RESEARCH ARTICLE

Biogenic Capped Silver Nanoparticles in Lablal Purpureus Pod Extract Exhibit Selective Antibacterial and Synergistic Anticancer Activity

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Biogenic Pod extract stabilized silver nanoparticles were synthesized by wet chemical route in negatively charged precursors. These green synthesized silver(0) nanoparticles with positive charges and neutralized by pod precursors had exhibited oval morphology, 21.5 nm. mean crystallite size and 462 nm. absorption band on the basis of UV-Vis., PXRD spectrometric and TEM analysis. The elemental composition and presence of silver(0) in pod extract flavonoid precursors of nanoparticles was proved on the basis of EDAX characterization. The positive surface capping and charges were estimated on the basis of FTIR and UV-Vis. Spectroscopy. The antibacterial testing of the nanoparticles had proved high surface interaction with gram negative bacteria than gram positive bacteria. The *in vitro* MTT assay for anticancer activity on MCF-7cells have been elaborated the better biocompatibility at 20 ppm. suspension than standard control drug and pod extract for nanoparticles, which were correlated to better positive charge on surface for interaction with highly negative charged breast cancer cells. Hence these biogenic stable silver nanoparticles had shown better ROS dependant biomedical property for cell particle interaction with synergistic and selective antibacterial and anticancer formulation property for selective biocompatibility.

Introduction

Metal and metal oxide nanoparticles are used nowadays in variety of applications, as these nanoparticles show better surface potentials for cell-particle interactions, these nanomaterials have free surface electrons and exhibit better electronic, and chemical potentials. Green synthesis of metal nanoparticles to obtain variety of shapes is the famous area of research in present era of nanoscience and technology. Such green synthesized metal nanoparticles like silver, gold, platinum, selenium in plant extract or in the bacterial cultures finds wide applications in chemical, biological and even in electronic fields due to their specific surface charges and activities. These metal nanoparticles posses zero neutral charges in surface coated state from precursor extracts, but many of them have special positive and negative surface charges after their biogenic synthesis inside their surface coats. These nanoparticles after their biogenic synthesis exhibits special surface activity related to their special bioactivities in biomedical areas such as antioxidant, antibacterial, anticancer potentials. Many researchers had formulated silver nanoparticles by biogenic methods with special surface charges and activities and set up synthetic characteristics for surface activity and biomedical applications.

Pal S. *et. al.*, had studied the role of shape of Ag nanoparticles on antibacterial activity [**1**], several research groups have estimated the surface activity and charge based cytotoxicity of silver and gold nanoparticles[**2-5**], Auffan M. and others have demonstrated size dependent activities of noble nanoparticles [**6**], many researchers have evaluated effects of surface charges, size and shapes on the bioactivities and toxicity of silver and other nanoparticles synthesized by chemical and green routes [**7-13**], such type of researches about functionalization and biological applications of silver and gold nanoparticles have been reported earlier [**14-22**], which posses limitations of specific surface activity related applications. More reports are available in the literature of synthesis and applications of silver nanoparticles in biomedical areas related to cell particle interaction [**15-28**], but few researchers have been studied the charge related activity of silver nanoparticles in biological fields. More literature data elaborates that Pod extract coating on Ag

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nanoparticles is superior than other biogenic plant extract coating as per references [**29-31**].

Also, as per references [**32,35**], the gold silver and zinc oxide nanoparticles synthesized in plant and pod extract have exhibits variety of shapes like oval, prisms or spherical morphologies, but all these nanoparticles have good surface activities due to phytochemical surface coatings. The surfaces of these nanoparticles are coated with phytochemicals like quecertin which activates the charges on their surfaces through chemical functionalities like carboxylate, amine or ketonic groups and neutralizes positive charges of nonparticles with negative functional charges. Overall, such nanoprticles when interacts with bacterial and cancer cells produces ROS inside cells after endocytosis and give best bioactivities.

But all these researches have been related to only surface charges of silver nanoparticles and their bioactivities [**29-31**], but these studies limits the research for specific and synergistic application study of these nanoparticles on the basis of surface activities. So here in this research work we had synthesized biogenic silver nanoparticles using rare plant extract to result positive charged surface of silver nanoparticles inside plant extract coat and set up synthetic parameters of 60°C temperature and pH = 6, to identify the surface activity related potential of these nanoparticles on antibacterial and anticancer biomedical areas. We have demonstrated the surface activity related ROS production ability of these biogenic silver nanoparticles in bacterial cells and highly negatively charged breast cancer cells, to evaluate the special, specific and synergistic bioactivity of these nanoparticles in biomedical fields.

Experimental section

Materials and instruments

All the chemicals used for synthesis of biogenic Ag(0) nanoparticles were of A. R. grade., $AgNO_3$, Conc. 11M HCl, KCl salt used for washing, MTT salt, solvents used in anticancer testing etc. all the chemicals were purchased from S. D. Fine Chem. Ltd. and Merck ltd and were used without further purification. The fresh Lablal Purpureus plant was collected from local forest area for phyto-biogenic synthesis of Ag nanoparticles. The double distilled water was obtained from Millipore system and was used throughout the biogenic synthesis and spectrometric characterization tests with this purified water as blank. The Systronics Model double beam spectrophotometer with scanning range 190 nm - 1100 nm was used for characterization, stability and synthetic parameter set up studies of Ag nanoparticles. The Perkin Elmer series model FTIR spectrophotometer is used to elaborate the surface functionalities and surface capping of biogenic Ag nanoparticles. The biogenic Ag nanoparticles synthesized using E-tronics magnetic stirrer with hot plate, centrifuge machine, sonicator, and drying oven. The PXRD patterns of biogenic Ag nanoparticles were estimated using the **JASCO-PXRD** technique with Cu Kα line source. The TEM images of nanoparticles were determined using Jasco type instruments with carbon mesh and Cu grid techniques. After EDAX analysis of nanoparticles the data was matched with PXRD and TEM image evidences. The surface charge and activity of Ag nanoparticles were determined on the basis of FTIR and UV-Vis. signals and data matched with TEM image evidences. The absorbances of cancer cell cultures after MTT assay were measured on double beam UV-Vis. spectrometer to determine cell viabilities for exposures of doses of standard drug control, plant extract and biogenic Ag nanoparticles.

Cell cultures

Bacterial cell cultures with gram positive *Staph. Aureus*, gram negative *E Coli.* Stains were obtained from Biotechnology center of Smt. K. W. College, Sangli, India and antibacterial studies performed by Agar well disc diffusion method in microbiology department of this college. The MCF-7 breast cancer cells were purchased from NCCS center of SPPU Pune University, Pune, India by aseptic carry technique and cell media from Himedia was purchased from same cell center in India.

Collection and preparation of lablal purpureus pod extract

Fresh and healthy Lablal Purpureus Pods were collected from a local forest area of district Kolhapur, Maharashtra, India and transferred to lab. About 50 gm. of these pods were taken, washed thoroughly with running tap water and then with double distilled water. These pods were then cut into small pieces and transferred to 500 ml. double distilled water and boiled for 20 minutes at 80°C. It was then allowed to cool and filtered using Whatmann filter paper No. 1. The filtrate thus obtained was stored at 4°C for further use.

Synthesis of Biogenic Silver (0) nanoparticles in Pod extract precursors

10 ml. filtrate of pod extract was mixed with 10 ml. of 10mM $AgNO_3$ and the reaction mixture was stirred well at 600 rpm. and 60°C for 8 hrs. Reaction mixture color was transformed from transparent pale yellow to dark brown color, which initially confirms the formation of biogenic AgNPs. Further confirmation of formation of AgNPs was done using UV-Vis. scanning on spectrophotometer which had showed a characteristic peak at 462 nm. Then the nanoparticles reprecipitated by adding 1mM solution of KCl to get positive charges on Ag inside pod extract flavonoids coating and particles were washed first by conc. HCl and then by double distilled water to get biogenic nano Ag(0). As synthesized biogenic capped Ag nanoparticles were stored in refrigerator and dried in oven whenever required. The effect pH, concentrations of precursors and temperature were studied on synthesis of Ag nanoparticles to set up the optimization conditions.

Physicochemical characterization for structure, morphology and surface capping

The formation, stability and surface capping of biogenic Ag nanoparticles were studied on the basis of UV-Vis. and FTIR spectrometric analysis. UV-Vis. spectra of nanoparticles was determined with 25 ppm. aliquot solution from suspension of ultrafine Ag nanopowder in Millipore double distilled water and water as blank. The FTIR spectra of dried biogenic Ag nanomaterial powder was determined on Perkin Elmer series spectrometer by palette KBr method. The surface capping had been proved by these estimations and then PXRD patterns of nanoparticles were determined by the PXRD technique with Cu kα source. The phase purity, packing of Ag atoms inside nanoparticles, surface capping, crystallite size had been estimated from PXRD data by matching with standard JCPDS card. TEM image and SAED pattern of Ag nanoparticles were obtained with Cu and carbon grid and mesh and related to study of morphology, structures and sizes. The SAED patterns of TEM analysis were matched with PXRD data.

Spectrometric stability studies and optimization of synthetic parameters

The stability of biogenic silver nanoparticles was studied along with setting the optimization parameters of synthesis of nanoparticles. The stability, effect of pH on synthesis, effect of temperature and concentration ratio of precursor plant extract and Ag salt were studied for synthesis of nanoparticles on the basis of UV-Vis. spectrometric real time screening by performing wavelength scans. The concentrations of Ag salt were varied from 6 to 10 mM. and with 1:1, 1:2, 2:1 pod plant extract and salt concentration ratios to decide the optimum stabilization of Ag nanoparticles. Also, the surface activity and capping over Ag nanoparticles was determined on the basis of these synthetic variations and UV-Vis. spectrometric scanning. The pH of synthesis varied from 5,6,7 to 8 to decide better stability at good surface capping of nanoparticles. The temperature of synthesis was varied from 50ºC to 80ºC to fix optimum temperature effect on stability of biogenic Ag nanoparticles. Overall, the synthetic parameters were varied during UV-Vis. spectrometric scanning and optimization for stability to estimate the possible surface capping, charges over neutral state of stable biogenic Ag nanoparticles.

Selective antibacterial activity

The antibacterial activity of biogenic AgNPs was studied against the bacterial strains of *E Coli* and *Staphylococcus aureus*. These bacterial strains were maintained on Nutrient Agar plates and Slants, sub-cultured and then stored at 4^0C. These strains were inoculated in the nutrient broth (peptone 5 gm., beef extract 1.5 ml., KCl 1.5 gm., pH 7.0) and incubated at 37ºC for 24 h. The strains of these gram negative and gram-positive bacteria were grown on Agar well plates. The wells bored for 2mm. size and drugs of 15, 20 and 25 ppm. of Ag nanoparticles suspension and plant extract were injected in wells. Then the culture plates were incubated by aseptic disc diffusion method. The zones of inhibition for plant extract and Ag nanoparticles were compared on gram positive and gram-negative bacteria. The selectivity of Ag nanoparticles for antibacterial activity was estimated on the basis of comparison of zones of inhibition of both types of bacterial cultures.

Anticancer activity on the basis of in vitro MTT assay

MCF-7 Breast cancer cell lines were purchased from NCCS center, Pune, India. All the cell lines were grown and maintained in suitable culture medium (DMEM (E) with FBS-10% Fetal Bovine Serum). All cells were trypsinated using trypsin, and exposed to five concentrations of Ag nanoparticles, pod plant extract and also standard drug 5-fluorouracil as 5, 10, 15, 20 and 25 ppm. The 96 well plate with material solutions and cell culture with cell density 1X 10^3 cells per well was incubated in a CO_2 incubator at 37^OC for 8 hours. At the end of the drug exposure period, 2 µL of MTT solution was added to all of the well and the plates were wrapped in aluminum foil, and incubated for another 4 hours in a CO_2 incubator at 37^OC. The culture medium was removed and from the wells the remaining MTT-formazan crystals were dissolved in to 100 µL of DMSO (dimethyl sulphoxide) to all of the wells. The absorbances of culture medium were recorded at 570 nm immediately. The Cell viability for drug exposure by MTT assay was calculated on the basis of optical density (OD) of culture solutions as with MCF-7 cells as control,

$$\textit{Cell Viability (\%)} = \textit{Mean OD of test material} / \textit{Mean OD of control} \times 100$$

The viability of cancer cells are related to the anticancer and apoptosis effects of nanoparticles and compared with standard drug 5-fluorouracil to estimate surface charge interaction of nanoparticles with highly negatively surfaces of MCF-7 breast cancer cells.

Results and discussion

Morphological and structural characterization

UV-Vis. Spectrometric stability and effect of pH on synthesis

As per **Fig. 1** to **Fig. 4** of UV-Vis. spectrometric wavelength scanning for synthetic parameter variations of Ag nanoparticles, the stability, surface activity and formation of neutral state in particles was determined with study of effect of pH for stabilization and optimization of synthesis. In **Fig. 1** better UV-Vis. absorption peak is observed for nanoparticles at 462 nm. with 10mM concentration of Ag salt. This evidence result into optimization of stability of Ag nanoparticles at 10mM salt concentration with characteristic maxima of Ag(0) state at 462 nm. In **Fig. 2** same peak of absorption maxima is obtained at pH=6 for nanoparticles elaborating the optimum pH of synthesis for 6. In **Fig. 3** such peak is obtained for 1:1 concentration ratio of pod plant extract and Ag salt proving 1:1 ratio for optimization of stable synthesis of nanoparticles with stable surface. In **Fig. 4** better absorption peak for nanoparticles is obtained only at 60°C temperature estimating the low temperature range for stability of nanoparticles. Overall, these observations can be related to stability and surface activity of Ag nanoparticles. At pH=6, 1:1 precursor ratio and 10mM salt concentration in the pod plant extract the stable biogenic nanoparticles are obtained elaborating the neutral charge in plant extract capped state, while containing some positive charges over surface inside coat due to surfactant like flavonoid interactions from extract over nanoparticles. Because as pH increases to 7 and 8 basic conditions shifts absorption maxima to blue side of high energy, and as precursor ratio increases for plant extract to 2:1 then absorption maxima shifts towards red side of low energy in figures. So, these evidences relates to surface chemistry of Ag nanoparticles in synthesis and stabilization. As acidic pH and optimum 1:1 precursor ratio in stabilization of nanoparticles play role for better positive surface neutralization by plant extract surfactant flavonoid coating resulting to Ag(0) nanoparticles. Hence these stable nanoparticles may contain positive charge surface potential hidden by capping of plant extract constituents.

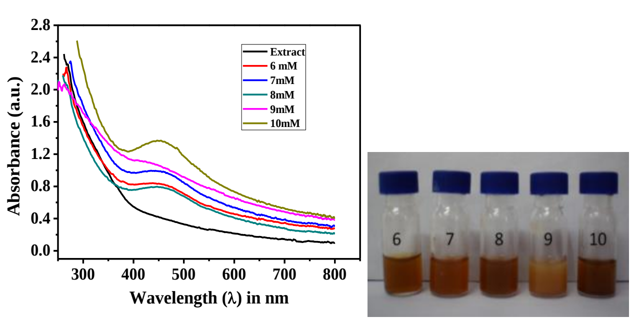


Fig. 1. Optimization of stability of nanoparticles for different concentrations of precursors on the basis of UV-Vis. absorption spectrum.

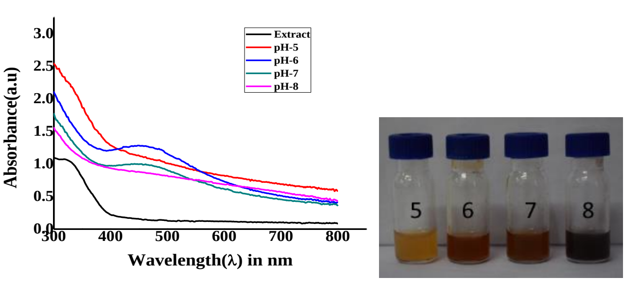


Fig. 2. Optimization of pH of synthesis on the basis UV-Vis. absorption spectrum.

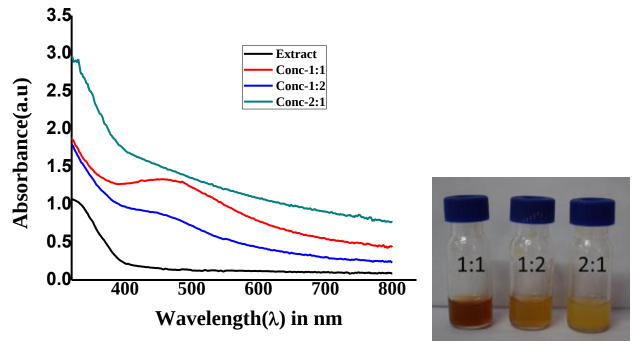


Fig. 3. UV-Vis. Absorption spectrum of biogenic pod extract capped silver nanoparticles for optimization of precursor ratio.

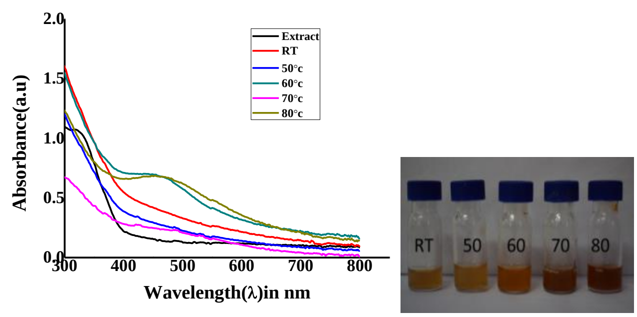


Fig. 4. UV-Vis. Absorption spectrum of biogenic pod extract capped silver nanoparticles for optimization of temperature of synthesis.

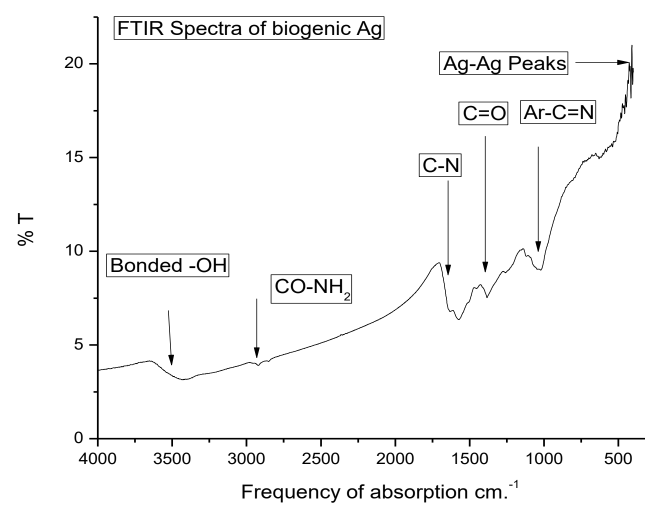


Fig. 5. FTIR spectrum of biogenic silver nanoparticles capped in pod extract flavonoids for surface activity.

FTIR spectrum proving surface capping

As per **Fig. 5** representing FTIR spectrum of Ag nanoparticles it is evident that the Ag neutral metal core contain flavonoid or surfactant like molecular capping. The FTIR signals in fingerprint region elaborates the presence of Ag-Ag metal core interactions with surface molecular capping. The signals in functional region of spectrum determines surface coat of flavonoid and surfactant like molecules from pod extract over Ag nanoparticles. Simultaneously these observations proves the surface charge activity of nano core silver inside molecular capping. Hence as per FTIR spectra it had been proved that, the surface of biogenic Ag nanoparticles have pure positive charges but coated with negative charges of quecertin like phytochemicals due to the functionalities like amide or carboxylic acids. Hence active surfaces are responsible for possible selective bioactivities of the nanoparticles.

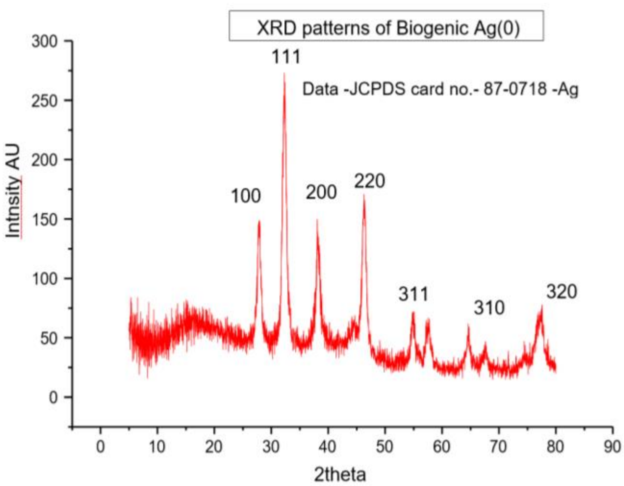


Fig. 6. XRD patterns of biogenic silver nanocrystallite particles proving crystallite lattice.

PXRD (Powder X-ray diffraction) pattern of biogenic silver nanoparticles

As per **Fig. 6** representing PXRD patterns of biogenic synthesized Ag nanoparticles, flavonoid or surfactant capped surfaces exhibit high intense, strong and some diffused peaks. The main diffraction peak was obtained in PXRD spectra for (111) plane at 32.5^o of 2 theta position. This XRD peak have proved the presence of single phase purity of nano ovals with FCC packing inside Ag nanoparticles. The crystallite size of the nanoparticles was then determined by Scherrer's formula as, $K = 0.9\lambda/\beta.COS\theta$ equal to 18-27 nm for biogenic nanocrystals (21.5 nm. from main diffraction peak) Where, β = FWHM of peaks, λ = 1.54 nm., θ = diffraction angle, and K = crystallite size. The surface capping of nano material had shown reduction in electron densities of nanostructure in diffraction at low intensity, because after flavonoid coating on surface of Ag nanoparticles the electron density of pure core nano Ag decreases to cause decrease in peak intensities from compared with JCPDS standard, hence in PXRD pattern the peaks are maintained at respective diffraction angles but intensities are slightly decreased in spectrum. The miller indices, lattice planes and constants representing FCC Ag in the nanoparticles are confirmed with the data of JCPDS card representing same packing and lattice constants as per **Table 1** as follows.

Table 1. Crystal parameters of biogenic Ag nanoparticles matched with standard JCPDS card.

Crystallite planes (Miller Indices) (h,k,l) FCC packing	d Calculated A^0 $d = a/\sqrt{(h^2+k^2+l^2)}$ or $2dSin\theta = n\lambda$	d Standard A^0 JCPDS card no. 87-0718	Lattice Constant a / b A^0 from main XRD peak of Ag nano ovals with FCC crystallite packing
100	2.9867	2.9834	a / b standard = 4.1021 JCPDS 87-0718 for FCC-Ag 2θ = 25 to 78^O
111 main diffraction peak/ plane giving Ag(0)	2.3738 d used in Scherer's calculation	2.3712 [2θ = 32.5]	
200	2.0334	2.0323	a/ b calculated = 4.1066 FCC Ag(0) nano ovals [biogenic Ag nano ovals as per TEM]
220	1.2298	1.2256	
311	1.1843	1.1823	

Hence the PXRD data reveals the phase purity, surface capping, free electron density over positive surface, FCC lattice packing, size below 30 nm., furthermore proving the surface activity of these biogenic Ag nanoparticles towards cell particle interactions.

EDAX analysis for elemental composition and particle characterization

EDAX graph of biogenic Ag nanoparticles in **Fig. 7** determines the composition and particle nature of nanoparticles. As per EDAX analysis it had been determined that these nanoparticles contain elemental composition as per synthetic protocol with elements as C, N, O, K and higher Ag. C, N, O may attributed from surface capping of pod plant extract flavonoids. Ag is present core of nanoparticles interacting with C, N, O molecules at surfaces. Slight K percentage may came from wasshing and purification in synthesis protocol. Overall this analysis proves elemental composition, purity, surface capping and particle state of Ag nanoparticles.

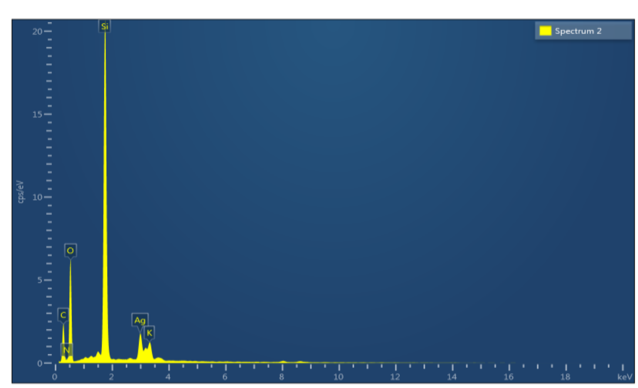


Fig. 7. EDAX graph of biogenic silver nanoparticles for elemetal composition.

TEM images and SAED patterns for Oval morphology estimation

The TEM image of nanoparticles in **Fig. 8(a)** and SAED patterns in **Fig. 8(b)** had proved the oval shapes and nanocrystallite nature of Ag nanoparticles. As per TEM image of nanoparticles the Ag nanocrystals exhibits more oval shapes and 20 to 23 nm sizes with actual particle diameters of 13 to 32 nm. The dot nature in SAED pattern proves crystallite planes of nanoparticles in FCC crystal packing as per XRD data. Here TEM and XRD data had matched with SAED patterns also elaborating crystallite nature, phase purity, FCC crystal system in surface capped nanoparticles.

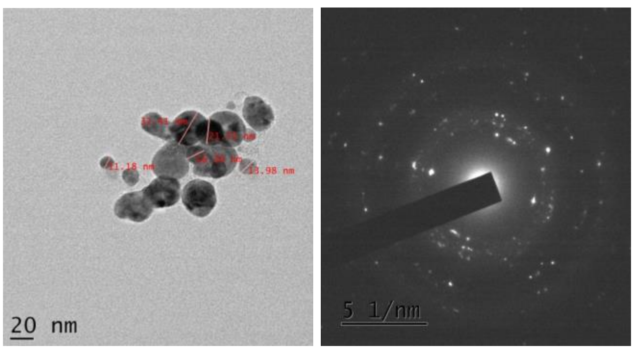


Fig. 8. (A) TEM image of biogenic Ag nanoparticles, (B) Corresponding SAED patterns.

Biomedical potential on the basis of selective antibacterial and synergistic anticancer activities

Antibacterial activity on gram positive and gram negative bacteria and selectivity

The biogenic Ag nanoparticles had exhibited higher and selective antibacterial potential on gram negative bacteria than gram positive bacteria (**Figs. 9(a) and 9(b)**).

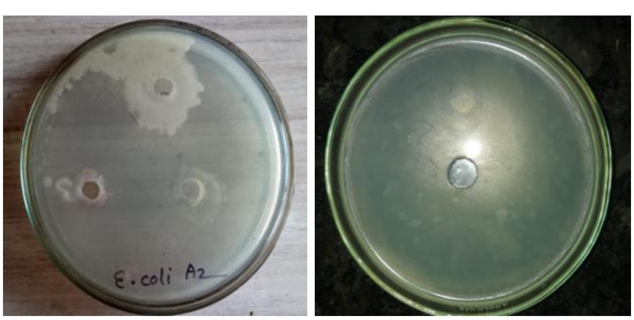


Fig. 9. (A) Zones of inhibition of *E coli.* (gram negative bacteria) culture for 20 ppm. suspension of Ag nanoparticles dose and plant extract, (B) Zones of inhibition for *Staph. Aureus* (gram positive bacteria) culture for 20 ppm. dose of Ag nanopartiles and plant extract.

Anticancer potential on MCF-7 breast cancer cells

The Ag nanoparticles at 20 ppm. concentration had exhibited significant anticancer activity with 42 % cell viability and 58 % apoptosis of MCF-7 cells than standard drug 5-FU. (**Figs. 10(a)** to **10(d)**).

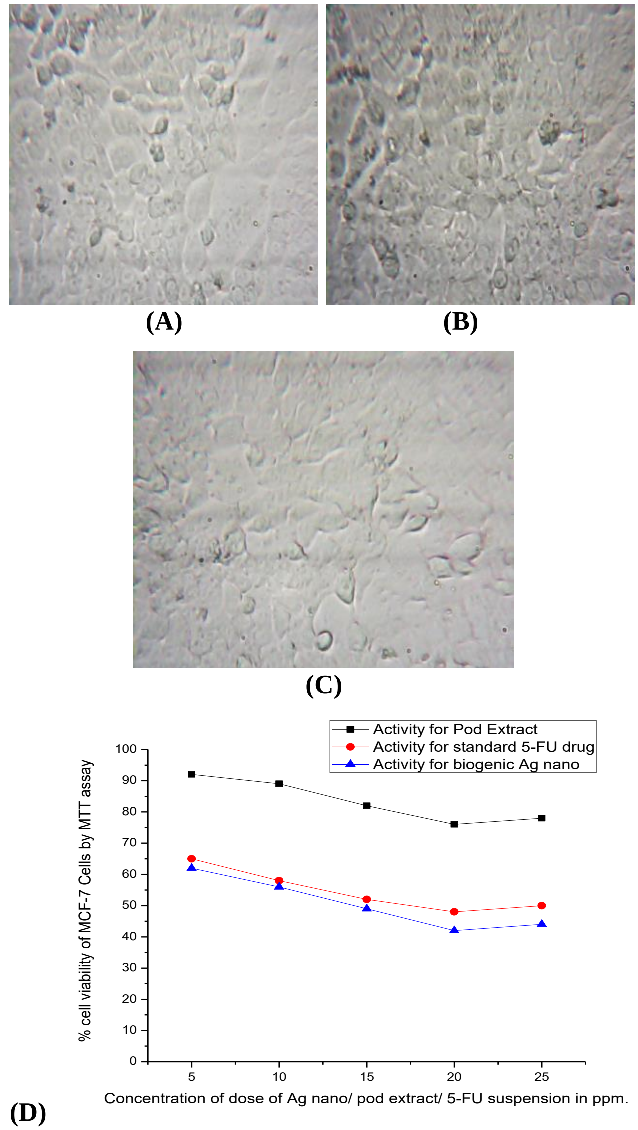
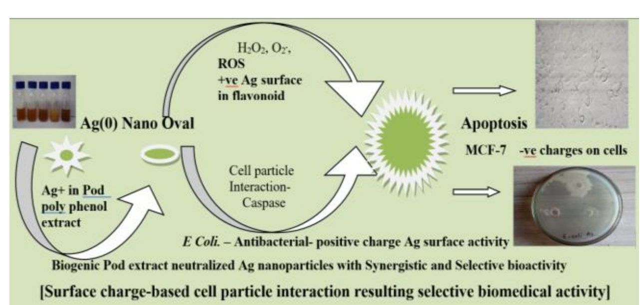


Fig. 10. (A) Anticancer potential on MCF-7 cells with inhibition from microscopic image after MTT assay of plant extract dosing of 20 ppm. on cancer cells, (B) Anticancer effects of standard 5-fluorouracil on MCF-cells at 20 ppm. after MTT assay, (C) Higher anticancer effects of biogenic Ag nanoparticles at 20 ppm. concentration on MCF-7 cancer cells after MTT assay, (D) Comparision of anticancer effects of standard control drug, plant extract and Ag nanoparticles doses on MCF-7 cells after MTT assay in terms of cell viability for 5, 10, 15, 20 and 25 ppm. concentrations.



Scheme 11. Mechanism for selective gram negative antibacterial activity and synergistic anticancer activity of the silver nanoparticles on the basis of surface activity and ROS production.

Mechanism for selective antibacterial and synergistic anticancer potentials on the basis of surface activity and ROS production [Scheme 11]

As per Scheme 11, **Fig. 9(a)** and **10(c)**, it is evident that the silver nanoparticles synthesized in pod plant extract by biogenic green method have some positive charges on surface and interact strongly with highly negative gram negative bacteria *E Coli.* and breast cancer MCF-7 cells. These positive surface charges on extract neutralized Ag(0) had been proved in UV-Vis. and FTIR spectral analysis, which are main features for selective and synergistic bioactivities of these nanoparticles for antibacterial and anticancer applications. As Pod plant flavonoids content like quecertin and surfactant like molecules are capped on Ag^+ ions to result Ag(0) with surface positive charges, which enhance the surface activity of these biogenic nanoparticles towards negative charged cellular species like *E Coli.* and MCF-7. Hence these nanoparticles shows the surface potential towards selective antibacterial activity on gram negative bacteria and synergistic anticancer property on MCF-7 cells. So, the positive charged surface activity and high ROS (reactive oxygen species) production in negatively charged cells by these nanoparticles are key factors of the mechanism of action for the selective and synergistic biomedical activity of the silver nanoparticles. As represented in figure of Scheme 11, these biogenic silver nanoparticles are more stable due to pod extract negative charged surface capping containing inside positive Ag surfaces. These highly stable Ag(0) when enters in negatively charged surface of cells like gram negative bacteria and breast cancer cells, they dissociate into positive surfaces of Ag and then go to organelles by endocytosis. Furthermore, these nanoparticles in dissociated phase releases free electrons in mitochondria or cell cytoplasm of cancer and bacterial cells. These nanoparticles hence produce free ROS like H_2O_2 or $O_2^{\cdot}$. These peroxide or superoxide species in cells produce caspases, which interacts with mutated DNA of cells and destroy cells to give Apoptosis or cell deaths resulting into anticancer and antibacterial activities showing cell lipid peroxidations []. As per antibacterial results these nanoparticles give higher zones of inhibition on gram negative bacteria and not for gram positive bacteria, so the antibacterial activity of these nanoparticles is selective on the basis of positive surface charges inside flavonoid capping like quecertin. The higher anticancer activity with higher selective antibacterial activity of these Ag nanoparticles is elaborating its synergistic biomedical potential and selective towards negatively charged cells like gram negative bacteria and breast cancer cells. So, these nanoparticles exhibit selective and synergistic biomedical activities on the basis of this mechanism. Hence as per this explanatory mechanism, these Ag biogenic nanoparticles have novelty for surface capping with positive charges through Pod extract coating and have selective antibacterial activity on *E Coli.* Furthermore, these nanoparticles exhibits synergistic bioactivity after testing the MCF-7 cell anticancer potential.

Role of pod extract on surface charge and relation to biomedical application

As per UV-Vis. absorption spectrum and FTIR analysis of the biogenic Ag nanoparticles and mechanism for surface activity discussed in Scheme 11, it is now clear that these nanoparticles show selective and synergistic biomedical activity towards negatively charged cells on the basis of some positive surfaces of Ag nanoparticles. The main role of surface activity of Ag(0) nanoparticles is played by constituents in pod plant extract. The pod plant extract contain plant flavonoids like quecertin and surfactant like species which stabilize the nanoparticles in the biogenic synthesis at pH=6 and $60^{O}C$ temperature to result stable nanoparticles. So, Ag^+ ions from precursors get capped with these molecules and neutralize the surface positive charges, but retain the charges inside capped state of the nanoparticles. Generally, at the slight acidic synthesis pH condition and low temperature these species stabilize silver nanoparticles, but when the nanoparticles interact with negatively charged cell surfaces or at high acidic pH of cancer cells, the surface positive charges on silver nanoparticles get developed inside cells at cell-particle interaction. So, pod plant extract species are having importance for stabilization of nanoparticles in synthesis as well as at cell particle interaction in biomedical application []. Hence these nanoparticles show selective and synergistic bioactivities. The surface activity and charge related biomedical potential of Ag nanoparticles synthesized by chemical and green routes from literature have been compared with our method and our Ag nanoparticles with literature data in **Table 2**, which gives idea about superiority of our Ag nanoparticles for selective and synergetic biomedical applications as per following summary reactions and the **Table 2**.

Reactions:

Ag^+ + Pod extract enzymes ⟶ Ag(0) ⟶ Cellular enzymes ⟶ Ag^+ with –ve surface
Ag^+ interaction with cellular organelles ⟶ Ag^+ to Ag(0) producing ROS

Reason for selection of Lablal Pod Extract and novelty of nanoparticles:

Lablal purpureus pod extract contain such enzymes which develops negative surface charges on positively charged Ag to neutralize to Ag(0) and facilitate endocytosis selectively to gram negative bacteria and breast cancer cancer cells as per previously reported in reference 30, 31, 32.

Biogenic plant extracts effects on capped surfaces of metal nanoparticles for selective bioactivities.

Table 2. Comparison of literature synthetic methods of silver nanoparticles and selectivity for bioactivity with our superior method for high selectivity of silver oval nanoparticles on gram negative bacterial biocompatibility and synergistic anticancer potential.

Synthesis of Ag nanoparticles by green and chemical route	Shape and size obtained with surface charge	Selectivity for Bioactivity	Reference
Chemical synthesis of positive and negative charged Ag nanoparticles	Spherical and circular shapes, +ve and -ve surface charges, 7 to 11 nm. sizes	Gram negative antibacterial activity by +ve charged and gram positive antibacterial by -ve charged Ag Nano spheres	29
Chemical route for synthesis using surfactant like coatings at high pH	Low to high negatively charged Ag nanoparticles, 10-20 nm. Sizes for 4 types of Ag nanoparticles, circular to oval shapes	Surface charge based antimicrobial bioactivity from less negative to highly negative Ag nanoparticles along with slight +ve surface charge	30
Chemical modified Ag nanoparticles compared with commercial Ag nanoparticles	Circular nanoparticles, 22 nm. Sizes, -ve surface charges	Better gram positive antibacterial activity and less cytotoxicity compared with commercial nano Ag	31
Chemically modified less and more aggregated Ag nanoparticles	Oval and circular shapes, 25 nm. Sizes, -ve surface charges	Charge specific gram positive antibacterial and antifungal activity	32
Green synthesized gold nanoparticles in plant extracts	Spherical and prism shapes, 15 to 50 nm. sizes	Positive surface charges and selective bioactivity	33
Pod extract synthesis of Ag nanoparticles	Oval and spherical shapes, about 40 nm. size	Good surface reactivity, antioxidant property	34
Plant extract synthesis of ZnO nanoparticles	Rod and oval type shapes	Effective antioxidant and antibacterial activity	35
Biogenic synthesis, Plant extract mediated surfactant like surface capping agents, pH=6 and 60^{O}C parameters	Oval shape, 22 nm. Size, +ve charged Ag, brown particles	20 ppm. dose gram negative bacteria and breast cancer bioactivity, surface charge-based ROS production, Selective and synergistic biomedical potentials	Present study

Conclusions

Very stable biogenic silver nanoparticles were synthesized in Pod extract using wet chemical bottom-up approach. Oval shape and crystallite nature of biogenic silver nanoparticles was elaborated on the basis of PXRD patterns and TEM image with SAED analysis. The surface flavonoid capping from pod plant and positive surface charges were determined using FTIR analysis related to UV-Vis. Spectrometric characterization. Selective antibacterial activity on gram negative *E coli.* was related to cell particle interaction and surface potential of nanoparticles. The anticancer activity of biogenic silver nanoparticles was compared with standard 5-fluorouracil drug on MCF-7 cells using MTT assay. Higher and synergistic along with selective biological activities of these nanoparticles was confirmed by these biomedical testing on the basis of key factor of ROS generation. Overall these pod extract capped silver nanoparticles are exhibited novel candidate for biomedical selective applications.

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Keywords

Biogenic; silver; pod extract; synergistic bioactivity.

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