

Biosynthesis and Characterization of Silver Nanoparticle (CPL-Agnps) From Carica Papaya Leaf, And Their Antibacterial Activities

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Abstract: This biosynthesis approach is cost effective, eco-friendly of Ag nanoparticles has become a vital branch of nanotechnology which is an increasing commercial demand for nanoparticles due to their wide and promising for applications in medicine. In the present study, an eco-friendly and economical way for the synthesis of silver nanoparticles (SNPs) using leaf extract Carica Papaya Leaf as a reducing agent from 1 mm silver nitrate ($AgNO_3$) has been investigated. Carica Papaya Leaf known as 'papaya' belongs to the family of Caricaceae. The Characterization SNPs Prepared carried out using UV-vis, TEM. Silver nanoparticles were synthesized within 24 hours of incubation period and synthesized SNPs showed an absorption peak at around 200 nm in the UV-visible spectrum. The morphological study of Silver nanoparticles using TEM suggests that the nanoparticles are spherical in shape with a diameter around 50 nm. This leaf is rapid, simple without any hazardous chemicals as reducing or stabilizing agents and economical to synthesized SNPs.

Keywords: Silver nanoparticle (CPL-AgNPs), UV-Vis, TEM, Carica Papaya Leaf, Green synthesis.

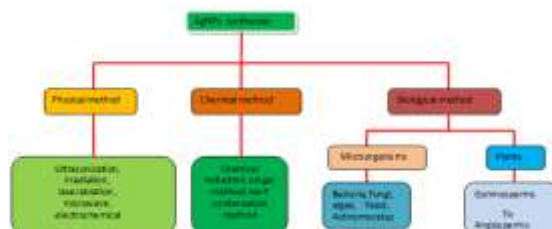
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I. Introduction

In India, food borne diseases occur frequently thereby causing higher morbidity and mortality rate. WHO estimates that 25% of the total 57 million annual deaths that occur worldwide are caused by microbes and this proportion is significantly higher in developing countries. India has a burgeoning urban population of over 1 million which is projected to increase by 15.5% in 2025. With the increase in urban population density, emerging and re-emerging food borne and waterborne diseases caused by multi-resistant pathogenic organisms constitute a major threat in India. Antimicrobial resistance is a major factor in virtually all infections and poses serious public health problems. These concerns have led to major research effort to discover alternative strategies for the treatment of bacterial infections. Hence, researchers are searching for "new antimicrobial agents" preferably as nanoparticles (Manonmani and Juliet, 2011). Nanotechnology is a reliable and enabling environment friendly process for the synthesis of nanoscale particles of structures ranging from approximately 1-100 nm (British Standards Institute, 2007; and SCENIHR, 2008). Nanosize results in specific physicochemical characteristics such as high surface area to volume ratio, which potentially results in high reactivity.

Synthetic Methods



Study Area: -The study area of green synthesis of nanosilver from papaya leaf is kota, Bilaspur district, Chattisgarh.



Materials: -C.papayawere taken from local area ofBilaspur, chattisgarh, India. The reagents such as silver nitrate were procured from Himedia laboratories, Bilaspur, India. Silver nitrate), Bacterial cultures (Escherichia coli) were procured from Clinical sample was obtained from Department of Chemistry, Bhilai institute of technology.

II. Methodology

Preparation of leaf extract: Fresh leaves of *C. papaya* (25 g) were diced into fine pieces and transferred to sterile 250 mL conical flask. Double distilled water 200 mL was added to the flask and heated at 70°C for 30 minutes to facilitate the formation of aqueous extract. The extract was filtered using Whatman No. 1 filter paper and the filtrate was stored at 4 °C for further use.



Figure1. Picture of *C. papaya* leaf extract with 1 mm AgNO_3 solution before and after the CPL-AgNPs.

Preparation of 0.01M silver nitrate solution

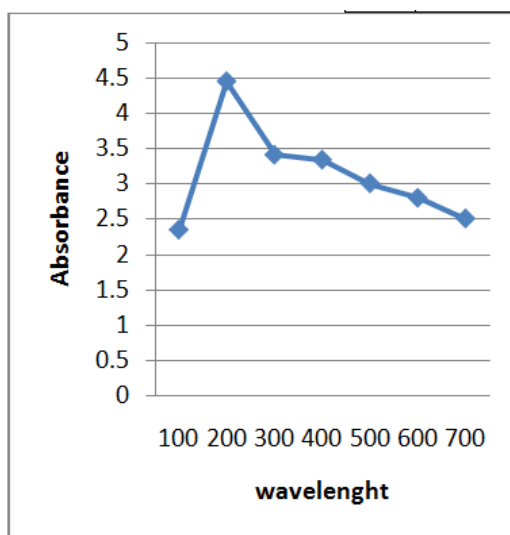
Silver nitrate 0.338gm was added to 200ml double distilled water, dissolved thoroughly. The solution obtained was transferred to an amber coloured bottle to prevent autoxidation of silver.

Determination,synthesis and characterization of silver nanoparticles:The aqueous leaf extract of *C.Papaya* and 0.01M AgNO_3 were mixed in the ratio of 1:4 (160ml leaf extract and 40 ml silver nitrate) and heated on a sand bath at 70°C for 20 min until change in colour was observed. The colour change indicated the formation of silver nanoparticles by *C. papaya* leaf extract (CPL-AgNPs). The extract of *C. papaya* leaves and fruit is rich in vitamins, phenols, proteolytic enzymes which acts as a good antioxidant and an excellent antimicrobial agent. The biosynthesis of nanoparticles was done using microbial strains, enzymes and metabolites, plant extracts and biodegradable products. Biosynthesis of nanoparticles by using *C. papaya* fruit and leaf extract had been previously reported to be having antimicrobial properties (Jain et al., 2009 ; Ratika and vedpriya, 2013). In the present study *C. papaya* silver nanoparticles (CPL-AgNPs) were biosynthesized using the biological approach. CPL-AgNPs were synthesized by mixing AgNO_3 solution with extract of *C. papaya* leaves. The chemical reaction involved in the formation of nanoparticles is the reduction of silver ions by the aqueous extract. The obtained nanoparticles were characterized by using UV–visible spectrophotometer. The reaction mixture was subjected to systronic UV- VIS double beam spectrophotometer 117 matched with 1 cm quartz cell. According to this technique many molecules absorb ultraviolet or visible light. The percentage of transmittance light radiations determines when light of certain frequency passed through the samples. This spectrophotometer analyses records the intensity of absorbance or optical density (O.D) as a function of wavelength. Absorption is directly proportional to the concentration of the absorbing species (Beer's law).Formation of silver nanoparticles is easily detected by spectroscopy because the coloured nanoparticle solution shows a peak ~200 nm. In this study, spectrophotometer was used to measure the optical density of solutions or suspensions.

The production of silver nanoparticles by reduction of silver ions due to the addition of *Carica Papaya* Leaf extract was followed by UV–Vis spectroscopy. The UV-Vis absorption spectrum of 'Green' silvernanoparticles in the presence of *Carica Papaya* leaf extract is shown in .The band in silver nanoparticles solutionwas found to be close to 200 nm throughout the observation period as the nanoparticles were dispersed

in this solution without possibility for aggregation in UV-Vis spectrum. The high OD of the solution suggests a high conversion of Ag^+ to Ag^0 as nanoparticle.

Sr. No	Cell no-name	Wavelength	Absorption
01	1-4	100.0	2.35
		200.0	4.45
		300.0	3.41
		400.0	3.34
		500.0	3.005
		600.0	2.8
		700.0	2.51



III. Result

SEM analysis

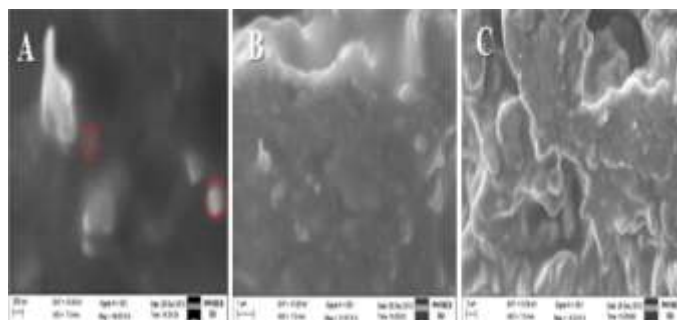


Figure: Scanning electron microscopy (SEM) micrographs of synthesized *C. papaya* leaf silver nanoparticles (CPL-AgNPs), (A) show the morphology of nanoparticles on 200 nm scale, (B) showing nanoparticles on 1 µm scale and (C) showing nanoparticles on 2 µm scale.

Gram negative strain	Disc1 CPL-AgNPs (1mg/mL)	Disc2 CPL-AgNPs (2mg/ml)	Disc3 CPL-AgNPs (3mg/ml)	Disc4 CPL-AgNPs (4mg/ml)
E.Coli	7	13	5	13

IV. Bacteria Culture

200ml solution of CPL-AgNPs was centrifused at 3000 rpm for 1hour and the precipitate was resuspended in ethanol and then in distilled water. The solution of 1mg/ml strength was prepared with the dry form using micropipette. Then an Agar medium of E.coli bacteria was prepared. Thereafter E.coli was confirmed by treatment of Streptomycin antibiotic. After that conformation E.coli was extracted and its solution was prepared. Then taking the solution on petridice, the silver nanoparticle solution of 1mg/ml strength was dropped on it (1 drop). Therefore an inhibition zone of 0.04cm was formed for E.coli.



Figure 5:Inhibition zone of E.Coli bacteria strain on nanoparticle solution.

V. Conclusion

The green synthesis and characterization of CPL-AgNPs was done and confirmed by UV-visible spectrophotometer techniques. The nanoparticles appeared to be spherical in shape with smooth surface and the size of the particles varied from 5 to 50 nm, but amongst them most of the particles obtained were sized in between 5 and 15 nm. The MIC and MBC of the CPL-AgNPs had exhibited inhibitory value $>25 \mu\text{g/mL}$ against both gram positive and negative bacterial species. In summary, the CPL extract mediated synthesis of silver nanoparticles was efficient and provides additional property such as bactericidal efficiency and might act as long searched alternative and could be the answer to antibiotic resistance. It has been demonstrated that the extract of papaya leaf extract are capable of producing silver nanoparticles extracellular and the silver nanoparticles are quite stable in solution. The flavonoid and terpenoid constituents which present in papaya leaf extract are the surface active molecules stabilizing the nanoparticles. Achievement of such rapid time scales for synthesis of metallic nanoparticles contributes to an increase in the efficiency of synthetic procedures using environmentally benign natural resources as an alternative to chemical synthesis protocols and low cost candidate as reductant for synthesizing silver nanoparticles.

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