

INVITRO EVALUATION OF ANTIOXIDANT, ANTIMICROBIAL, ANTICANCER ACTIVITIES AND CHARACTERISATION OF BRASSICA OLERACEA. VAR. BORTRYTIS. L SYNTHESIZED SILVER NANOPARTICLES

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ABSTRACT

Objective: Vegetable mediated synthesis of nanoparticles is a green chemistry approach that links Nanotechnology and Biotechnology. The present study is focussed on the biosynthesis of silver nanoparticles using aqueous fresh Cauliflower floret extract and to investigate the free radical scavenging potential, antimicrobial activity of the nanoparticles against different human pathogens and its cytotoxic activity against MCF-7 breast cancer cell line.

Methods: It was found that aqueous silver ions when treated with aqueous extract of fresh Cauliflower floret are reduced in solution there by leading to the formation of silver nanoparticles under optimum conditions at pH 6. The formation of silver nanoparticles was indicated by the colour change from colourless to reddish brown. Biosynthesized nanoparticles was characterised using several techniques, viz- UV-Vis spectroscopy, FT-IR, XRD, TEM, SEM and EDAX analysis. The free radical scavenging potential was measured by DPPH assay, antimicrobial activity against four microorganisms was tested using disc diffusion method and cytotoxicity of the nanoparticles was determined against MCF-7 cell line at different concentrations by MTT assay.

Results: Water soluble antioxidant constituents present in the Cauliflower floret extract were mainly responsible for the reduction of silver ions to nanosized Ag particles. UV-Vis spectral analysis showed silver Surface Plasmon Resonance band at 425nm. The presence of active proteins and phenolic groups present in the biomass before and after reduction was identified by FT-IR. The presence of elemental silver was characterised by EDS. The crystalline morphology and size of the nanoparticles were determined by TEM, SEM, and X-ray diffraction studies which showed the average size of the nanoparticles in the range 40 -50nm, as well as revealed their FCC structure. The biologically synthesized nanoparticles efficiently inhibited pathogenic organisms such as Klebsiella Pneumonia, Staphylococcus Saprophyticus and E. Coli. The biosynthesized nanoparticles might serve as a potent antioxidant as revealed by DPPH assay. Further these nanoparticles efficiently showed reduced viability and increased cytotoxicity on MCF-7 breast cancer cell line in a dose dependent manner.

Conclusion: The present investigation revealed that the fresh aqueous Cauliflower floret extract are capable of producing silver nanoparticles extracellularly through green synthesis and the reduction process was proved to be good, competent, convenient, easy to handle and considered ecofriendly as an alternative route to physical and chemical methods. The biosynthesized nanoparticles are quite stable in aqueous solution for a month without any sign of precipitation.

Keywords: Eco-friendly, Silver nanoparticles, Brassica oleracea var.botrytis.l, UV-Vis, FT-IR,EDS, SEM, Cytotoxic activity, MCF-7 cell lines, Antimicrobial activity, Antioxidant.

INTRODUCTION

Nanotechnology is an emerging, interdisciplinary area of research with important commercial applications and would be a dominant technology in new world economics. Nanoparticles are being viewed as fundamental blocks of Nanotechnology. Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. The most effectively studied nanoparticles today are those made from Noble metals, in particular Ag (1), Au (2), Pt (3) and Palladium. Metal nanoparticles find vast applications in various fields ranging from medical to physical fields (4,5,6).

Because of the unique physicochemical characteristics of metal nanoparticles including catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties (7- 9) they are gaining the interest of scientists for their novel method of synthesis.

Among the four, silver nanoparticles play a significant role in the field of biological system, living organism and medicine (10-12). Conventionally, nanomaterials are synthesised using either chemical or physical methods which include micelles(13) sol process, chemical precipitation, mechanical shaking, hydrothermal method and chemical vapour deposition method (14). Unfortunately many of these methods have several disadvantages such as high cost, consumption of high energy and chemical method of synthesis involving carcinogenic chemicals impart genotoxic effect in the medical applications. There has been a resurgence of interest in

plants and plant derived products as a source of medicine in the last few decades. Plant, fruit and vegetable mediated synthesis found to take place extracellularly would make the nanoparticles biocompatible and the reaction time has been reported to be very short compared to that of chemical method and microbial method of synthesis.

This promotes the growing need to develop environmental eco-friendly processes through green synthesis and other biological approaches. It has been reported that silver nanoparticles are nontoxic to human and most effective against bacteria, virus and other eukaryotic microorganism at low concentration without any side effects.

The most widely used applications of silver and silver nanoparticles include tropical ointments and creams containing silver to prevent infection of burns and wounds (15). The role of silver nanoparticles as an antiproliferative agent should create a new era in the field of medicine.

In general extensive research has been carried out on the biosynthesis of silver nanoparticles exploring its antimicrobial activity (16-20) rather than its anticancer activity (21-23). Cancer is a disease in which abnormal cells divide without control and are able to invade other tissues.

As there is increasing demand for anticancer therapy (24) on the health perspective and *invitro* cytotoxicity testing procedures reduces the use of laboratory animals (25) the usage of cultured tissues and cells have scientifically proved the significance of urgent

need to identify novel active chemotherapeutic agents from such natural sources.

White colour Cauliflower is a popular cruciferous vegetable which belongs to the family Brassicaceae, is considered as a food of high nutritional value. Cauliflower can be included in the diet regularly as it is a low calorie food. Cauliflower although not green, white head Cauliflower floret contains power house of antioxidants viz vitamin C and polyphenols. It is not only enriched with phytonutrients and antioxidants, it also contains significant amount of cancer fighting compounds such as sulforaphane and indole -3-carbinal, which boosts DNA repair in cells and appears to blocks the growth of cancer cells. The antioxidants play a chemo protective key role in human against risk of oxidative stress-related diseases such as cancer and cardiovascular diseases.

Keeping this in view, we have explored for the first time the green synthesis of silver nanoparticles using fresh aqueous cauliflower floret extract and an investigation is also focussed to outline the potential use of fresh aqueous Cauliflower floret extract as reducing, stabilizing and capping agent in the reaction that converts silver ions in to silver nanoparticles. AgNPs were characterised by UV-VIS, SEM, TEM, XRD, FT-IR techniques. Further for the first time the anticancer activity of biosynthesized nanoparticles against MCF-7 breast cancer cell line and antimicrobial activity against four human pathogens namely Klebsiella pneumonia, Staphyococcus Saprophyticus, Bacillus cereus and Escherichia coli were also explored. The free radical scavenging potential of biosynthesized nanoparticles are evaluated by DPPH assay.

MATERIALS AND METHODS

Cauliflower used for the preparation of the extract was procured from local supermarket (Fig.1a). The silver nitrate was supplied by Sigma-Aldrich Chemicals. The bacterial strains employed in this work were procured from Microbial Type Culture Collection Centre (MTCCC) located at the Institute of Microbial Technology, Chandigarh, India. For anti -cancer studies, cell lines are procured from National Centre for Cell Science, Pune, India. All chemicals and reagents used in the study were of Analytical grade.



Fig. 1: Photograph of a fresh cauliflower floret.

Preparation of Sample Extract

25gm of Cauliflower florets (*Brassica Oleracea*. var. *botrytis*.I) were accurately weighed, thoroughly washed under running tap water followed by washing it with double deionised water to remove the surface impurities. They were crushed using a blender and finely macerated. After homogenisation 100 ml of double deionised water was added and heated over a water bath maintained at 80°C for 15 minutes. The extract obtained was filtered through muslin cloth and then through Whatmann No.1 filter paper (pore size 25 μ m) and used immediately for the biosynthesis of AgNPs.

Pharmacognostic evaluation of aqueous extract

Fresh Cauliflower floret extracts were used for the following analysis:

Qualitative Phytochemical Analysis

Preliminary phytochemical screening was carried out for the identification of carbohydrates, proteins, phenols, flavanoids,

terpenoids, coumarines, steroids, phlobatannins, quinones, saponins and tannins using standard phytochemical methods (26).

Quantitative determination of Ascorbic acid

Quantitative determination of ascorbic acid content was done using UV- VIS spectrophotometer. Ascorbic acid content was determined using 2,6- dichlorophenol indophenol spectrophotometric method (27). 5g of Cauliflower florets are crushed, soaked in 100 mL of 4% Oxalic acid for 5 hrs at room temperature and filtered through Whatmann No.41 filter paper. The filtrate (1ml) was mixed with 5 ml of 2,6- dichlorophenol indophenol shaken well and added 4 ml of 4% Oxalic acid. The absorbance was immediately measured at 516nm against a blank. Amount of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.020-0.120mg/ml).

Quantitative determination of Total Phenolic Content

Total phenolic content was determined using FOLIN -CIOCALTEAU reagent (28). 100gm of fresh Cauliflower florets are crushed, soaked in 100ml double deionised water and filtered through Whatmann No.41 filter paper. 1ml of filtrate was mixed with 5ml of FOLIN-CIOCALTEAU reagent and added 15ml of saturated solution of Na₂CO₃ after three minutes and made up to 100ml with double deionised water. The reaction mixture was kept in the dark for 60 minutes. Absorbance was measured at 760nm in a spectrophotometer. Amount of phenolic content was calculated on the basis of the calibration curve for authentic Gallic acid (50-500mg/ml). The results are expressed as mg of gallic acid (GAEs) equivalents per 100gm of extract.

Determination of Total Antioxidant Capacity

The total antioxidant capacity (TAOC) was evaluated by Prieto (29). An aliquot of 1ml of concentration (1mg/ml) was mixed with 9ml of reagent solution (600mM sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes are capped and incubated over a boiling water bath maintained at 95°C for 90 minutes. Once the sample attains room temperature the absorbance of the solution was measured at 695nm against a blank. The results are expressed as mg of ascorbic acid (AAEs) equivalents per 100g of the extract

Determination of Total Reducing Power

The reducing power was determined according to the method of Oyaizu (30). 1ml of the extract (1mg/ml) was mixed with 1ml of 200mM of sodium phosphate buffer (pH- 6.6) and 1ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and 1ml of 10% trichloroacetic acid (w/v) was added. The mixture was centrifuged at 2000rpm for 10min. The upper layer solution (2.5ml) was mixed with 2.5ml of double deionised water and 1ml of fresh ferric chloride solution (0.1%). The absorbance was measured at 700nm. A higher absorbance indicates a higher reducing power.

Synthesis of Silver Nanoparticles

Aqueous solution of 1mM AgNO₃ was prepared and used for the synthesis of silver nanoparticles. 20ml of aqueous Cauliflower extract is mixed with 80ml of AgNO₃ for the synthesis of silver nanoparticles. The formation of silver nanoparticles is confirmed by colour change from colourless to reddish brown and by UV-Visible spectroscopy.

Fixation of parameters for Biosynthesis of Silver Nanoparticles

Biosynthesis of AgNPs using different compositions (Volume ratio of extract:AgNO₃)

The biosynthesis of the AgNPs was carried out for different compositions of the extract and AgNO₃ solution (1:1, 1:2, 1:3,1:4 and 1:5). Time taken to record an absorption peak at 425nm was noted.

Biosynthesis of Silver Nanoparticles at two different Room Temperatures

The bio synthesis of silver nanoparticles was done at two different room temperatures namely; 27°C and 34°C, and the absorbance at the absorption maximum was measured spectrophotometrically.

Microwave Assisted Biosynthesis of Silver Nanoparticles

The biosynthesis was carried out under microwave condition maintained at 100W power. The colour change as well as the absorbance of the reaction mixture was monitored spectrophotometrically for every 30 sec.

Water bath assisted Biosynthesis of Silver Nanoparticles

The biosynthesis of silver nanoparticles was carried out over a water bath maintained at 80°C till the resultant solution changes to yellowish red colour and the UV-Visible spectrum was recorded.

Autoclave assisted Biosynthesis of Silver Nanoparticles

Synthesis was carried out under autoclave condition maintained at 125°C and 5-7 lbs pressure, till there is appearance of reddish brown colour. The UV-visible spectrum was recorded.

Presence of capping agent

The synthesis of AgNPs was carried out in the presence of 1% freshly prepared rice starch and the change in colour was observed and UV-Visible spectrum recorded.

Stability of Silver Nanoparticles

The stability of the silver nanoparticles was determined at room temperature at an interval of 12 hrs for 30 days. The pH of the biosynthesized silver nanoparticles was monitored regularly.

Characterisation of Biosynthesized Silver Nanoparticles

Visual inspection

The bio reduction of silver nitrate using aqueous cauliflower floret extract was monitored and the appearance of reddish brown colour indicates the formation of silver nanoparticles.

UV-Vis Spectroscopy

The reduction of silver nitrate to silver using aqueous cauliflower extract was monitored by measuring the UV-Visible spectrum of the reaction mixture after diluting a small aliquot of the sample with deionised water. The measurements are recorded on Shimadzu Dual Beam Spectrometer (Model UV-1650 PC) operated at a resolution of 1nm.

FT-IR Analysis of Biomass before and after bio reduction

FT-IR measurement was carried out for both the extract and silver nanoparticles to identify the possible bioactive molecules responsible for the reduction of the Ag⁺ ions and the capping of the bio reduced silver nanoparticles by the cauliflower extract, in the diffuse reflectance mode at a resolution of 4cm⁻¹ using KBr pellets and the spectrum was recorded in the wavelength interval 4000 to 400cm⁻¹.

X-ray Diffraction Studies

X-ray diffraction (XRD) measurement of the cauliflower reduced AgNPs was carried out using powder X-ray diffractometer instrument (SEIFERT JSO DEBYEFLEX -2002) in the angle range of 10° -70° operated at a voltage of 40KV and a current of 30mA with Cu K α radiation in a θ -2 θ configuration. The crystallite domain size was calculated by using Debye -Scherer formula.

Scanning Electron Microscopy (SEM)

The sample was prepared by placing a drop of colloidal solution of AgNPs on carbon coated copper grid and subsequently drying in air, before transferring it to the microscope operated at an accelerated voltage of 130KV (Hitachi -S 3400N).

Transmission Electron Microscopy (TEM)

TEM technique was employed to visualise the size and shape of silver nanoparticles. The 200KV high resolution transmission electron microscope (FEITECNAI F- 20) was used. TEM grid was prepared by placing a drop of the particle solution and drying under a IR lamp.

Energy Dispersive X-ray Spectroscopy (EDAX)

The presence of elemental silver was confirmed through EDS. Energy dispersive analysis X-ray spectrometer takes advantage of the photon nature of the light. In the X-ray range the energy of a single photon is just sufficient to produce a measurable pulse X-ray. A semiconductor material is used to detect the X-ray along with processing electronics to analysis the spectrum. The EDS observations were carried out by instrument coupled with TEM.

Pharmacognostic Evaluation of Silver Nanoparticles

Determination of Free Radical Scavenging Activity by DPPH assay

The ability of the AgNps to annihilate the DPPH radical (1,1-diphenyl-2-picrylhydrazyl) was investigated by the method described by Harbone and Baxter 1995 (31). Stock solution of sample was prepared to the concentration of 1mg/ml. 50 μ g 100 μ g and 150 μ g of each sample were added to 100 μ l of metabolic solution of DPPH (0.1%). The reaction mixture was incubated for 30 min at room temperature and the absorbance (A) was recorded at 517nm. The experiment was repeated for three times. BHT (Butylatedhydroxytoluene) was used as standard control. The annihilation activity of free radicals was calculated as % inhibition according to the following formula

$$\% \text{ of Inhibition} = (A \text{ of control} - A \text{ of test}) / A \text{ of control} \times 100$$

Determination of Antibacterial Activity

The Antimicrobial activity of silver nanoparticles synthesized using fresh aqueous cauliflower extract was determined on Muller & Hinton Agar (Hi-Media Pvt. Ltd. Mumbai) using Kirby-Bauer disk diffusion method (32). Test pathogens were spread on the test plates- Muller Hinton agar (MHA) for bacteria using sterile swabs. Sterile wells were made with the help of a sterile cork borer at aseptic conditions. Samples (1500 μ g) were added to the wells at aseptic conditions. Stock solutions of the extracts were prepared using DMSO. The test plates were incubated and the zone of inhibition (in mm diameter) was read and taken as the activity of the extract against the organisms.

Determination of *In vitro* Assay of Cytotoxic Activities

Cytotoxic effect of the silver nanoparticles was determined by the MTT assay (33). Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine serum, at 37°C in humidified atmosphere with 5% CO₂. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2 X 10⁴ cells/well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the extract for 24 hours. After the incubation, medium was discarded and 100 μ l fresh medium was added with 10 μ l of MTT (5mg /ml). After 4 hours, the medium was discarded and 100 μ l of DMSO was added to dissolve the Formosan crystals. Then, the absorbance was read at 570nm in a micro titre plate reader. Cell survival was calculated by the following formula.

$$\text{Viability}\% = (A_t/A_c) \times 100$$

$$\text{Cytotoxicity}\% = 100 - \% \text{ Viability.}$$

Where A_t is the absorbance of the test sample, A_c is the absorbance of the control.

RESULTS AND DISCUSSION

Pharmacognostic evaluation of aqueous cauliflower floret extract

The results of the phytochemical analysis of cauliflower floret extract are shown in Table.No.1, which indicate the presence of secondary metabolites such as carbohydrates, proteins, tannins, flavanoids, phenols, phytosteroids, steroids, terpenoids, and cumarines etc. The presence of phenolic compounds constitutes a major group of compounds that act as primary antioxidants which are mainly responsible for the reducing property of the cauliflower floret extract. Numerous analysis have shown that adequate intake of ascorbic acid is effective in lowering the risk of developing cancer of breast, cervix, colon and rectum. Hence the ascorbic acid present

in cauliflower floret extract was determined and it is found to be 400 mg /100g of florets. Phenolic compounds are very important plant constituents because of the scavenging ability of their -OH groups. The antioxidant property of phenolic compounds is mainly due to the redox property which allows them to act as reducing agents. The amount of phenol content was found to be 600µg in 100gm of florets. The total antioxidant capacity of the aqueous cauliflower extract was found to be 300 mg of ascorbic acid/ 100g of floret. The reducing power of the aqueous extract shows higher absorbance at 700 nm indicating its high reducing property

Visual Characterisation

As the aqueous cauliflower extract was mixed with aqueous solution of 1 mM silver nitrate, it started to change colour from colourless to reddish brown due to reduction of silver ions; which indicates the formation of silver nanoparticles (Fig.1b). Different parameters were optimized viz- ratio of volume of extract and AgNO₃, temperature, different modes of heating viz, microwave, water bath, autoclave and in the presence of capping agent (starch) which have been identified as factors affecting the rate of formation of silver nanoparticles.



Fig. 1b: Photograph of a fresh aqueous Cauliflower floret extract and Formation of Silver Nanoparticles.

Table 1: Qualitative phytochemical screening of fresh cauliflower floret extract

S. No.	Phytochemicals	Results
1	carbohydrates	+
2	Tannin	+
3	Saponin	-
4	Flavonoid	+
5	Alkaloid	-
6	Protein	+
7	Quinone	+
8	Glycoside	-
9	Cardiac glycoside	-
10	Terpenoid	+
11	Phenol	+
12	Coumarine	+
13	Steroid&phytosteroid	+
14	Pholabatannin	-
15	Antraquinone	-

Indication of sign (+)present and (-) absent.

Table 2: Rate of formation of Silver Nanoparticles using different ratios of volume of fresh aqueous cauliflower floret extract and aqueous 1mM AgNO₃

S. No.	Composition	Ratio	Time in minutes
1	20ml extract+20ml AgNO ₃	1:1	90
2	20ml extract+40ml AgNO ₃	1:2	60
3	20ml extract+60ml AgNO ₃	1:3	60
4	20ml extract +80ml AgNO ₃	1:4	30
5	20ml extract +100ml AgNO ₃	1:5	90

1) Ratio of volume of extract: AgNO₃

The time taken for the formation of AgNPS depends on the ratio of volume of extract to AgNO₃ solution and the results are given in Table.No.2. The time taken for the formation of silver nanoparticles was found to be less for 20 ml of extract and 80 ml of 1mM AgNO₃ solution. This ratio was found to be ideal because biosynthesized nanoparticles showed maximum absorption at 425 nm, which is in agreement with reported value (34) (Fig.2a).

2) Effect of temperature and different modes of heating on biosynthesis of AgNPs

The effect of temperature on the rate of formation of AgNPS was studied for the composition 20 mL of the extract and 80 mL of AgNO₃. The AgNPs were formed within one hour at 27°C however, at 34°C the AgNPs are formed within 30 minutes and under water bath

condition it was formed within 10 minutes (Fig.2b).Hence higher temperature favours the formation of AgNPs (Fig.2b).The rate of formation of AgNPs was still higher under microwave condition which is found to be 2 min. However, under autoclave condition though there is visual colour change, the UV-VIS spectrum shows a decrease in the absorption intensity at 425 nm as indicated in (Fig.3).

3) Stability of AgNPs

The UV- Visible spectrum for the biosynthesis of AgNPs using 20 mL of extract and 80 mL of AgNO₃ was recorded over a period of time for 30 days. There was no change in the UV-visible spectrum.The biosynthesised nanoparticles were found to be stable for 30 days at pH 6-7 without any sign of precipitation and without any change in λ max value as indicated in the (Fig.4)

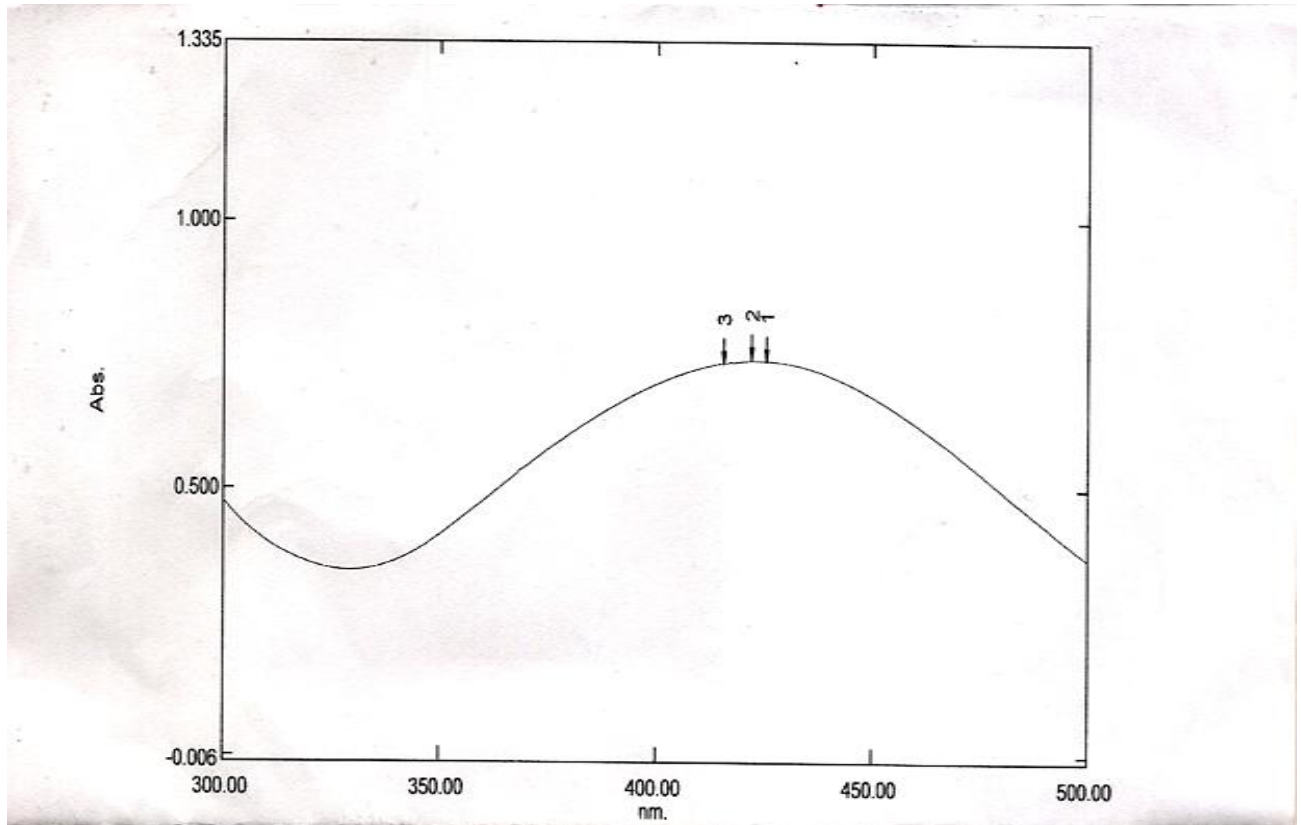


Fig. 2a: UV-Vis. Absorption spectrum of Ag Nanoparticles synthesized under optimum conditions from fresh aqueous cauliflower floret extract and 1mM AgNO₃

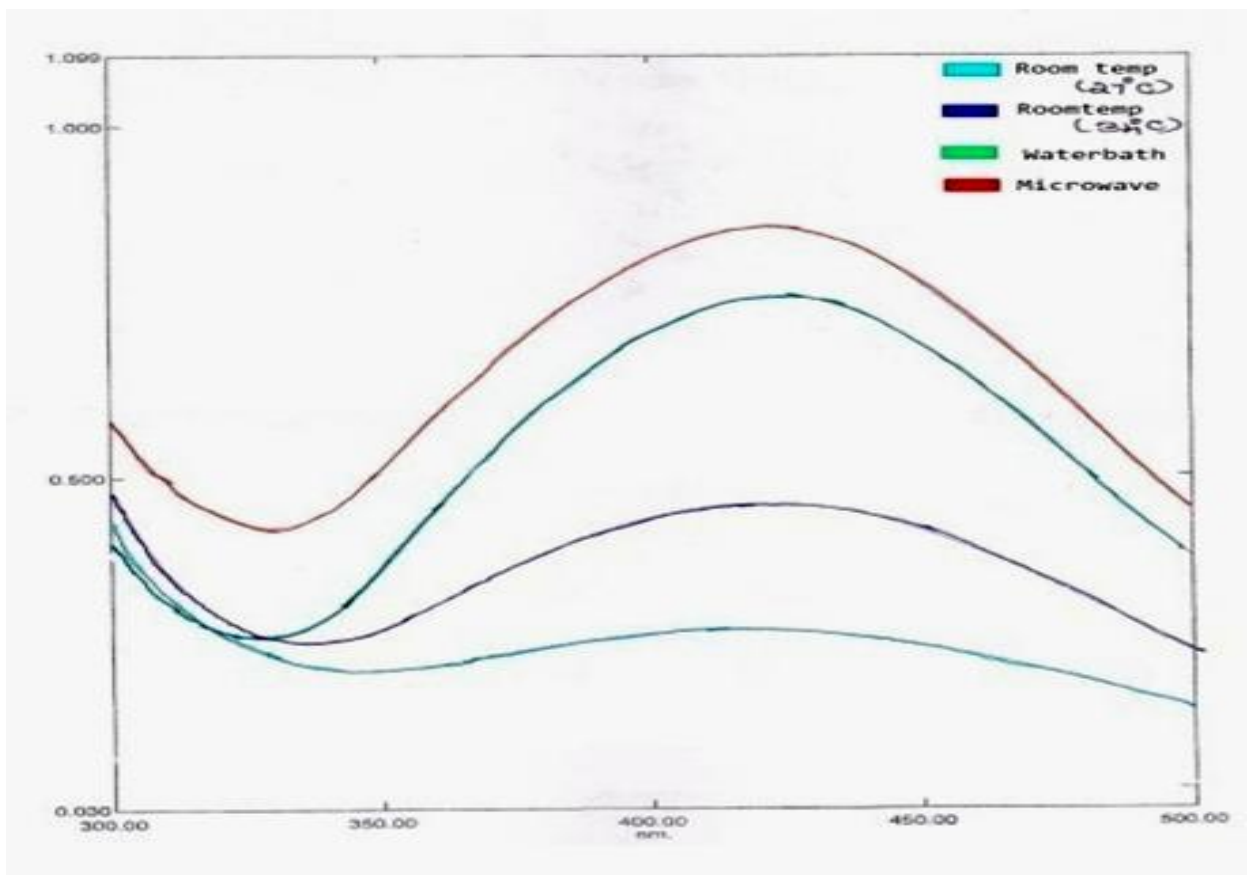


Fig. 2b: UV-Visible absorption spectra of Silver Nanoparticles synthesized at different temperatures and under different heating conditions

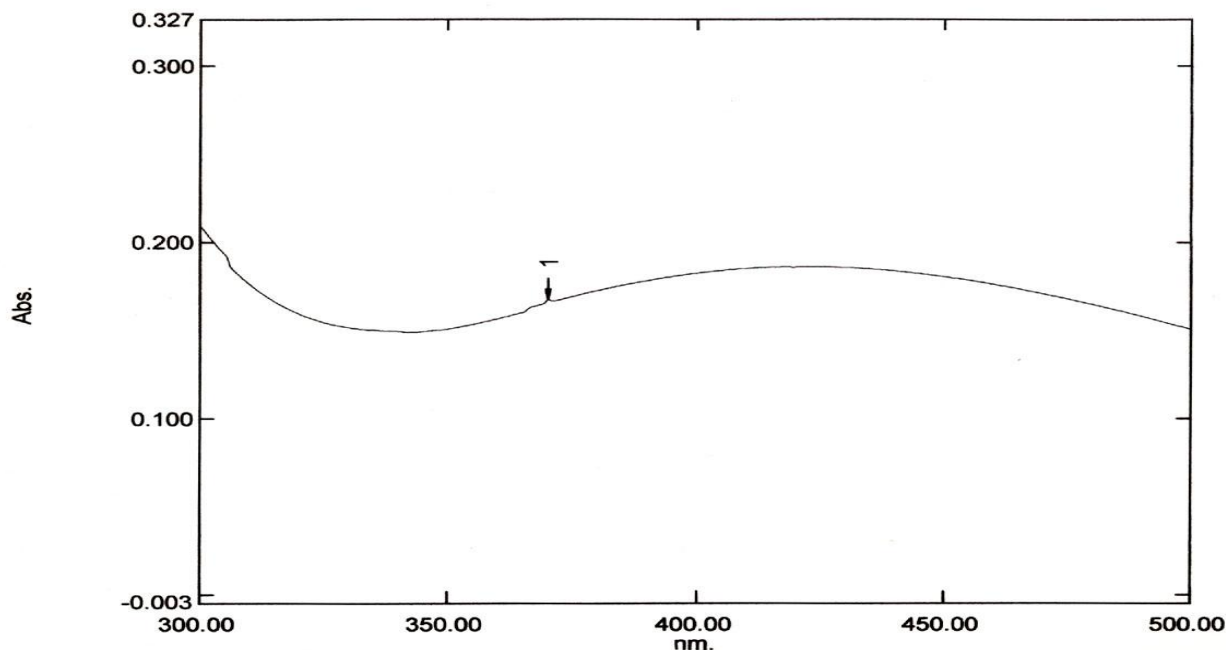


Fig. 3: UV - Visible spectrum of Silver Nanoparticles synthesized using fresh aqueous cauliflower floret extract under autoclave conditions

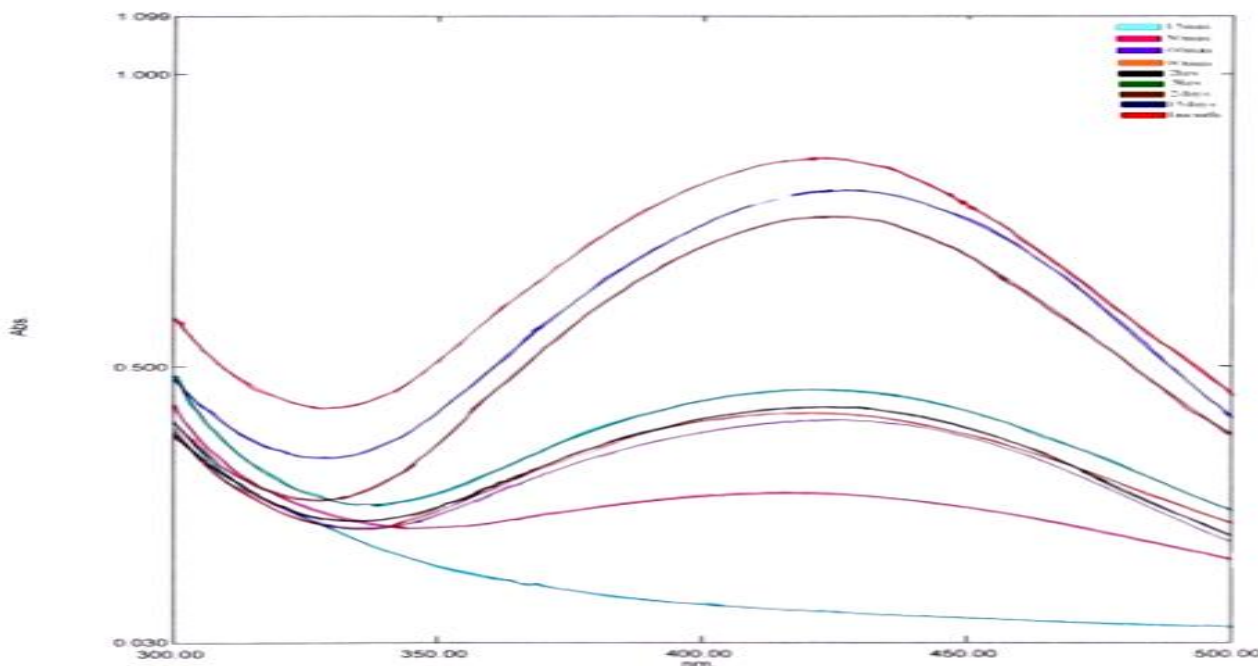


Fig. 4: UV-VIS spectra of Biosynthesized Silver Nanoparticles taken at different period of time indicating its stability.

Characterisation of Biosynthesized Silver Nanoparticles by spectral methods

UV-VIS spectroscopy

UV-VIS spectroscopy could be used to examine the size and shape of silver nanoparticles in aqueous suspensions (35). For an ellipsoidal particle there are two peaks whereas for spherical particle there is only one peak centered at 420 nm, in the UV-VIS spectrum. The Absorption spectrum of silver nanoparticles formed in the reaction has an absorption peak at 425nm which indicates the particles are spherical in shape. The absorption peak maximum is attributed to the Mie scattering by silver metal (36).The appearance of yellow

colour indicated the formation of silver nanoparticles in the reaction mixture, as it is well known that AgNPs exhibit striking colours (light yellow -brown) due to the excitation of surface Plasmon Vibrations in the particles (37). In the present study there is only one peak at 425 nm indicating that the AgNPs are spherical in shape.

FT-IR

The FT -IR spectrum of the cauliflower floret extract was taken before and after the synthesis of AgNPs is shown in (Fig.5a and5b). The spectrum was recorded in the wavelength region between 400cm^{-1} to 4000cm^{-1} . The IR spectrum of the cauliflower extracts shows sharp peaks at wave numbers 3632cm^{-1} , 3745cm^{-1} , 3577

cm^{-1} which shows the presence of $-\text{OH}$, $-\text{NH}_2$, $-\text{NH}$ group in the compound. The band at 3417 cm^{-1} , 3431 cm^{-1} are characteristic of the hydrogen bonded N-H group in secondary amide. The band at 1639 , 1623 cm^{-1} corresponds to the $>\text{C}=\text{O}$ group in secondary amides. The bands in the range 2359 to 2924 cm^{-1} correspond to C-H stretching from methyl or methylene groups and $-\text{C}\equiv\text{N}$ bond. The

position of these bands was close to that reported for native protein (38). The C-S stretching appears as a weak band in the region between 700 - 600 cm^{-1} . The IR spectrum of the AgNPs indicates the absence of many fundamental groups and peaks of lower intensity. The disappearance of the bands and the decrease in intensity is attributed to reduction of silver ions.

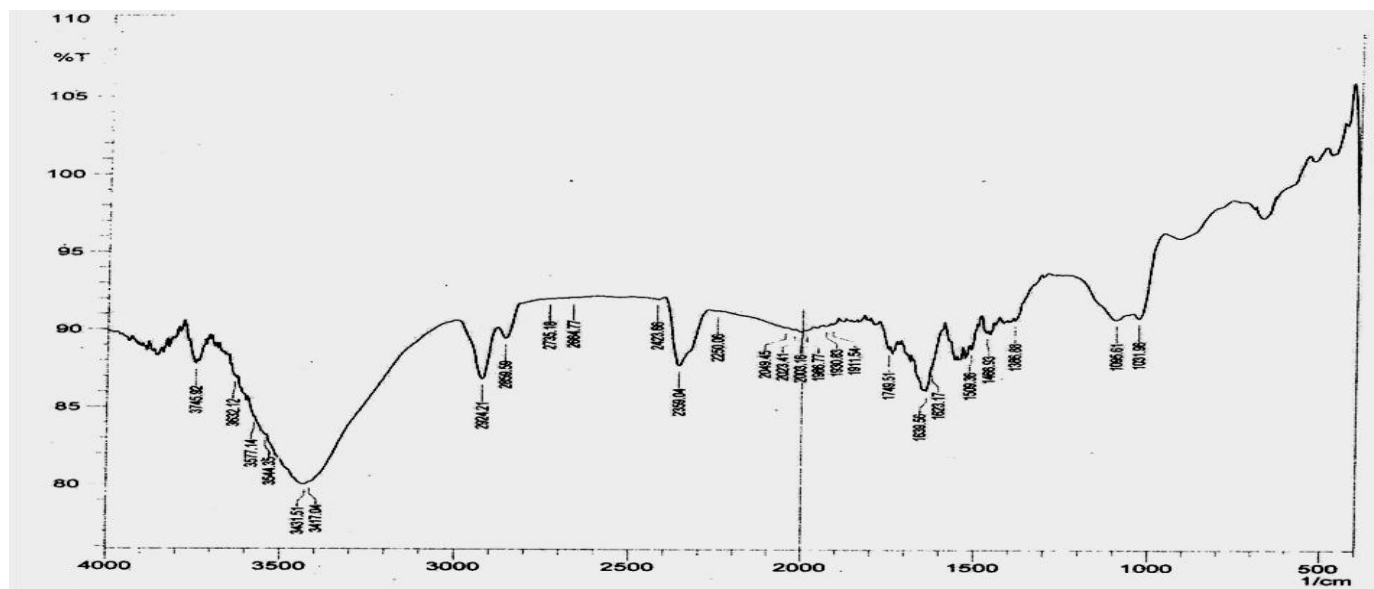


Fig. 5a: FT-IR spectrum of Cauliflower floret extract

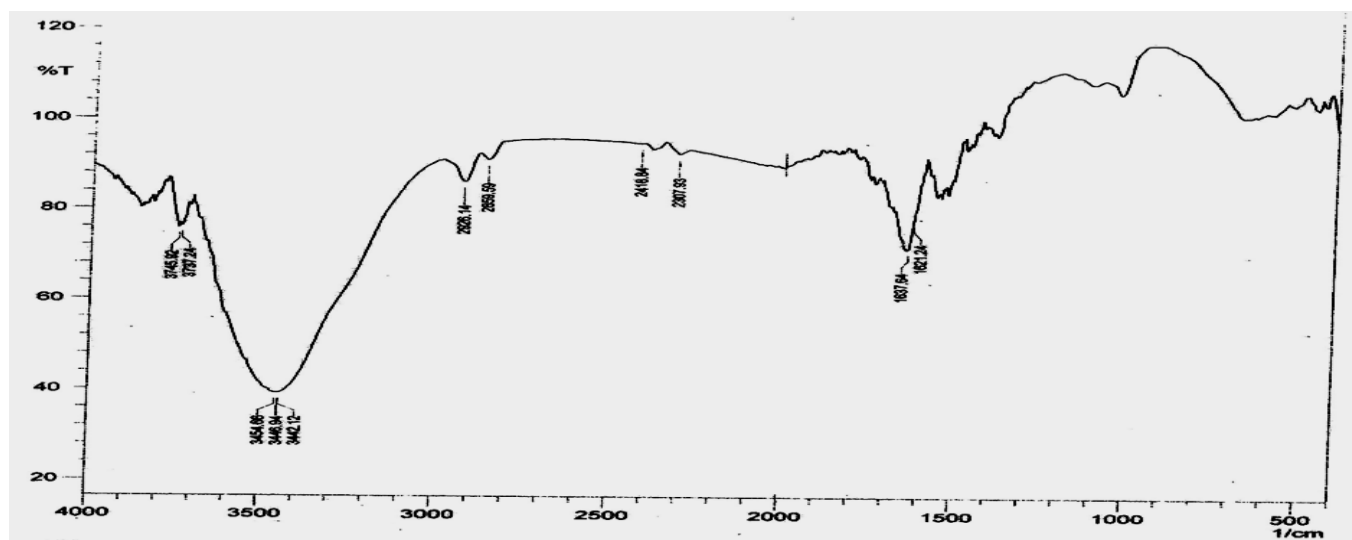


Fig. 5b: FT-IR spectrum of Biosynthesized Silver Nanoparticles.

XRD

XRD patterns taken using powder X-ray diffractometer instrument (SEIFERT JSO DEBYEFLEX -2002) in the angle range of 10° - 70° of the AgNPs at 2θ , scan axis:2:1 sym is shown in (Fig.6). A number of Bragg reflections corresponding to (111), (200) and (220) sets of lattice planes are observed, which can be indexed to face-centered cubic silver. The peaks match with the Joint Committee on Powder Diffraction Standards (file No. 04-0783), which further proves the formation of crystalline AgNPs (39). Furthermore, the average diameter of the silver nanoparticles is calculated as 48 nm by Scherer formula using FWHM obtained from the diffraction peaks

$$\text{peaks } D = 0.89\lambda / \beta \cos\theta$$

Where D is main grain size, λ is the wavelength for Cu target, β is the FWHM of diffraction peak and θ is the diffraction angle. Thus XRD is

commonly used to determine the chemical composition and crystal structure of a material (40).

SEM Studies

Scanning electron microscopy provided further insight into the morphology and size details of the silver nanoparticles. Experimental results showed that the diameter of prepared nanoparticles was about 40-70nm and the shape was spherical as shown in (Fig.7). A similar phenomenon has been reported (41, 42).

TEM Studies

TEM analysis reveals that the Ag nanoparticles are predominantly spherical (Fig.8a). The overall morphology of the silver nanoparticles produced by reduction of Ag^+ ions with 1mM AgNO_3 is composed of almost uniform nanoparticles.

Further, the capping ability of Cauliflower floret extracts was observed (Fig.8b). TEM image shows selected area electron diffraction pattern (SAED) of the silver nanoparticles. The Ag particles are crystalline as can be seen from the selected area

diffraction pattern recorded from one of the nanoparticles in the aggregate. SAED spots correspond to the different crystallographic planes of face-centered cubic (fcc) structure of elemental silver as seen in (Fig.8c).

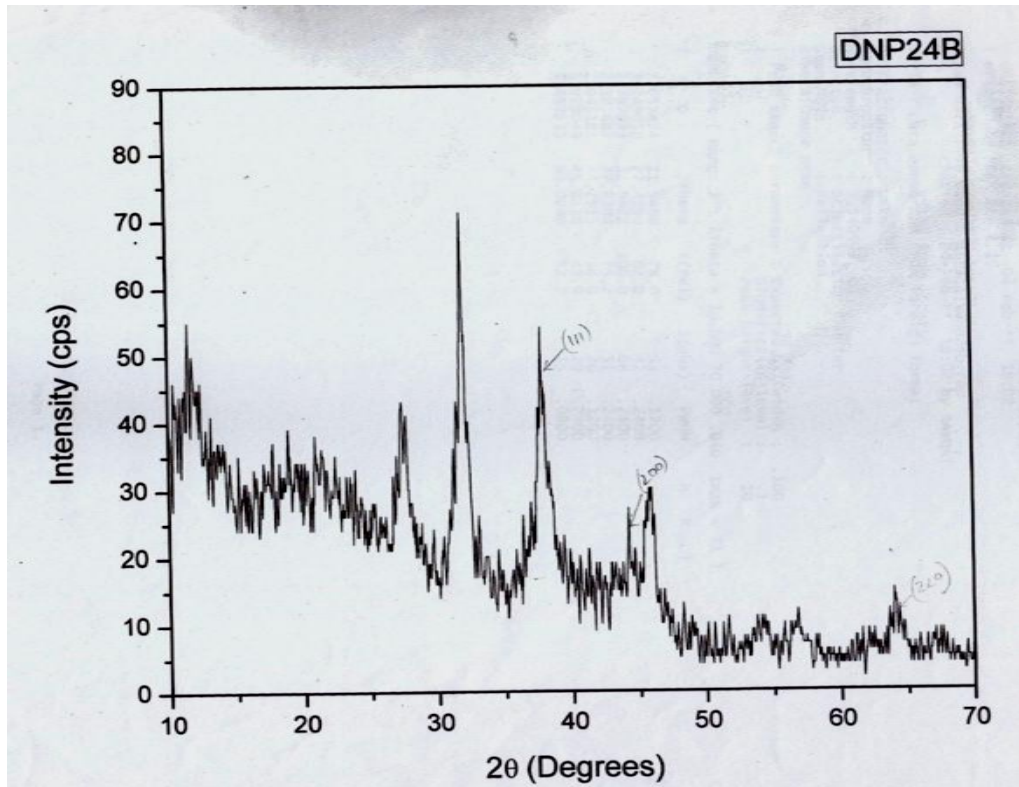


Fig. 6: XRD pattern of Biosynthesized Silver Nanoparticles at 2θ

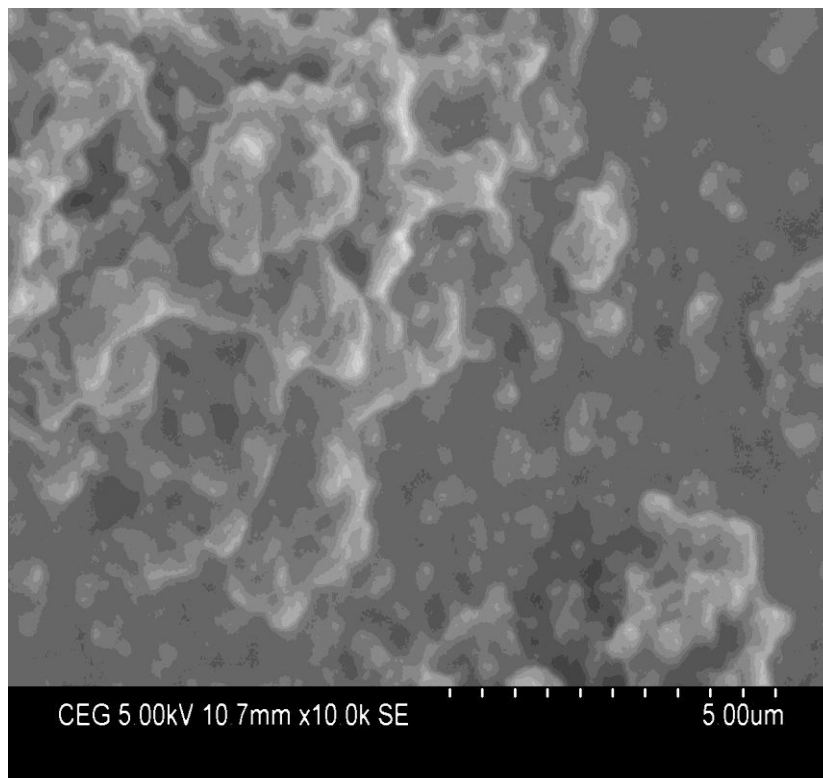


Fig. 7: SEM Micrograph of Biosynthesized Silver Nanoparticles.

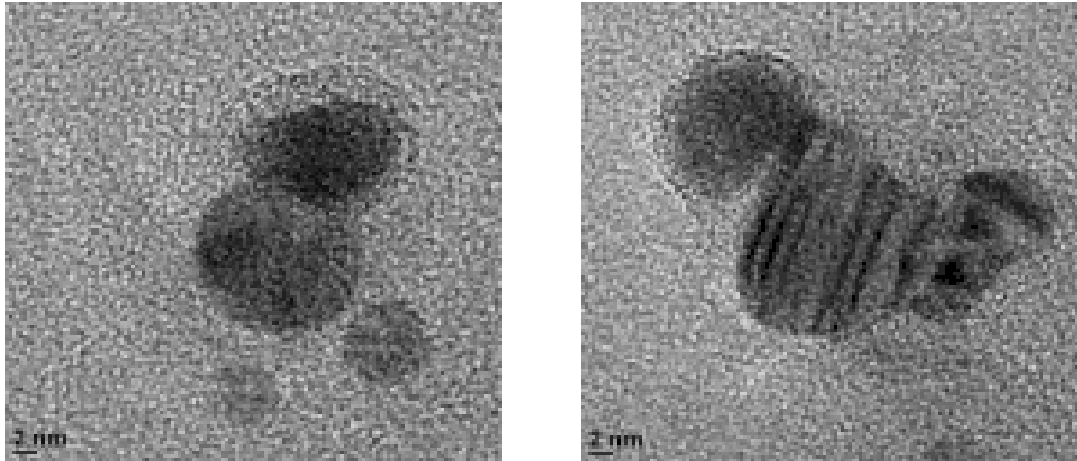


Fig. 8a: TEM images of Biosynthesized Fig. 8b: Biosynthesized Nanoparticles Silver Nanoparticle showing capping ability of aqueous cauliflower floret extract.

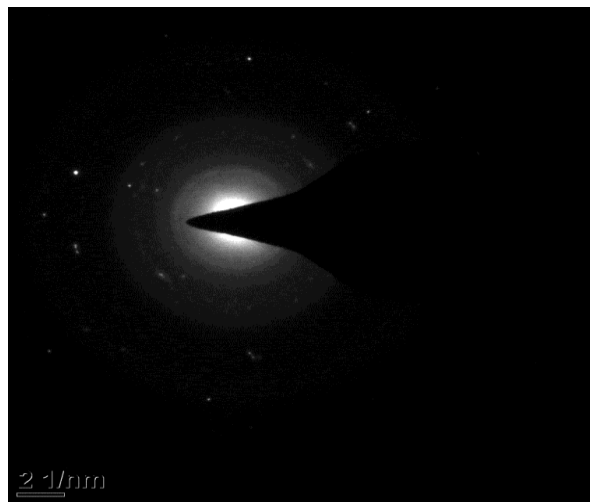


Fig. 8c: Selected area electron diffraction showing the characteristic crystal planes of elemental silver

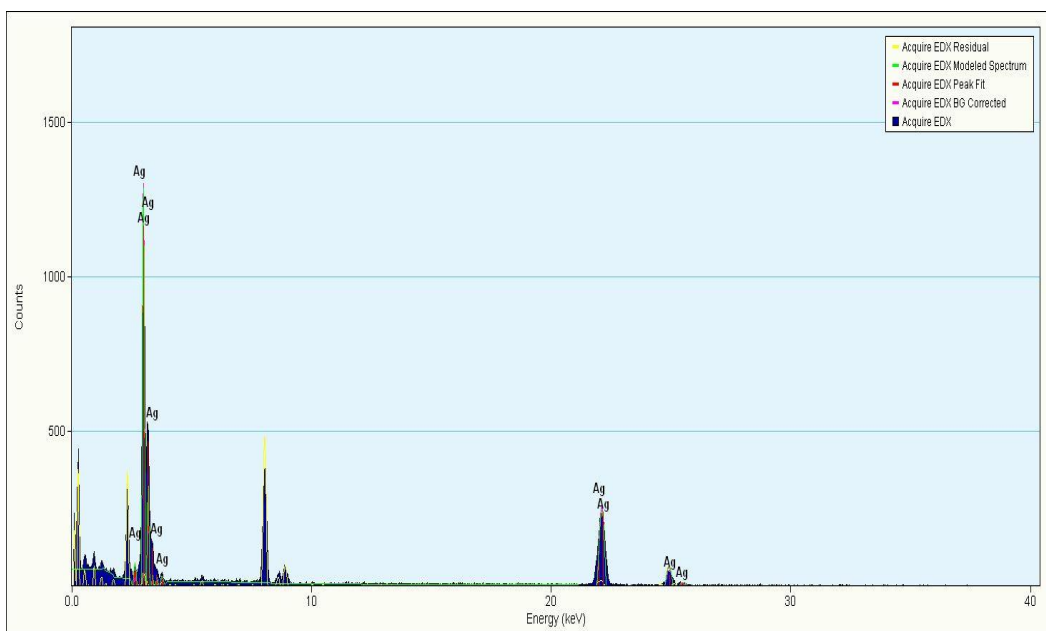


Fig. 9: EDX Profile of Biosynthesised Silver Nanoparticles showing strong peaks (signals) indicating the presence of elemental silver.

EDAX

The EDAX pattern also clearly shows that silver nanoparticles are crystalline in nature by the reduction of silver ions made in this study using fresh cauliflower broth. The EDAX spectrum of the solution containing silver nanoparticles observed at 3KeV, which is typical for the absorption of metallic AgNPs. Strong signals obtained in the present study also confirmed the presence of elemental silver without any peaks of impurities (Fig.9). The EDAX analysis confirmed the weight percentage of silver as 100 against earlier

reports demonstrating the weight percentage as only 33.52 by using Shoa tumbuggala (43) and 91.05 weight percentage obtained by using cauliflower broth stored at 4°C.

Pharmacognostic Evaluation of Silver Nanoparticles

The free radical scavenging property as measured by DPPH method showed that the percentage of inhibition increases with increase in concentration of synthesized silver nanoparticles as indicated in Table No.3. This confirms the antioxidant activity of biosynthesized silver nano particles (Fig.10).

Table 3: DPPH Free radical scavenging activity of biosynthesized Silver Nanoparticles.

Concentration of the sample	control	(i)	(ii)	Average	% of Inhibition
50µg	1.1379	0.8139	0.8132	0.8135	28.50866
100µg	1.1633	0.7679	0.7642	0.766	34.15284
150µg	1.1637	0.6889	0.6889	0.6889	40.80089

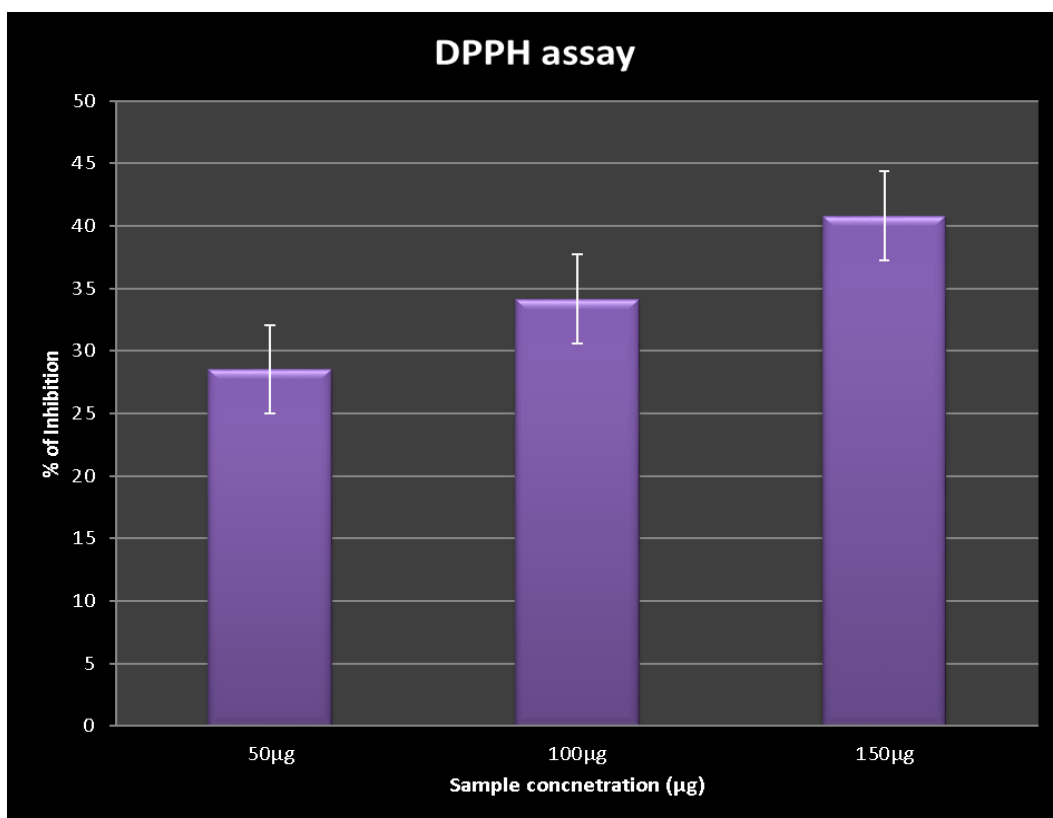


Fig. 10: DPPH Scavenging activity of biosynthesized Silver Nanoparticles.

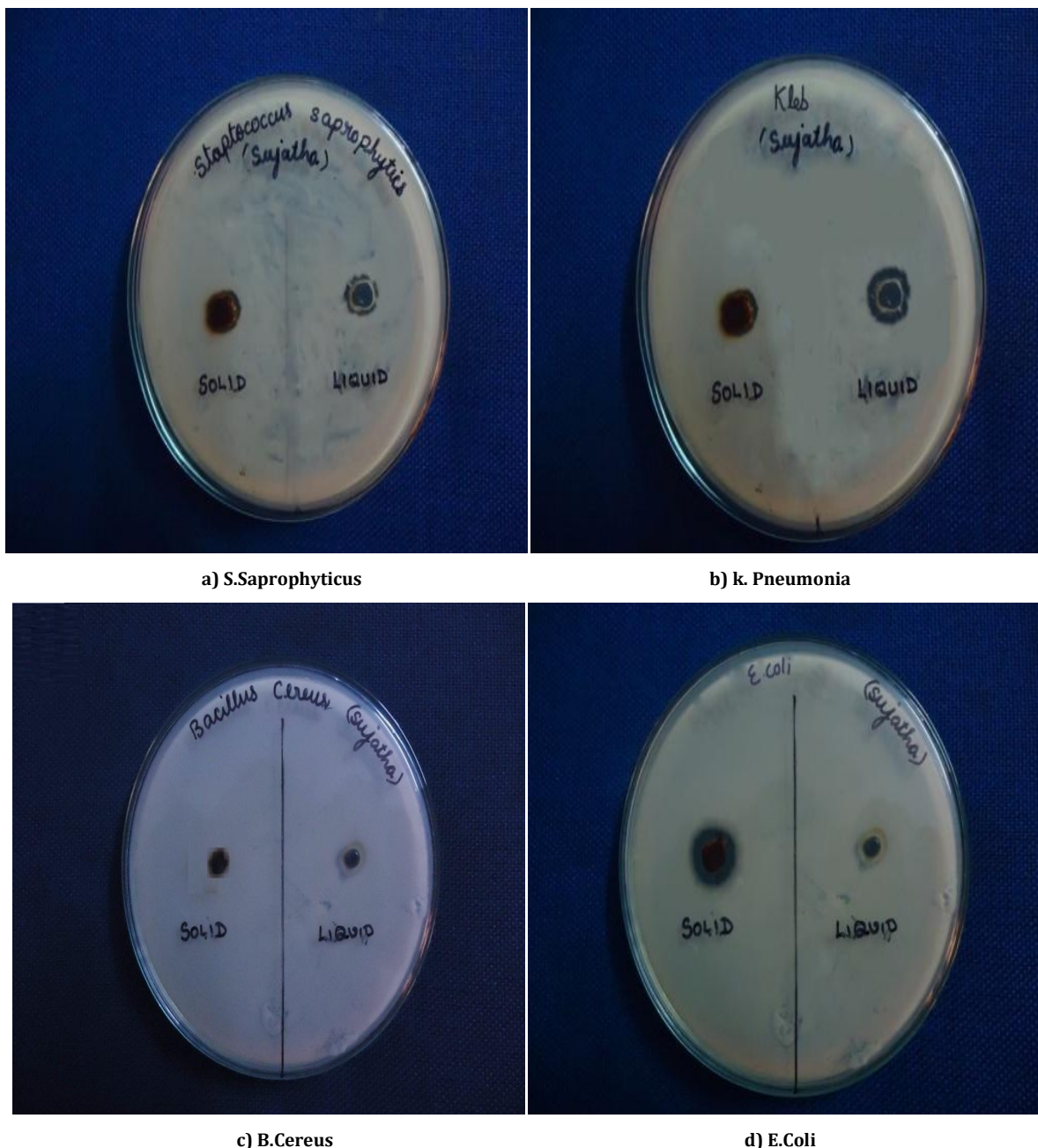
Antimicrobial Activity

The antimicrobial activity of biosynthesized silver nanoparticle was carried out on four human pathogens such as klebsiella Pneumonia, Escheria Coli, Staphylococcus Saprophyticus and Bacillus Cereus. K.Pneumonia and E.Coli are gram -ve bacteria and S. Saprophyticus and B.Cereus are gram +ve bacteria (Fig11.a,b,c,d).

Biosynthesized AgNPs showed clear zone of inhibition as indicated in the Table-4 against E.coli, klebsiella Pneumonia and S.Saprophyticus. It is reported that Ag nanoparticles attach to the surface of the cell membrane, disturb its function and penetrates directly with the bacterial outer membrane and releas Ag⁺ ions. Ciprofloxacin 25 µg/ml was used as +ve control. AgNPs may show antimicrobial activity against B.Cereus at higher concentration.

Table 4: Antimicrobial activity of biosynthesized Silver Nanoparticles against four human pathogens showing zone of inhibition

S. No.	Name of the microorganism	Zone of inhibition (mm)
1	Klebsiella pneumonia	12
2	Escheria coli	10
3	Staphylococcus saprophyticus	8
4	Bacillus cereus	R



a) S.Saprophyticus

b) k. Pneumonia

c) B.Cereus

d) E.Coli

Fig. 11: Plates showing diameter of zones of inhibition of a) S. Saprophyticus b) k. Pneumonia c) B.Cereus d) E.Coli against biosynthesized Silver Nanoparticles.

Cytotoxicity of Silver Nanoparticles

The *in vitro* cytotoxicity of the AgNPs was evaluated against MCF-7 breast cancer cell line at different concentrations (Table No. 5 and 6). The samples demonstrated a considerable cytotoxicity against the MCF-7 cell line. The result showed that MCF-7 cells proliferation was significantly inhibited by AgNPs with an IC_{50} value of $190.501 \mu\text{g/ml}$ of the concentration. Cyclophosphamide is used as standard

control. The % toxicity increases with increase in concentration of silver nanoparticles suggests that biosynthesized silver nanoparticles could be of immense use in medical field to certain extent as anticancer agent (Fig. 12). From the results indicated in Table 6 it is seen that percentage viability decreases with concentration whereas cytotoxicity increases with concentration demonstrating a direct dose dependent relationship.

Table 5: Anticancer activity of Biosynthesized silver nanoparticle on MCF-7 cell line

Absorbance	Control	50 μg	100 μg	150 μg	Positive control
Absorbance at 570nm	1.786	1.476	1.324	1.098	0.467
Absorbance at 570nm	1.842	1.489	1.309	1.102	0.398
Absorbance at 570nm	1.876	1.472	1.298	1.089	0.412
Average	1.8347	1.479	1.310333	1.096333	0.425666667

Table 6: Percentage cell viability and toxicity of Biosynthesised Ag nanoparticle against MCF-7 cell line.

	Control	50µg	100µg	150µg	Positive control
% of viability	100	80.6141	71.42078	59.75654	23.20130814
% of toxicity	0	19.3859	28.57922	40.24346	76.79869186

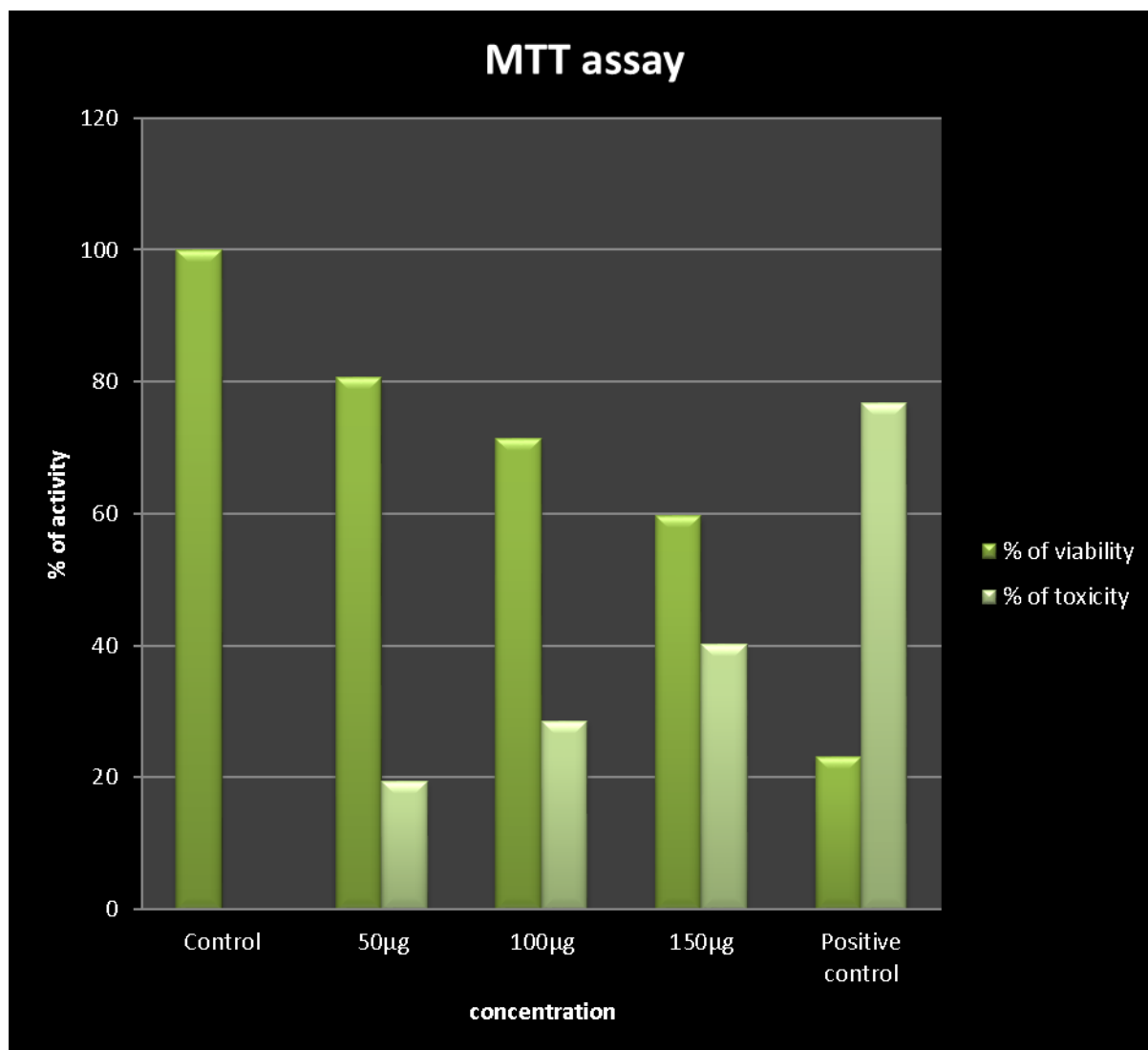


Fig. 12: Cell proliferation using MTT method for synthesized nanoparticles.

CONCLUSION

The development of reliable and ecofriendly process for the synthesis of metallic nanoparticles is of great importance in the field of nanotechnology. Here we have reported a simple reproducible and low cost approach for the preparation of stable Ag nanoparticles by using aqueous cauliflower floret extract as the reducing, stabilising and capping agent. The Biosynthesised nanoparticles have been characterized by SEM, TEM, EDS, FT-IR, XRD and UV-VIS spectroscopy. The AgNPs are crystalline in nature and the size of silver nanoparticles is 48nm. The biologically synthesised AgNPs showed excellent antioxidant potential, antimicrobial activity and possessed considerable cytotoxic effect on MCF-7 cell line. The biosynthesized silver nanoparticles proved to be high potential candidates for medical applications where antioxidant, antimicrobial and cytotoxic activities are highly essential. Hence the biosynthesized nanoparticles would be more efficient in the drug delivery process. Therefore further studies are needed to fully

characterise the toxicity and the molecular mechanisms involved with the antimicrobial and anticancer activity of these nanoparticles.

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