BIOSYNTHESIS OF SILVER NANOPARTICLES (AgNPs) BY EXTRACT OF *Annona Squamosa* WASTE: CHARACTERIZATION AND THEIR ANTIMICROBIAL ACTIVITY

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Abstract. Nanoparticles have been applied to food, agriculture, medicine for preservation, prevention, diagnosis, treatments. Biosynthetic rout is used for production of non-toxic, eco-friendly particles. Biosynthesis (Green synthesis) of Silver nanoparticles was studied using *Annona Squamosa* (Custard Apple) waste. *Annona Squamosa* (Custard Apple) was used to prepare various food product such as juice, shakes, sweets, etc. The peel and seed become waste which has various bioactive components. These bioactive components act as a reducing agents as well as capping agent during biosynthesis of Silver nanoparticles. In biosynthesis process Silver Nitrate was used as precursor with 1:1 proportion. Silver nanoparticles were characterized by UV- visible spectroscopy and X-ray Diffraction. Antimicrobial activity was checked against E-coli were significant activity against E-coli was observed.

Key words: *Annona Squamosa* (Custard Apple) waste, Biosynthesis, Silver nanoparticles, Antimicrobial activity.

1. Introduction: Nanotechnology is developing field of science in which particle size vary from 1 – 100 nanometers. Nanoparticles can be synthesized by physical, chemical and biological rout of synthesis. This study focused on biological synthesis of nanoparticles.

Silver has very different and unique properties like antimicrobial activity, protection against cancer cells, anti-viral activity etc. activity against microorganisms helps to preserve food for longer time. Biological way of silver nanoparticles synthesis is cost effective and eco-friendly. Also biosynthesis is very simplest way to produce nanoparticles.

Now day's food waste utilization is important to minimizing generated waste and waste related problems. The waste which is generated during food processing has many important components. That's why waste of custard apple which is collected after removing pulp for process used for biosynthesis process.

2. Materials and method:

2.1 Material: Custard apple peel and seeds were collected from home or from ice cream parlors, where only pulp used eating purpose. Peel and seeds gently washed under tap water before use and dry properly.

2.2 Extract preparation: Prepare powder of dried peel and seed. Then add water in it. After proper mixing filter out whole mixture and increase its volume by 1:5 proportion by distilled water. Store it in cool and dark place and use it as early as it is fresh.

2.3 Silver Nanoparticle Synthesis: Prepare 1 ml of silver nitrate solution. Immediately treat it with extract in 1:1 proportion. Continue the process of mixing with continuous stirring. Color change of solution shows that reaction takes place and nanoparticles were formed.

After completion of reaction particles will settle at bottom. By centrifuging the solution discard supernatant and drying sediment part gives silver nanoparticles. Centrifuge the solution with high speed that is 10000 rpm for 10 minutes. Sediment will dry at 40 – 50 ^{0}c for avoiding heat reaction.

2.4 Characterization Techniques: UV-Vis absorption spectra were measured using Shimadzu UV-1601 spectrophotometer. Crystalline-metallic silver nanoparticles were examined by X-ray diffractometer (Shimadzu XRD-6000) equipped with Cu Kα radiation source using Ni as filter and at a setting of 30 kV/30 mA. All XRD data were collected under the same experimental conditions. Field emission scanning electron microscopy (FE-SEM) analysis of silver nanoparticles analysis was done.

2.4 Antibacterial Assay: Silver nanoparticles biosynthesized from bamboo leaves extract were tested for antimicrobial activity by Kirby{Bauer method against pathogenic bacteria Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive). The pure bacteria cultures were sub cultured on nutrient agar media. Both strains were swabbed evenly onto the single plates using sterile glass rods. After incubation at 37±C for 24 hours, the levels of zone diameter inhibition of bacteria were measured.

3. Result and discussion:

3.1 Silver nanoparticle synthesis: When precursor of silver nitrate and extract that reducing agent were mixed thoroughly solution get reduced form silver (fig 1). After that stable nano-sized silver particles settled at bottom of the solution. Further processing like centrifugation of colloidal solution, drying at 40^0c to obtain silver particles in dry form and size reduction for even particle size of silver nanoparticles

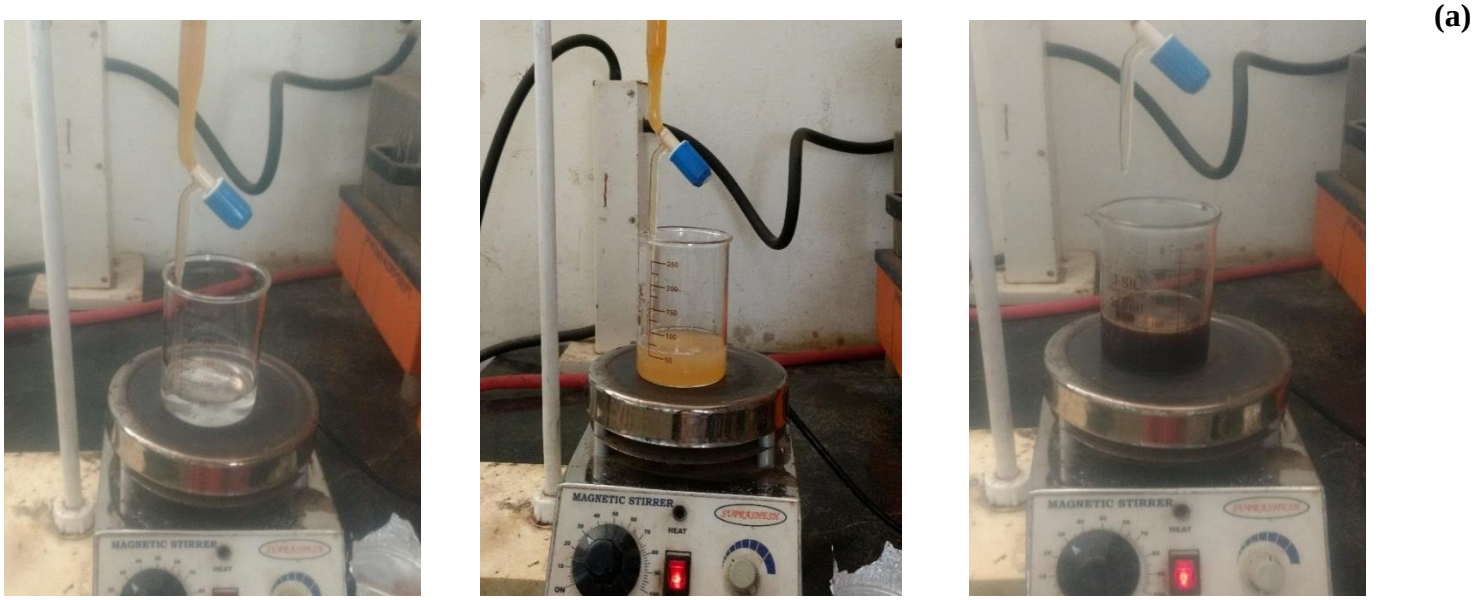

Figure 1. color change from a to c as silver particles formed

3.2 Characterization of Silver nanoparticles: To confirm the formation of silver nanoparticles characterization is most important. Ultra-violet visible spectroscopy (UV), X-ray diffraction (XRD), FE-SEM were characterization technique held in this study.

3.2.1 UV analysis: Visual Observation and UV-Vis Spectral Study Formation and stability of AgNPs in sterile distilled water is confirmed using UV-vis spectrophotometer in a range of wavelength from 200 to 800 nm. As soon as, *Annona Squamosa* peel extract was mixed in aqueous solution of silver ion complex, the reduction of pure Ag+ ions to Ag was monitored by measuring UV–vis spectrum of the reaction media at regular intervals. UV–vis spectra were recorded, Fig. 2. We observe that there is peak showing surface plasmon resonance of silver at 360nm.

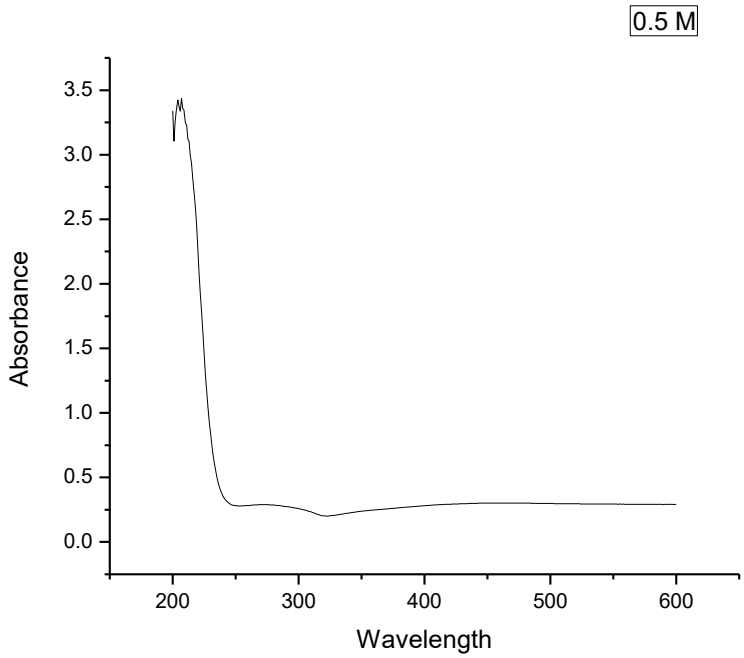

Figure 2. UV-vis analysis of aqueous solution of silver NPs (from *Annona Squamosa* peel extract)

3.2.2 X-ray diffraction (XRD) Studies: Analysis through X-ray diffraction was carried out to confirm the crystalline nature of the particles, and the XRD pattern showed numbers of Braggs reflections that may be indexed on the basis of the face cantered cubic structure of silver. A comparison of our XRD spectrum with the standard confirmed that the silver particles formed in our experiments were in the form of nano-crystals. The X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag+ ions by the *Annona Squamosa* peel extract are crystalline in nature. The presence of structural peaks in XRD patterns and average crystalline size around 20 nm clearly illustrates that AgNPs synthesized by our green method were nano-crystalline in nature. The XRD patterns thus clearly illustrates that the AgNPs synthesize by the present green method are crystalline in nature. The average particle size of silver nanoparticles synthesized by the present green method can be calculated using Debye-Scherrer equation

The presence of structural peaks in XRD patterns and average crystalline size around 23 nm clearly illustrates that AgNPs synthesized by our green method were nanocrystal-line in nature. The XRD patterns displayed in this work are in good agreement with the earlier research reported for green synthesis of silver nanoparticles.

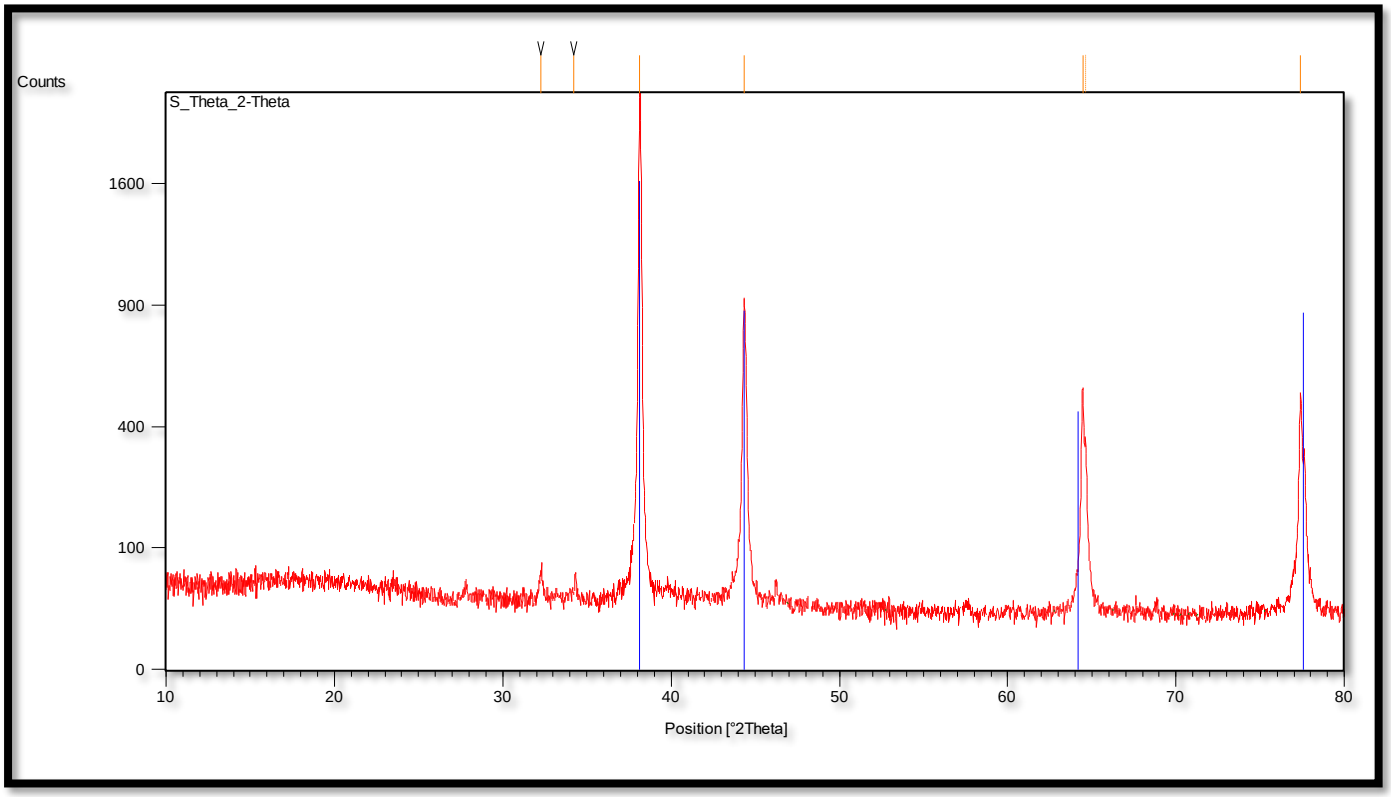

Figure 3. XRD pattern of AgNPS synthesized by *Annona Squamosa* peel extract

3.2.3 FE-SEM Analysis of Silver Nanoparticles (AgNPs): The suspended silver nanoparticles in sterile distilled water were used for field emission scanning electron microscope analysis by fabricating a drop of suspension onto a clean electric stubs and allowing water to completely evaporate. The FE-SEM image of silver nanoparticles, Fig. 4 showed spherical and relatively uniform shape of nanoparticle formation with diameter range 20-40 nm. The larger silver particles may be due to the aggregation of the smaller ones, due to the FE-SEM measurements.

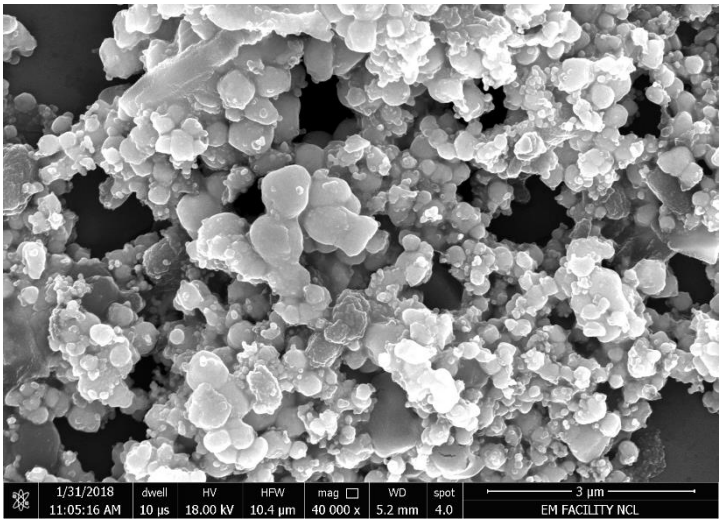

Figure 4. FE-SEM of silver nanoparticles

3.2.4 Antimicrobial Activity Study of Silver Nanoparticles (AgNPs): From the preliminary screening by disc diffusion method, it was observed that silver nanoparticles have antibacterial activities at concentration of 50µl/disc. This was observed on *Staphylococcus aureus* and *E.coli*. The zone of inhibition ranged from 19 to 39 mm in diameter, Fig. 5.

Figure 5. Antimicrobial activity of silver nanoparticles

4. Conclusion: Green chemistry approach towards the synthesis of nanoparticles has many advantages such as, ease with which the process can be scaled up and economic viability. We have developed a fast, eco-friendly and convenient method for the synthesis of silver nanoparticles using agro based food waste of *Annona Squamosa* peel extract leaves extract. These particles are mono-dispersed and spherical. No chemical reagent or surfactant template was required in this method, which consequently enables the bioprocess with the advantage of being environmental friendly. Color change occurs due to surface plasmon resonance during the reaction with the ingredients present in the fruit peel and seed extract results in the formation of silver nanoparticles which is confirmed by UV–vis, XRD and FE-SEM. The antibacterial activity of biologically synthesized silver

nanoparticles was evaluated against *Staphylococcus aureus* and *E-coli* showed effective antimicrobial activity. The synthesized nanoparticles can be used in the packaging material for increasing the shelf life of the packaged foods.

5. References:

Appendini, P., and Hotchkiss, J. H. (2002). Review of antimicrobial food packaging. Innovative Food Science and Emerging Technologies, 3(2): 113-126.

Ghosh, P. R., Fawcett, D., Sharma, S. B., and Poinern, G. E. (2017). Production of High- Value nanoparticles via biogenic processes using aquacultural and horticultural food waste. Materials, 10(8): 852.

Guzmán, M. G., Dille, J., and Godet, S. (2009). Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. Int J Chem Biomol Eng, 2(3): 104-111.

Hasan, S. (2015). A review on nanoparticles: their synthesis and types. Research Journal of Recent Sciences ISSN: 2277, 2502.

Hussain, I., Singh, N. B., Singh, A., Singh, H., & Singh, S. C. (2016). Green synthesis of nanoparticles and its potential application. Biotechnology letters, 38(4), 545-560.

Lin, C. S. K., Pfaltzgraff, L. A., Herrero-Davila, L., Mubofu, E. B., Abderrahim, S., Clark, J. H., and Thankappan, S. (2013). Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. Energy and Environmental Science, 6(2): 426-464.

Maynard, A. D. (2007). Nanotechnologies: overview and issues. In Nanotechnology– Toxicological Issues and Environmental Safety and Environmental Safety (pp. 1-14). Springer, Dordrecht.

Radusin, T. I., Ristić, I. S., Pilić, B. M., and Novaković, A. R. (2016). Antimicrobial nanomaterials for food packaging applications. Food and Feed Research, 43(2): 119- 126.

Rao, K. G., Ashok, C. H., Rao, K. V., Chakra, C. S., and Rajendar, V. (2015). Synthesis of Tio2 Nanoparticles From Orange Fruit Waste. Synthesis, 2(1): 1.

Rao, K. S., El-Hami, K., Kodaki, T., Matsushige, K., and Makino, K. (2005). A novel method for synthesis of silica nanoparticles. Journal of Colloid and Interface Science, 289(1): 125-131.

Shobha, G., Moses, V., and Ananda, S. (2014). Biological Synthesis of Copper Nanoparticles and its impact. International Journal of Pharmaceutical Science Invention, 3(8): 2319-6718.

Tankhiwale, R., and Bajpai, S. K. (2009). Graft copolymerization onto cellulose-based filter paper and its further development as silver nanoparticles loaded antibacterial food-packaging material. Colloids and Surfaces B: Biointerfaces, 69(2): 164-168.

Tankhiwale, R., and Bajpai, S. K. (2009). Graft copolymerization onto cellulose-based filter paper and its further development as silver nanoparticles loaded antibacterial food-packaging material. Colloids and Surfaces B: Biointerfaces, 69(2): 164-168.