GREEN SYNTHESIS OF SILVER NANOPARTICLES BY PAPAYA PEEL EXTRACT

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Abstract : This study was to investigate an efficient, cost effective and sustainable route of silver nanoparticles (AgNP) from silver nitrate. In this natural green synthesis, papaya peel extract which act as natural reducing agent to synthesize silver nanoparticles. The characteristic color is changed to reddish brown in the reaction due to reduction. The AgNPs were characterized by UV-visible (vis) spectrophotometer, FTIR and scanning electron microscopy (SEM) with EDAX. The synthesized silver nanoparticles (AgNPs) were visually confirmed with surface plasmon resonance peak at 440 nm upon UV–Vis spectroscopy analysis. Fourier transform infrared spectroscopy (FT-IR) affirmed the role of fruit extract as a reducing and capping agent of silver ions. Furthermore, the nanoparticles formation were characterized with different pH, temperature and stability of the nanoparticles. AgNPs displayed strong antibacterial potentials against Gram-positive and Gram-negative bacteria. Papaya peel -AgNPs also displayed with strong free radical scavenging and reducing power. Based on strong antibacterial and antioxidant capacities, PP-AgNPs are anticipated to have potential applications in the biomedical industries.

***IndexTerms* - Silver** nanoparticles, Papaya peel extract, FTIR, SEM- EDAX**.**

I. INTRODUCTION

Nanotechnology is the practice of science to regulate matter at the molecular level. It produced various types of materials at nanoscale level ie less than 100 nanometers. Nanoparticles (NPs) are of great scientific interest because of its extremely small size and the large surface to volume ratio. This property leads both physical and chemical differences (e.g. mechanical properties, biological and sterical properties, catalytic activity, thermal and electrical conductivity, optical absorption and melting point) when compared to the bulk of the same chemical composition1, 2. NPs exhibit size and shape-dependent properties which are of interest for applications ranging from biosensing and catalysts to optics, antimicrobial activity, computer transistors, electrometers, chemical sensors, and wireless electronic logic and memory schemes. These particles also have many applications in different fields such as medical imaging, nanocomposites, filters, drug delivery, and hyperthermia of tumors3, 4.

Moreover, biosynthesis of metal nanoparticles is an environmentally friendly method (green chemistry) without the use of harsh, toxic and expensive chemicals5. Recently, biosynthetic methods employing either biological microorganisms such as bacteria and fungus or plants extract6,7,8, have emerged as a simple and viable alternative to more complex chemical synthetic procedures to obtain Nanomaterials. Different types of Nanomaterials like copper, zinc, titanium9, magnesium, gold10, alginate11 and silver have come up but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms12. Of these, silver nanoparticles are playing a major role in the field of nanotechnology and Nanomedicine. Colloidal silver is of particular interest because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activities13.

In this regard, there is a growing need to develop reliable, non-toxic, clean, eco-friendly, and green experimental protocols for the synthesis of NPs. One of the options to achieve this objective is to use natural processes such as the use of enzymes, microbial enzymes, vitamins, polysaccharides, biodegradable polymers, microorganisms, and biological systems for synthesis of NPs. One approach that shows immense potential is based on the biosynthesis of NPs using plant source (a kind of bottom-up approach)14.

Silver nanoparticles have drawn the attention of researchers because of their extensive applications in areas such as integrated circuits, sensors, biolabelling, filters, antimicrobial deodorant fibers, cell electrodes15 and antimicrobials16. Antimicrobial properties of silver nanoparticles caused the use of these nanometals in different fields of medicine, various industries, animal husbandry, packaging, accessories, cosmetics, health, and military. Silver nanoparticles show potential antimicrobial effects against infectious organisms such as Escherichia coli, Bacillus subtilis, Vibrio cholera, Pseudomonas aeruginosa, Syphillis typhus, and Staphylococcus aureus15,16.

2. **Experimental Methods**

2.1 **Chemicals**: All the experiments were conducted at room temperature. Materials used for the synthesis of silver nanoparticles are AR grade silver nitrate (AgNO3) purchased from Merck, India.

2.2 **Preparation of Plant Extract** :

The Fruits such as Papaya, Sapota, and Pineapple were collected from the local market, the peels were collected and cut into small pieces. The peels were washed thrice with sterile distilled water to remove adhering salts and other associated contaminants. The fruit peels are dried on paper toweling. About 25g of fruit peels was taken and boiled with 100ml of sterile distilled water at 100 °C

for 10minutes and filtered through Whatman No 1 filter paper. The resultant filtrate was stored in the refrigerator at 4°C for further studies 17.

2.3 **Synthesis of silver nanoparticles**:

For the Ag nanoparticles synthesis, 5 mL of peel extract was added to 20 mL of 1 mM aqueous AgNO3 solution in a 250 mL Erlenmeyer flask. The flask was then incubated in the dark at 5 hrs (to minimize the photoactivation of silver nitrate), at room temperature. A control setup was also maintained without extract. The Ag nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water17.

2.4 **UV and FTIR Spectroscopic Analysis**:

The reduction of pure Ag+ ions was examined under UV-Vis spectrophotometer analysis. For UV and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper. The sample was diluted to 1:10 with the sterile distilled water. The reduction of pure Ag+ ions was scanned in the wavelength ranging from 300-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using the Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm-1 and their functional groups. Each and every analysis was repeated twice for the spectrum confirmation.

2.5 **SEM and EDX Analysis of Silver Nanoparticles**:

For morphological characteristics, the AgCl-NPs were uniformly spread and sputter coated with platinum using an ion coater for 120 s, then observed under a SEM (S-4200, Hitachi, Japan). The particle size of the synthesized nanoparticles was calculated from the enlarged SEM image. The elemental composition of the powdered AgCl-NPs was subsequently analyzed using an EDX detector (EDS, EDAX Inc., Mahwah, New Jersey, USA) attached to the SEM machine.

2.6 **Effect of different pH, temperature and concentration of extract on the synthesis of Ag nanoparticles.**

The synthesis of Ag nanoparticles was monitored at different pH intervals. The reaction was monitored using the different concentration of silver nitrate (1 mM, 2 mM, 3 mM, 4 mM and 5 mM) and also by varying leaf extract solution (1–5 mL) and their absorbance was measured. By varying the temperature such as 0, 10, 20, 30 and 40˚C for the synthesis of nanoparticles and the concentration of the extract is also been changed for the synthesis of nanoparticles.

2.6. **Assessment of antimicrobial assay**

The antibacterial assays were done on human pathogen by using standard good diffusion method. Mackonkey broth (HiMedia) medium was used to subculture bacteria and was incubated at 37 °C for 24 h. Fresh overnight cultures were taken and spread on the Mackonkey agar plates to cultivate bacteria. Sterile wells were made by using the sterile cork borer of 5 mm diameter, silver nanoparticle and double distilled water (as control) were placed on each plate and incubated again at 37 °C for 24 h and the antibacterial activity was measured based on the inhibition zone around the well with plant extract and synthesized silver nanoparticle 18

RESULTS AND DISCUSSION

The UV–visible absorption spectra recorded for the formation of silver nanoparticles using different fruit peel extract like Papaya, Sapota, and Pineapple as a reducing agent was carried out (Fig. 4). The UV-Visible spectrum of silver nanoparticles of papaya peel extract clearly indicates the color change as well as absorption band at 410 nm (Fig. 5). All the extract were able to change the colors but papaya peel extract exhibited higher intensity and also sharp peaks at 410 nm was observed 19. Hence, papaya peel extract was selected for further studies, there are no peaks located around 400 to 450nm. This indicated the absence of nanoparticle aggregation and the color intensity is also high compared to that of the other peel extract. Hence, Papaya extract was selected for further studies.

CHARACTERIZATION OF NANOPARTICLES SYNTHESIZED BY PAPAYA PEEL EXTRACT.

Effect of pH on the biosynthesis of Ag Nanoparticles.

The biosynthesis of nanoparticles was analyzed at different pH (2, 4, 6, 8 and 10) (Fig 5). The formation of color intensity of the reaction mixture was pH dependent. At pH 2.0, 4.0 and 8.0 no color change was observed. The highest color intensity was obtained at pH 6.0 and 10.0. In the agreement, pH 2.0 and pH 4.0 no color change was observed20. The extremely acidic conditions (pH 2.0) inactivated by the variety of biomolecules involved in the synthesis of nanoparticles. Highly monodispersed nanoparticles were obtained at pH 11 with an average size of 23 ± 2 nm21. The variation in the dissociation constants (pKa) of functional groups are responsible for the differences in the color formation22.

Effect of concentration of extract on the biosynthesis of Ag Nanoparticles.

The reaction mixtures containing 0.1, 0.5 and 1.0 of silver nitrate developed a light to dark reddish brown color, while those containing 1.5 to 2.0 developed darker reddish brown color. The peaks were proportionally more intense and the maximum peak intensity was observed 1.0mM (Fig. 1c). It was also observed that the intensity of absorption peaks increases with increase in the concentration of the silver nitrate salt. Similar results were already reported in Cochlospermum religiosum extract23 and by Pithophoraoe dogonia extract 24.

Effect of Temperature on the biosynthesis of Ag nanoparticles.

The temperature of incubation was also important for the biosynthesis of nanoparticles. Light reddish brown color and a very less pronounced peak were observed in the reaction mixture at temperature 0, 40 and 50ºC whereas dark reddish brown color and a more intense peak was exhibited at 40 and 80ºC. At higher temperature, the reduction process is faster and the color was developed earlier. In agreement Park25 also exhibited that when the temperature is increased, the reactants are consumed rapidly leading to the formation of smaller nanoparticles. The size of silver nanoparticles was decreased with an increase in incubation temperature was employed with the fungus Trichoderma viride26 .

3.3 **Fourier Transform Infra-Red Spectral Analysis FTIR**

FTIR spectrum of Ag nanoparticles analyzed to identify the possible biomolecules responsible for capping and efficient stabilization by papaya peel extract NPs. The absorption peaks should be owing to aryl di sulfide (473.5 cm-1) alkanes C-N(1374.34 cm-1), aromatics c=c(1603.88 cm-1) , arenes C-H bond (668 cm-1), C==C(2414.02 cm-1) ,aliphatic iodo compound (545.88 cm-1) alcohol(O_H) (3391.08cm-1)suggest the presence of flavonoids and phenols adsorbed on the surface of Ag nanoparticles. The results of FTIR analysis confirmed the presence of alcohol, phenol, alkanes, aldehydes, carboxylic acid, aromatics and aliphatic amines compound are bounded along with Ag NPs. AgNP's provided an idea about biomolecules having different functional group's which are present in the underlying system. It is well known that proteins can bind to Ag NP through free amine groups in the proteins 27, and, therefore, stabilization of the Ag NP by surface-bound proteins is a possibility.

SCANNING ELECTRON MICROSCOPY:

SEM and EDX analysis are done to visualize the shape, size and purity of the bio-synthesized Ag nanoparticles was studied. Fig. 5(a) shows the scanning electron microscope of synthesized Ag nanoparticles. The SEM images of the silver oxide size about 100 nm obtained by the biosynthesis process. Most of the nanoparticles aggregated and only a few of them were scattered, as observed under SEM. The SEM analysis showed the particle size between 10-40 nm nanoparticles. Similar results were reported for phyto-synthesized silver nanoparticles28, 29. This result strongly confirms that papaya peel extract might act as a reducing and capping agent in the production of silver nanoparticles. The EDX (energy-dispersive X-ray) analysis shows the chemical composition of the synthesized Agnos. Fig. 5(b) shows the EDX after 12 h incubation. The Fig. 5(b) shows the strong signal for silver. The strong peak at 3 keV indicated the presence of the elemental Ag nanoparticles as evident from previous observations30. Apart from Ag, other existing elements revealed by the EDX analysis included carbon, oxygen, phosphorous and chlorine which might be due to the X ray emission from the proteins/enzymes present in the PE extract. Since PE-AgCl-NPs were synthesized using the aqueous extract of peach outer peels, the presence of these elements confirmed that the organic metabolites present in the peel extract were responsible for capping and stabilization of the nascent nanoparticle30.

SILVER NANOPARTICLES AS ANTIBACTERIAL AGENT:

Antibacterial activity of the biosynthesized AgNPs was determined qualitatively by testing the zone of inhibition and level of inhibition was estimated by using different concentrations of silver nitrate solutions. The AGNP's exhibited toxicity against Enterobacter aerogenes, Staphylococcus aureus, Escherchia coli, Proteus vulgaris, Klebsiella pneumonia and Salmonella typhimurium in different concentrations like 100µl, 200µl and 300µl and control as silver nitrate solute on by measuring the inhibitory zone (Fig 15). Similarly the synthesis of different fruit peel was used for the synthesis of silver Nanoparticles like banana and orange peel and lemon peel31 and their antimicrobial activities were tested against the several microbes like Klebsiella, E.coli, Pseudomonas, and Salmonella. The antibacterial activity may be because of the decrease in the size of particles formed, which can cause the increase in the distinct outer surface of a specimen, enhancing their ability to infiltrate the cell membrane and induce its antimicrobial action32 . The bacterial activity is assumed to be due to the changes caused in the membranal structure of microbial cell because of its interaction with the rooted AgNPs which leads to the increase the permeability of the cell membrane and consequently, leading to their death33.

CONCLUSION

We have reported Papaya peel extract as an agricultural waste material was utilized for the successful synthesis of silver nanoparticles. It is fast, simple, efficient cost-effective and non-toxic method and an alternative to the conventional method. It would be suitable for developing a biological process for large-scale production. The synthesized products were characterized by FT-IR, UV–Vis Spectrophotometer and Fe- SEM. The synthesized AgNPs had a surface plasmon resonance at 433 nm with an average particle size of 33.95 nm calculated from the FE- SEM analysis. It suggests that the particles are irregular shaped. The FT-IR results suggest that proteins present in the extract were largely responsible for the biosynthesis of the silver nitrate particles. Also, the antimicrobial activity of synthesized Ag NPs has confirmed that these can be used as a potent antimicrobial agent against human pathogenic bacteria. Overall, AgNPs possess prospective applications in the biomedical industries.

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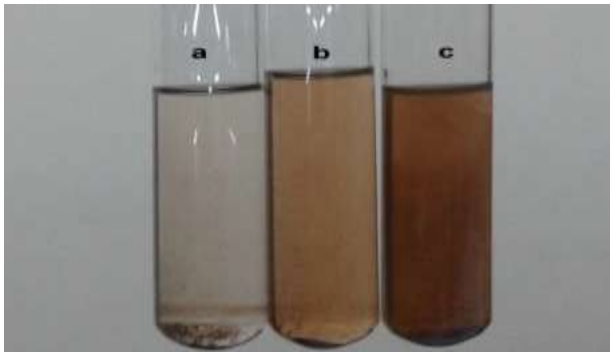
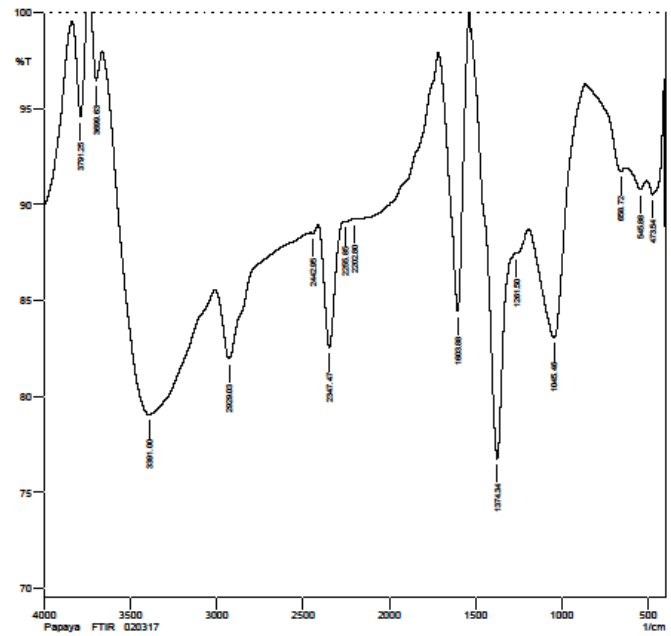


Fig. 1 Synthesis of Silver nanoparticles by papaya peel extract.



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	473.54	90.52	2.49	509.23	407.96	3.91	1.07
2	545.88	90.77	0.68	622.07	510.19	4.43	0.16
3	658.72	91.73	0.84	861.25	623.03	6.17	0.19
4	1045.46	83.06	9.04	1193.99	862.22	17.48	6.15
5	1261.5	87.45	0.08	1265.36	1194.95	3.92	0.05
6	1374.34	76.77	15.52	1526.72	1266.32	16.15	8.04
7	1603.88	84.43	14.92	1716.72	1534.44	6.57	5.83
8	2202.8	89.23	0.03	2204.73	1751.44	18.61	3.68
9	2255.85	89.12	0.01	2259.7	2205.7	2.69	0
10	2347.47	82.54	6.48	2413.05	2260.67	9.77	2.09
11	2442.95	88.52	0.14	2455.49	2414.02	2.17	0.02
12	2929.03	81.96	4.02	3009.08	2456.45	35.38	2.19
13	3391	79.04	13.74	3664.91	3010.05	50.4	25.28
14	3699.63	96.47	3.06	3741.1	3665.87	0.62	0.51
15	3791.25	94.57	5.91	3838.51	3742.06	1.03	1.24

Fig. 2. FTIR analysis of papaya peel extract

The results of FTIR analysis confirmed the presence of **alcohol, phenol, alkanes, aldehydes, carboxylic acid, aromatics and aliphatic amines compound** are bounded along with Ag NPs.

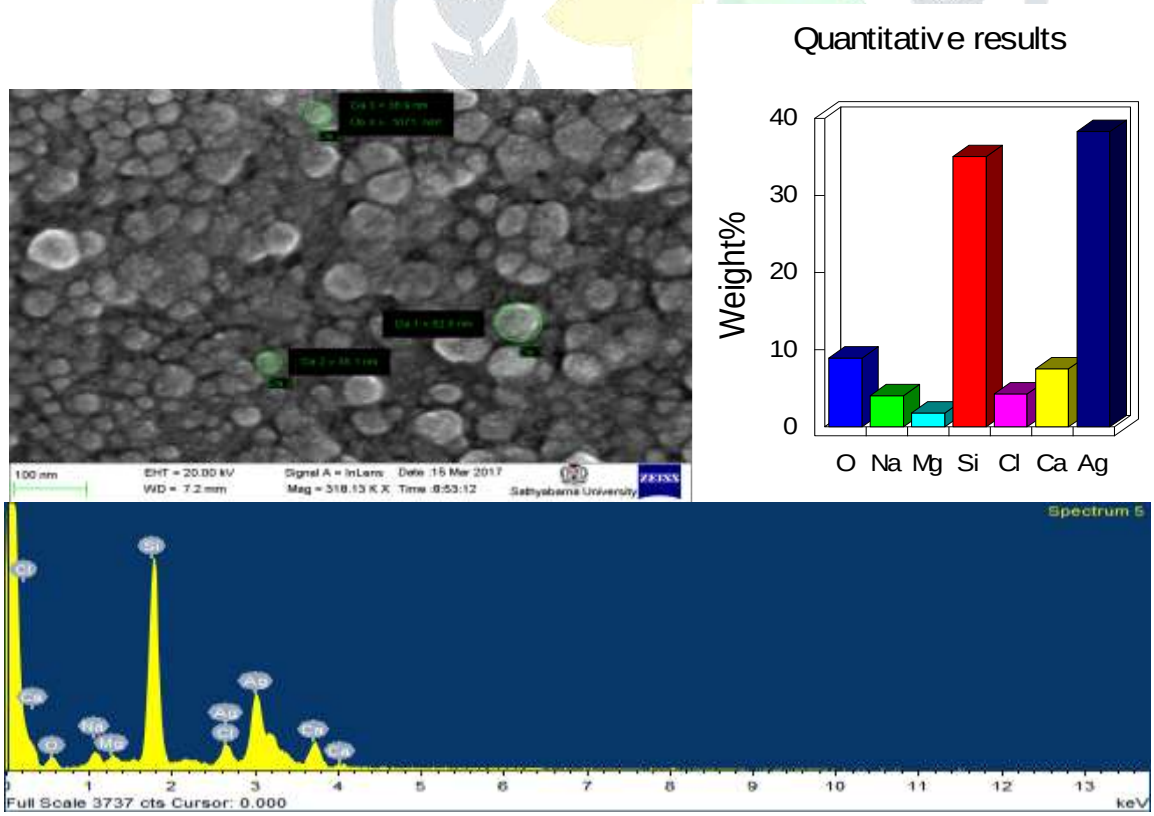


Fig. 3 SEM and EDAX analysis of Ag nanoparticles

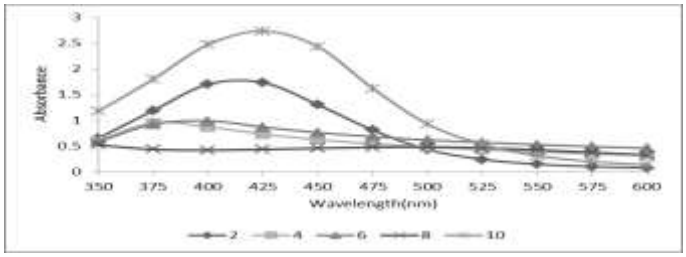


Fig 4. Effect of pH on the synthesis of Ag nanoparticles.

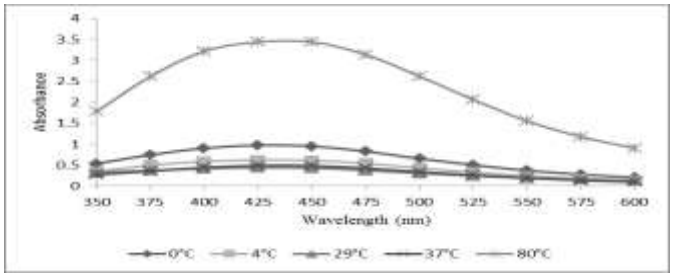


Fig. 5 Effect of different temperature on the synthesis of Ag nanoparticles.

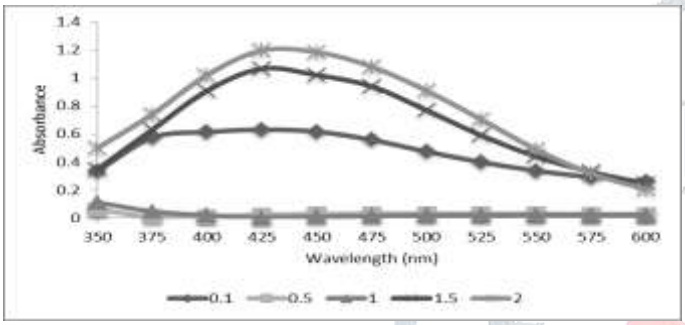


Fig. 6 Effect of different concentration of extract on the synthesis of Ag nanoparticles.

Table. 1 Antibacterial activity of Ag nanoparticles synthesized by papaya peel extract.

	Zone of Inhibition (mm)				
CONCENTRATIONS	**$AgNo_3$**	0.1 M	0.5 M	1 M	2 M
PATHOGENS					
Enterobacter aerogenes	18	15	18	20	21
Staphylococcus aureus	21	21	22	23	25
Escherichia coli	18	15	17	18	20
Proteus vulgaris	14	-	12	13	14
Klebsiella pneumonia	18	17	18	18	19
Salmonella typhimurium	18	18	19	19	20

Table. 2 Antioxidant activity of the Ag nanoparticles synthesized by papaya peel extract.

Concentrations (µg/mL)	**Ag- nanoparticles**	**Papaya peel extract**	**Ascorbic acid**
25	39±0.135	37±0.120	36±0.014
50	53±0.124	48±0.132	45±0.011
75	70±0.110	51±0.012	63±0.015
100	93±0.105	67±0.017	81±0.104

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