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Research article

Biosynthesis, characterization antibacterial effects of silver nanoparticle by using *Carica papaya* fruit extract and it's interaction with an anticancer drug (5-fluorouracil)

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Key words: Silver Nanoparticles, 5-Flurouracil drug, *Carica papaya*, Anti microbial activity.

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Abstract

Silver nanoparticles (AgNp) has been synthesised using *Carica pappya* as a biological reduction technique. From the optical measurements, the synthesized silver nanoparticles exhibit high mono dispersed in nature it's an evident from SEM analysis. The concentration of silver nanoparticles determined by the Dude model using absorption spectrum. The interaction of AgNp with 5-Flurouracil (5- FU) drug was studied using UV–Vis absorption spectra, fluorescence spectra, FT-IR and life time fluorescence spectra. The powdered form of silver nanoparticles was synthesised and characterized by XRD pattern to measure the size of nanoparticle, their Antibacterial activity was screened against both gram-negative and gram positive microorganisms. Thus, this method can be used for rapid and eco-friendly synthesis of biocompatible silver nanoparticles possessing Antibacterial activity suggesting their possible application in medical industry.

Introduction

Nanotechnology is a rapidly expanding field today due to the multidisciplinary support. According to the latest update in March 2005, the 2006 NNI budget request for nanotechnology research and development across the federal government is $1.05 billion [1]. It is a promising arena and has exposed a panorama of applications in industrial, mechanical and medical field, in general nanoparticles are defined as particles with size<100 nm [2]. Among several nanoparticles, recently silver nanoparticles have been extensively used in electronics, engineering, textiles, paints, food industry, cosmetics, bio-sensing, chronic wounds, and even in medicine and biology [3-5]. Therefore, design and development of simple, one-step, reliable, low-cost, non-toxic and eco-friendly method for the fabrication of multifunctional silver nanoparticles is the greatest importance to expand their biomedical applications. And it focuses on formulating therapeutic applications of nanoparticles based on their mechanical agents in biocompatible nanocomposites such as micellar nanoparticles, nanocapsules, micellar systems, and conjugates [6]. The first most report of synthesis of silver nanoparticles, by the cell free aqueous extract of unripe papaya fruit [7] has several advantages than conventional chemical methods eco- friendly due to avoidance of many toxic chemicals, convenience of bio-resources such as plants, fungi, algae, microorganism that act as reducing as well as stabilizing/capping agent, universally acceptable solvent like water etc., [8]. And the Antibacterial activity of silver nanoparticles has been investigated against gram negative and positive bacteria [9]. The nanoparticles in general can be used to provide targeted (cellular / tissue) delivery of drugs, to improve oral bioavailability, to sustain drug/gene effect in target tissue [10]. 5-Fluorouracil (5-FU) with the chemical name 2, 4-dihydroxy-5-fluoropyrimidine is a cytotoxic agent, interferes with nucleic acid synthesis and inhibits DNA synthesis has been used for the treatment of solid tumours, which employed most extensively in clinical chemotherapy for the treatment of carcinomas of the colon or rectum and also precancerous dermatomes [11]. However, like other drugs used for chemotherapy, it affects the growth of normal body cells and often causes side effects such as hair loss, fatigue, birth defects, mouth sores and ulcers, liver disease, and a temporary drop in bone marrow function [12].

In the present study silver nanoparticles were synthesised by (green) biological reduction technique using ripe *Carica papaya* as a reducing and capping agent. Such reduced silver nanoparticle characterized by ultraviolet-visible spectroscopy (UV–Vis), Fluorescence spectroscopy, Fourier transform infrared (FTIR) spectroscopy and powder X-ray diffraction (XRD) and FESEM, then the AgNp was interacted with anticancer drug 5- Flurouracil and characterized through, UV–Vis , Fluorescence and FTIR spectroscopy. The evolution of

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antibacterial activity for both gram negative and gram positive organism have been studied by well diffusion method.

Experimental

Materials

Silver nitrate ($AgNo_3$), 5- Flurouracil was purchased from Sigma Aldrich, Whatman no. 1 filter paper, tryptic casein agar, Muller-Hinton broths (MHB) were supplied by Himedia Laboratories. All chemicals used in this study are analytical grade, and the solutions are prepared using double distilled water.

Synthesis of silver nanoparticles from *Carica papaya* fruit extract

The papaya extract was prepared, previously reported by Jain *et al.,* (2009) [7]. In brief, 25 *g* ripened *Carica Papaya* was weighed and thoroughly washed with distilled water, dried, cut into fine pieces. It was smashed into 100 ml sterile distilled water and filtered through Whatman No.1 filter paper, pore size 0.45 µm and was further filtered through 0.22 µm sized filters. The extract was stored at room temperature for further experiments. The aqueous solution of 1mM silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. *Carica Papaya* extract was added to an aqueous solution of silver nitrate; in 1:9 ratio, and kept at room temperature for 5 hours to occur reduction reaction of Ag^+ ions. Here the filtrates act as reducing and stabilizing agent for 1mM of AgNO3.

5-FU Interaction method

50µm 5-FU drug was dissolved in distilled water, used as a stock solution. From the stock solution (1% w/v) of drug was used as a working standard (WS) solution [13]. Before carrying the experiment, the nanoparticles concentration was matched with the concentration of 50µl 5-FU drug by matching the optical density of absorption spectra by UV-Visible spectroscopy (Perkin Elmer Lambda-35 UV-Visible spectrophotometer).The sample was prepared by taking 2ml 5-FU (WS) drug into a series of tubes having 9 test tubes labelled as S_1 to S_9. 10 to 80 µl of standard silver nanoparticle was added to $S_2 - S_9$ tubes in an ascending concentrations. And 2 ml of 5-FU drug alone (WS) was taken in S_1 was considered as control. The samples were making up into 3 ml using distilled water and kept test tubes at 4°C for 24hours to occur the interaction between drug and nanoparticles.

Bacterial cultures and evaluation of antibacterial activities

The bacteria were kept in tryptic casein agar in stock culture plates till the experiment started. Before the experiment the microorganisms were grown in Muller-Hinton broth (MHB) (Himedia-India) at 37°C for 16h. They were then harvested by centrifugation and washed three times by normal saline (NS). Subsequently the bacteria were diluted in 0.9% NS at an optical density of 0.05 and 0.06 at 600 nm for *S. aureus* and *P. aeruginosa* respectively [14]. All Petri plates and test tubes were sterilized in an autoclave before the experiments. The petriplates used in the tests were prepared using sterile L-rod, after allowing the bacteria to dry (within 5–10 min) and made the well to load the nanoparticles by using gel puncture [15]. The test solutions of silver nanoparticles in different concentrations (1, 0.5 and 0.1mM) were loaded within a well of 8mm diameter. The zone of inhibition was measured after 24 h incubation [16].

Results and Discussion

Characterization of silver nanoparticles and drug interactions

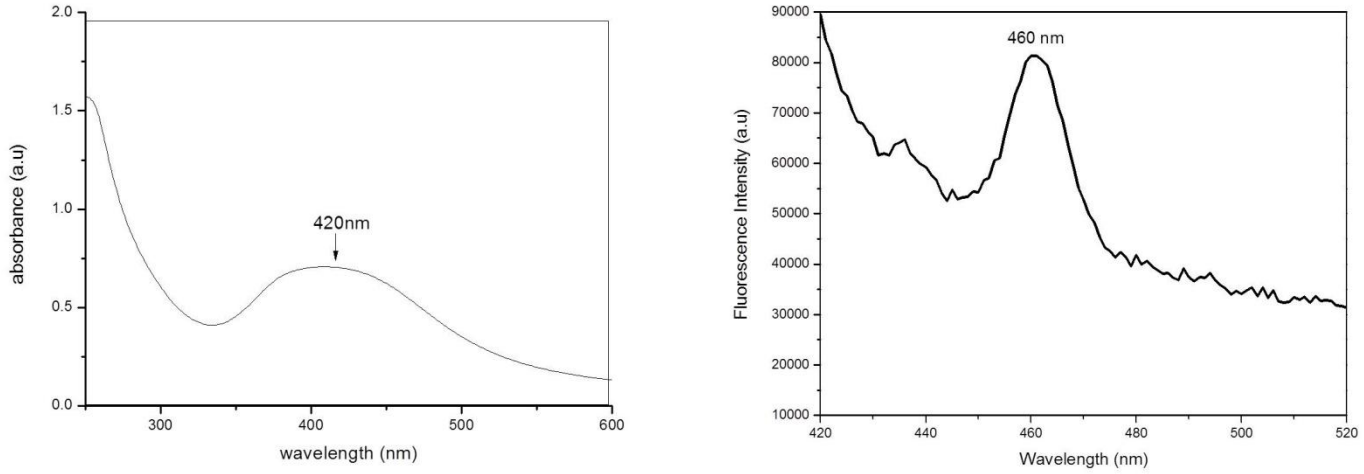
UV-Vis and fluorescence Spectra analysis - silver nanoparticles

When the frequency of the electromagnetic field becomes resonant with the coherent electron motion, hence the color of the prepared silver nanoparticles was yellowish brown after an hour and it turns to reddish brown color after 5 hours and remains stable. The stability of silver nanoparticles is observed for 2 weeks and it shows a SPR peak at the same wavelength. UV-Vis spectral analysis was done by using Perkin Elmer Lambda-35 UV-Visible spectrophotometer [17]. The silver nanoparticles which are having surface Plasmon resonance were synthesized using biological reduction and capping technique and measured their absorption spectra maximum at 420 nm (Figure 1 a). The absorption strongly depends on the particle size, dielectric medium and chemical surroundings. Small spherical nano particles (< 50nm) exhibit a single surface plasmon band. The absorption peak (SPR) was obtained in the visible range at 420 nm. The fluorescence emission of silver nanoparticles shows a strong peak at the wavelength of 460nm with 420 nm excitation. The fluorescence emission of silver nanoparticles (Figure 1 b) shows a strong peak at the wavelength of 460nm with 420 nm excitation. The AgNp interaction with drug helps to enhances the activity of targeted delivery on the desired cancer therapy and also took part in reducing an adverse effect while on cancer therapy drugs [18, 19].

FESEM Analysis

The suspension of AgNp in distilled water was used for FESEM analysis by fabricating a drop of suspension onto a clean electric stubs and allowing water to dry completely. The FESEM observation was carried out on a FEI Quanta250 FEG [16]. The mono dispersion of AgNp was showed in Figure 2. Through the SEM result the size

of the nanoparticle have been calculated as ~30- 40 nm approximately. The size of AgNPs are became high when compared to the previously reported literatures [7].



a **b**

Figure 1. a) Absorption spectra of silver nanoparticle (inset bio reduced AgNp solution) a broad spectrum has been shown at 420nm b) Emission spectra of silver nanoparticle was observed in 460nm.

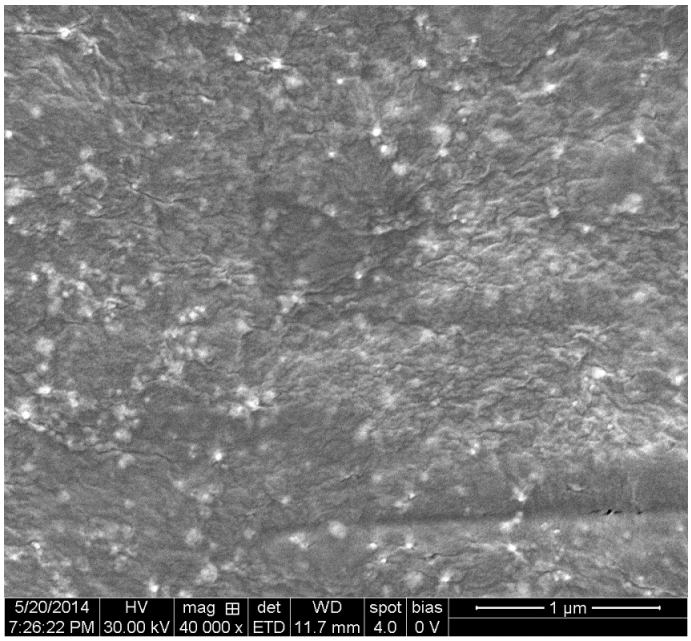


Figure 2. FESEM images of AgNp synthesized from Carica papaya fruit extract.

X-RD Analysis

1mM concentrated silver nanoparticles were dried at 100°C on hot plate till the black coloured particles obtained. Sample was collected from the petridish by scratching method. Finally the sample was fully dried in hot air oven and purified Ag- NPs, XRD patterns of silver nanoparticles gives X-ray diffraction (XRD) was performed with BRUKER D8 ADVANCE diffractrometer with Cu_{α} monochromator [20,21]. The analysis was run at room temperature with operating voltage of 40 kV and a current intensity of 30 mA. Figure 3 shows the X-RD spectrum of AgNp. The crystalline size can be calculated approximately from Deby- Scherer equation $D=0.89\lambda /\cos\theta$ [22]. It was confirmed by the characteristic peaks observed in the XRD image at (111) (200) (220) and (311) has θ=19.03, marked with (111). A number of Bragg reflections corresponding to the (111) sets of lattice planes are observed which may be indexed based on the face-centred crystal structure of silver. The XRD pattern thus clearly shows that the AgNPs are crystalline in nature.

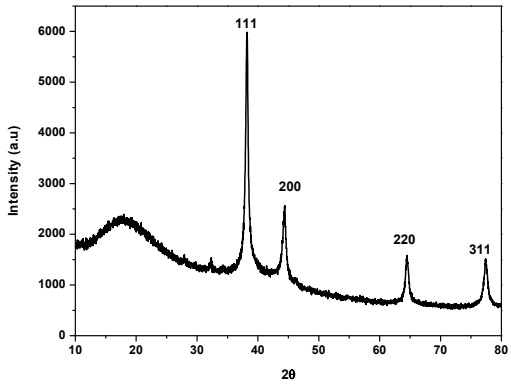


Figure 3. XRD patterns recorded for the silver nanaoparticles synthesized from pappya extract

UV-Vis Spectra analysis – standard drug 5- FU

5-Fluorouracil is a predominant anticancer drug that interferes with the growth of cancer cells which can be used to treat many types of cancers such as colon, rectum, breast, stomach, head and neck. It has a strong absorption at 267 nm in the UV region (Figure 4), which arises due to the n-pi* transition in the molecule.

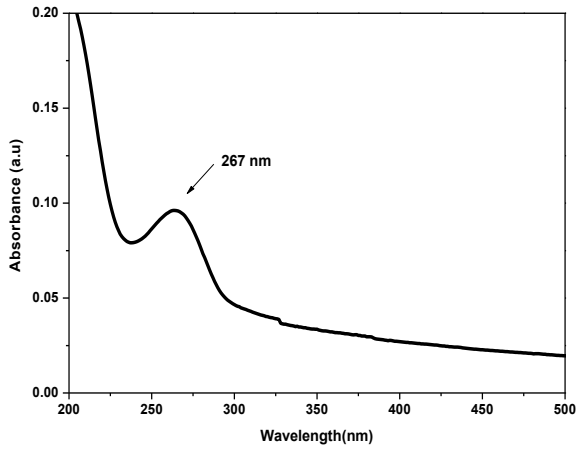


Figure 4. Absorption spectra of Anti cancer drug 5-FU observed at 267nm.

UV-Vis and fluorescence Spectra analysis – 5- FU interacted silver nanoparticles

UV- Visible absorption spectroscopy is simple method to obtain the structural changes and identify the complex formation to the different small molecules**.** Figure 5a shows the UV-Visible absorption Spectra of individual 5-Fluorouracil along with its combination with various concentration of silver nanoparticles. In general, the absorption wavelength of silver nanoparticles having greater absorptive at 420 nm and some of the factors can alters the absorption spectra such as pH, ionic strength etc., Due to the increase in the concentration of the nanoparticles in the finite concentration of 5-FU, we observed that there is an increase in the absorbance in both drug and silver nanoparticle regions at 267 and 420 nm. Papin stabilized silver nanoparticles exhibit a negatively charged surface rendering yellow stable dispersions in water due to the electrostatic repulsion between nanoparticles. Both the free fluorophore and the silver nanoparticle undergoes some photochemical reaction and it results in slight rise in the fluorescence emission in the AgNp emission region. Our results show that plasmonic-controlled fluorescence can lead to a novel physical mechanism to enhance fluorescence intensity. These effects can increase the field incident on the fluorophore and cause the changes in quantum yields, lifetime and photo stability [23]. There is an indication of 2 peaks in drug and nanoparticles region at 350 nm and 460 nm were noticed, after excited it at 280 nm (Figure 5b).

Fluorescence life time spectroscopy

The final implementation of the described TCSPC (Horiba USA) used in various measurement modes to verify basic functionality and timing accuracy on silver nanoparticles and its combination with 5-Fluorouracil drug with various concentration. Those combination lifetimes was determined by recording the lifetimes of each excited photon with respect to LED pulse. The average lifetime of silver nanoparticles was observed as 4.6244ns with 420 nm excitation, [24]. **Table 1** shows the fluorescent lifetime spectra for series of the samples AgNp, S_2, S_4 S_6 S_8 and S_9. That is now agreed that the metal-enhanced fluorescence occurs via near field interaction of fluorophore with the metal substrate, which can be described as localizing a dipole fluorophore in the electric field near a metal particle. The rate of photon emission by an excited fluorophore can be modified by changing the photonic mode density at the emitter position. Lifetime measurements support the coupling mechanism between the fluorophore and metal particle. Thus, the use of these ultra-bright and stable fluorophore/metal complexes has great potential for applications in the fields of medical diagnostics and biotechnology which have high background emission.

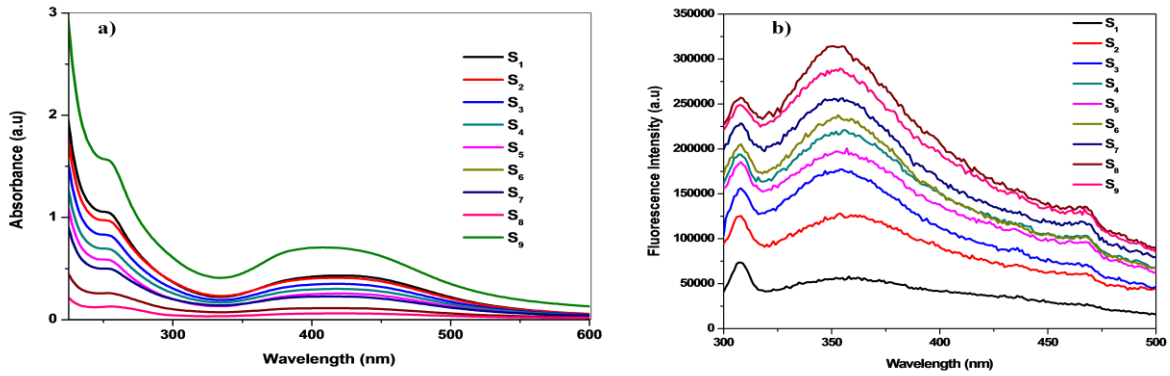


Figure 5. a) Absorption spectra of drug interacted nanoparticle samples (S_1- S_9) was observed at 267nm and 420nm for drug and nanoparticles respectively, b) Emission spectra of drug interacted nanoparticle samples (S_1 - S_9) was observed at 350nm and 460 nm respectively.

Table 1. Measurement of Average Fluorescence Lifetime spectra

Sample	a1	a2	a3	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	Average Life Time (ns)	Chi Sq
AgNp	13.08	43.07	43.85	1.1248	2.839	7.422	4.624428	1.041717
S_2	4.48	32.60	62.92	1.3487	2.125	5.866	4.444059	1.1593
S_4	35.80	8.60	55.60	2.228	0.92636	6.3432	4.40411	1.157
S_6	13.81	42.34	43.85	1.3209	3.1990	6.8642	4.546825	1.0312
S_8	44.57	5.55	49.88	2.234	0.3073	6.677	4.343237	0.98008
S_9	31.27	19.83	48.90	3.327	0.153	6.5882	4.292323	1.1190

FTIR analysis

The lyophilized nanoparticle samples were analyzed in FTIR to identify the possible bio molecules responsible for the reduction of the Ag^+ ions by the fruit extract filtrate, 5-FU (WS) S3 and S8. Those FTIR spectrums were represented in Figures 6 a, b, c, d, then the characteristics bonds shifting and functional groups were listed in Table 2. The representative spectra of silver nanoparticles (Figure 6a) obtained manifests absorption peaks located at about 3313.42 cm^{-1} (–OH stretch H-bonded) alcohol or phenol as functional group, 2141.76 cm^{-1} (–C≡C– stretch). The papaya fruit extract has a peak on C–O group band of polyols such as hydroxyflavones and catechins have been shifted to 1634.26 cm^{-1}(N–H bend 1^0 amines) from 1226 cm^{-1} that may possibly a cause of synthesis of bio-reduced silver nanoparticle [25].

Table 2. FTIR spectra characterstic absorption and functional group

	Characteristic Absorption	Functional Group
AgNp	3313.42	O-H stretch
	1634.26	stretching vibration of (NH) C=O group
	1062.18	C-N stretch vibration of amines
	796.81	CH2 C–Cl stretch
	656.3	NH2 & N-H (1^o amines)
5-FU (WS)	3286.94	–NH stretching
	1636.33	Stronger aminde than amines
	1366.38	α-CH3 bending
	1227.1	O-C Carboxylic Acids & Derivatives
	1036.21	-N stretching amines
S3	3315.33	–NH stretching 2^o amines
	1637.53	Stronger aminde than amines
	1226.82	C- N stretch aromatic amines
	697.33	C- H bending and ring puckering
	633.16	C- H deformation
S8	3327.12	–NH stretching
	1637.98	C=O carbonyl stretching
	1225.01	α-CH3 bending
	784.49	O-C Carboxylic Acids & Derivatives
	626.53	-N stretching amines

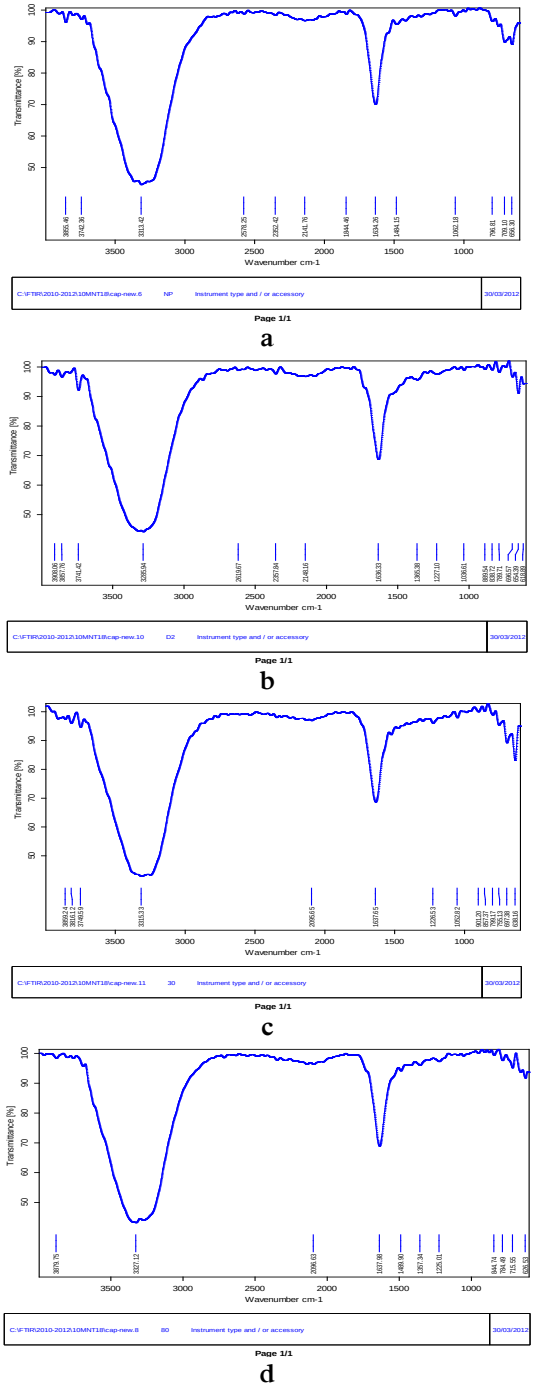


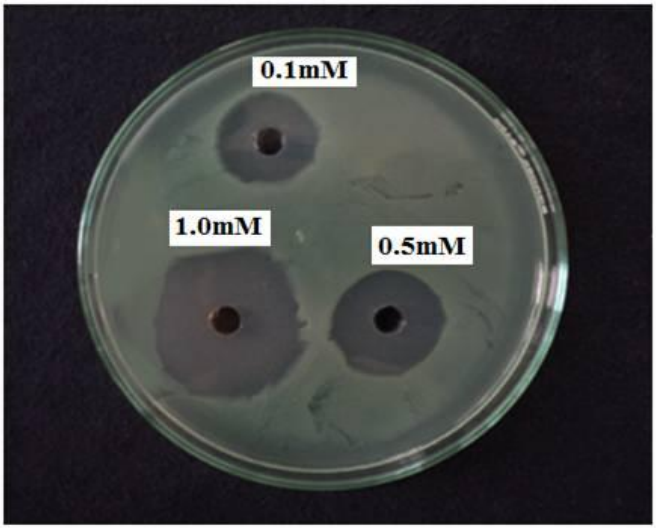
Figure 6. a) FT - IR spectra of silver nanoparticle (AgNp), b) 5- Flurouracil, (5- FU) c) S_3 and d) S_8 and their shifted functional group.

Anti bacterial activity of silver nanoparticles

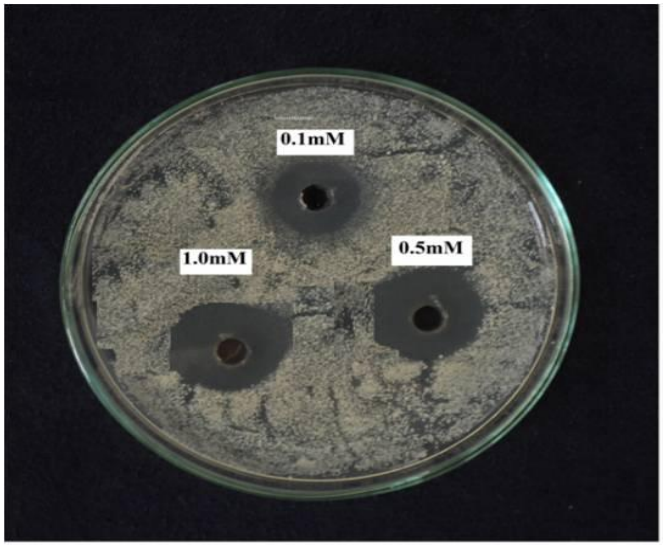
In an anti bacterial activity, the diameters of the zone inhibition for both gram negative and gram positive microorganisms *P. aeruginosa* and *S. aureus* respectively has been evaluated at the concentrations of 1mM, 0.5mM and 0.1mM of AgNp. However the highest inhibition rate of silver nanoparticles was observed for 1mM concentration in both organisms shown in Figure 7a and 7b respectively, and Table 3 showed the diameters of inhibition zone.

Table 3. Zone inhibition of silver nanoparticle at different concentrations for *S. aureus* and *P. aeruginosa*

Silver nanoparticle (mM)	Zone of inhibition (mm) *S. aureus*	*P. aeruginosa*
1	9	14
0.5	7	9
0.1	3	5



a



b

Figure 7. a) Zones of inhibition of silver nanoparticle against *P. aeruginosa*_b) Zones of inhibition of silver nanoparticle against *S. aureus.*

Conclusion

In conclusion we introduce a simple, fast, and economical biological procedure to synthesize Ag nanoparticles using *Carica papaya* fruit extract. Water-soluble bioactive compounds from papaya fruit extract that act as bio-reductants of silver nitrate to form AgNPs were most likely ascorbic acid and other phenolic compounds. We characterized these nanoparticles using FESEM, XRD, UV-visible, Fluorescence, Life time, and FTIR spectroscopic techniques, The SEM analysis revealed that size of the nanoparticles ranges from 50 to 70 nm, and then the synthesized nanoparticles were well dispersed. The interactions between AgNp and – 5- FU with different concentration of AgNp, have been evaluated through the UV-visible, Fluorescence, Life time, and FTIR spectroscopic techniques. An Antibacterial agent was investigated and exhibited better Antibacterial activity against *S. aureus and P. aeruginosa* for 1mM, AgNp. However, the Antibacterial effect was dose-dependent, and more pronounced against gram-negative bacteria than gram-positive bacteria. This green synthesized nanoparticle could be used in the medical field against human diseases due to their high efficiency as antibacterial agent.

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