Conclusion

The sample II showed good absorbance peak of 430nm in UV-visible spectrophotometer and the samples were tested with antimicrobial activity showed zone of inhibition against *Staphylococcus* and *Pseudomonas* species. The shape of the synthesized silver nanoparticles was analysed by SEM, which is agglomerated in nature. Indicating the particle agglomeration in the order of few hundred nanometres. The sample coated film covered over cut tomato, mango and papaya showed good results.

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