ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 6, Issue 4, 2013

ISSN - 0974-2441

Research Article

BIOSYNTHESIS OF SILVER NANOPARTICLES USING AQUEOUS BROCCOLI EXTRACT- CHARACTERIZATION AND STUDY OF ANTIMICROBIAL, CYTOTOXIC EFFECTS.

G.CAROLING*[1], SUNITA KUMARI TIWARI[2], A.MERCY RANJITHAM[3], R.SUJA[4]

[1]**Department of Chemistry, Ethiraj College for Women (Autonomous), Chennai- Tamilnadu, India, Email:** gcaroling@yahoo.com

Received: 9 July 2013, Revised and Accepted: 2 September 2013

ABSTRACT

OBJECTIVE– The synthesis of metal nanoparticles is a growing area of research due to its potentiality in the application and development of advanced technologies. In general, nanoparticles are synthesized by using chemical methods which are not eco-friendly. Vegetable mediated synthesis of nanoparticles is a green chemistry approach that connects the nanotechnology and biotechnology. In the present investigation we report a green chemistry approach, for the biological synthesis of silver nanoparticles using Broccoli floret aqueous extract under optimum conditions

METHOD- Here we have used a fast, convenient and environment friendly method for the synthesis of silver nanoparticles by biologically reducing $AgNO_3$ with aqueous extract of Broccoli florets (Brassica Oleracea L. var. Italica) under optimum conditions (pH-6-7). The formation of silver nanoparticles was indicated by the colour change from colourless to reddish brown. Biosynthesized nanoparticles were characterized by UV-VIS, FT-IR, XRD, SEM, TEM and EDAX analysis. The free radical scavenging activity was assessed by DPPH assay. These biologically synthesised Ag nanoparticles were tested for antimicrobial activity against four human pathogens viz. Klebsiella Pneumonia, Staphylococcus Saprophyticus, Bacillus Cereus and Escheria Coli. These nanoparticles were assessed further for cytotoxic activity on MCF-7 cell line.

RESULT- The reduction process was simple and convenient to handle and monitored by UV-Vis spectroscopy which showed surface plasmon resonance at 425nm. The presence of active proteins and phenolic groups present in biomass before and after reduction was identified by FT-IR. The crystalline morphology and size of the nanoparticles were determined by TEM, SEM AND X-ray diffraction studies, which showed the average particle size of silver nanoparticles was in the range 40- 50nm as well as revealed their FCC structure. Presence of elemental silver was revealed by EDAX analysis. These biologically synthesised Ag nanoparticles were found to be effective in controlling growth of human pathogens viz. Klebsiella Pneumonia, Staphylococcus Saprophyticus and Escheria Coli. These nanoparticles showed high % toxicity against MCF-7 cell line. The reducing property of aqueous extract is due to the presence of antioxidant viz. ascorbic acid, polyphenols which is confirmed by quantitative assay and scavenging effect of free radicals proved by DPPH scavenging activity.

CONCLUSION- The present investigation revealed that the fresh Broccoli floret aqueous extract is capable of producing silver nanoparticles that are quiet stable for 15 days at room temperature without any sign of precipitation.

Keywords: Brassica oleracea L.var.italica, SEM, TEM, XRD, EDAX, FT-IR, Scherrer formula and Cytotoxic effect.

INTRODUCTION

Nanotechnology is the application of science to control matter at nano level. This technique increases the scope of investigation and regulation of nanoparticles at cellular stage [1], drug delivery [2], diagnostics imaging cancer detection [3], artificial implant [4], HIV inhibition [5], water filtration [6] . A number of approaches are available via chemical and photochemical reactions for synthesis of nanoparticles, such as reverse micelles, thermal decomposition of metallic compounds, radiation assisted, electrochemical, sonochemical and microwave assisted [7]. These methods of preparations are the building blocks of nanotechnology which involves hazardous chemicals, low material conversion, high energy requirements anddifficult wasteful purifications. There are multiple opportunities to develop greener processes for the manufacture of these materials. The green synthesis is more advantageous over chemical and physical methods as it is cost effective and environment friendly[8,9]. In recent years, noble metal nanoparticles have been the subject of focused research due to their unique optical, electronic, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials. Silver nanoparticles play a profound role in the field of biology and medicines due to their attractive physiochemical properties. Silver have long been known to have strong inhibitory and bactericidal effects ,as well as a broad spectrum of antimicrobial activities which have been used for centuries to prevent and treat various diseases most notably infections[10]. Silver nanoparticles are reported to possess anti- fungal[11], anti- inflammatory[12], anti-viral[13], anti-angiogenesis [13] and antiplatelet activities[14] .Recently plant mediated biological synthesis of noble nanoparticles is gaining importance due to its simplicity, ecofriendlyness and it eliminates the elaborate process of maintaining cell cultures. Although biosynthesis of silver nanoparticles by plants viz. papaya fruit [15], Jatropa curcus latex [16], lantana camera fruit [17], Coriandrum sativum [18], Cauliflower [19], Portulaca oleracea [20], have been reported, the potentiality of the plant as biological materials for synthesis of nanoparticles is yet to be fully explored.

Broccoli is a cruciferous vegetable which belongs to Brassica family. It is classified in the Italica cultivar group of the species Brassica Oleracea. Broccoli is considered as a good source of nutrients because it is rich in Vitamin C, Carotenoids, Fibre, Calcium, and folate. Broccoli contains several compounds called isothiocyanates including sulforaphane and indole-3-carbinol, which have been touted as possible anticancer agents. These substances may act as anti- oxidants and may boost detoxifying enzymes in the body.

Extensive work has been carried out on the biosynthesis of silver nanoparticles exploring its antimicrobial activity rather than its anticancer activity. Cancer is an abnormal growth of cells which tends to proliferate in an uncontrolled manner and in some cases metastasizes. As there is increasing demand for anticancer therapy on the health perspective and invitro cytotoxicity testing procedures reduces the use of laboratory animals, the usage of cultured tissues and cells have scientifically proved the significance of urgent need to identify novel active chemotherapeutic agents. As such, the need to search for new potent, effective, relatively safer, reliable remedies for the treatment and management of cancer is still paramount. Hence, green synthesis paves a way to identify the noble anticancer agents.

Here, in this work we report vegetable assisted synthesis of silver nanoparticles, reducing the silver ions by the aqueous extract of broccoli florets, characterized by UV-Vis spectroscopy, FT-IR, XRD, SEM, TEM and EDAX. Further, these biologically synthesized nanoparticles were found to be toxic against multidrug resistant human pathogens and also against breast cancer cell line (MCF-7) for the first time. The investigation is also focused for the first time to outline the potential use of Broccoli extract as reducing, stabilising and capping agent in the reaction.

The reducing property of broccoli extract is due to the total anti – oxidants viz. ascorbic acid, polyphenols present, which is confirmed by quantitative assay and scavenging effects of free radicals, which is proved by DPPH scavenging activity studies.

MATERIALS AND METHOD-

Materials- Broccoli used for the preparation of extract was procured from a local supermarket Fig 1(a). 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, Chandigrah, 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.

Preparation of Sample Extract- 25gm of Broccoli florets (Brassica Oleracea L.var. Italica) were accurately weighed, thoroughly washed under running tap water followed by washing it with double deionised water to remove surface impurities. They were crushed using a blender and finely macerated. After homogenisation 100ml double deionised water was added and heated over 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 $25\mu m$) and used immediately for the biosynthesis of AgNPs.

Pharmocognostic Evaluation of Aqueous Extract – Fresh extract of florets of Broccoli were used for the following analysis:

Phytochemical Screening – Preliminary phytochemical screening was carried out for the identification of carbohydrates, proteins, phenols, terpenoids, alkaloids, coumarines, steroids, phlobatannins, quinones, saponins, flavanoids and tanins using standard phytochemical methods[22]

Quantitative Determination of Ascorbic Acid - Quantitative determination of ascorbic acid was done using UV-VIS spectrophotometer. Ascorbic acid content was determined using 2,6 dichlorophenol- indophenol spectrophotometric method[23]. 5g of broccoli florets are crushed, soaked in 100ml of 4% Oxalic acid for 5hrs at room temperature and filtered through Whatmann No. 41 filter paper. The filtrate (1ml) was mixed with 5ml of 2,6 dichlorophenol-indophenol shaken well and added 4ml of 4% Oxalic acid. The absorbance was immediately measured at 516nm against 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 [24]. 50gm of fresh Broccoli florets are crushed, soaked in 50ml 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 Na_2CO_3 after 3min and made upto 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.

Quantitative Determination of Total Antioxidant Capacity – The total antioxidant capacity (TAOC) was evaluated by method of Prieto et.al[25]. 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 [26]. 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 (0.1%). The absorbance was measured at 700nm.A high absorbance at 700nm indicates a higher reducing power.

Synthesis of Silver Nanoparticles – Aqueous solution of 1mM $AgNO_3$ was prepared and used for the synthesis of silver nanoparticles.5ml of aqueous Broccoli extract is mixed with 95ml of $AgNO_3$ 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: $AgNO_3$)-The biosynthesis of the AgNPs was carried out for different compositions of the extract and $AgNO_3$ solution (1:3, 1:5, 1:10, 1:15 and 1:19). Time taken to record an absorption peak at 425nm was noted.

Biosynthesis of Silver Nanoparticles at two different Room Temperatures – The biosynthesis 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 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 AgNPs – The stability of the AgNPs was determined at room temperature at an interval of 12 hrs for 15 days. The pH of the biosynthesized silver nanoparticles was monitored regularly.

Characterisation of Biosynthesized Silver Nanoparticles –

Visual inspection – The bioreduction of silver nitrate using aqueous broccoli extract was monitored and the appearance of reddish red colour indicates the formation of silver nanoparticles. Photograph of the silver nanoparticle are shown in Fig. 1(b).

UV-VIS Spectroscopy – The reduction of silver nitrate to silver using aqueous broccoli extract was monitored by measuring UV-VIS 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 (ModelUV-1650 PC) operated at a resolution of 1nm.

FT-IR Analysis of Biomass before and after bioreduction – 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 bioreduced silver nanoparticles by the Broccoli extract, in the diffuse refluctance mode at a resolution of $4cm^{-1}$ using KBr pellets and the spectrum was recorded in the wavelength interval 4000 to 400 cm^{-1}.

X-ray Diffraction Studies – X-ray diffraction (XRD) measurement of the Broccoli 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 40KVand a current of 30mA with CuKα radiation in a θ-2θ configuration. The crystallite domain size was calculated by using Debye –Scherrer 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 (FEI TECNAI 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.

Pharmocognostic 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[27]. Stock solution of sample was prepared to the concentration of 1mg/ml. 50µg, 100µg and 150µg of each sample were added to 100 µl 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 517 nm. The experiment was repeated for three times. BHT (Butylated hydroxytoluene) was used as standard controls. The annihilation activity of free radicals was calculated as % inhibition according to the following formula

% of Inhibition = (A of control – A of test)/A of control X100

Determination of Antibacterial Activity- The Antibacterial activity of silver nanoparticles synthesised using fresh aqueous Broccoli extract was determined on Muller and Hinton Agar (Hi –Media Pvt.Ltd. Mumbai) using Kirby-Bauer disk diffusion method [28]. 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 (1500µg) 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 Invitro Assay of Cytotoxic Activities – Cytotoxic effect of the silver nanoparticles was determined by the MTT assay [29]. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine serum, at 37°C in humidified atmosphere with 5% CO_2. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2 X 10^4 cells/well and allowed to attach overnight at 37^0C.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 100µl fresh medium was added with 10µl of MTT (5mg /ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtitre plate reader. Cell survival was calculated by the following formula

Viability% = (A t/Ac) x100 Cytotoxicity % = 100 - % Viability

Where At is the absorbance of the test sample, Ac is the absorbance of the control.

RESULTS AND DISCUSSION

Pharmocogonostic Evaluation of Extract-The results of phytochemical analysis of the Broccoli extract are shown in Table -1 which indicate the presence of secondary metabolites such as carbohydrates, proteins, tannins, saponins, phenols, phytosteroids, steroids, coumarins and flavonoids 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 Broccoli extract.

Table1: Phytochemical Analysis

Components	
Carbohydrates	+
Tanin	+
Saponin	-
Flavanoid	+
Alkaloid	+
Quinone	+
Glycoside	-
Cardiac Glycoside	+
Terpenoid	+
Phenol	+
Coumarine	-
Steroid and phytosteroid	+
Pholabatanin	-
Anthraquinone	-

. 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 Broccoli floret extract was determined and it is found to be 400mg per 100gm 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 phenolic content was found to be 450µg in 50gm of florets. The total antioxidant capacity of the aqueous Broccoli extract was found to be 600mg of ascorbic acid/ 100gm of florets. The reducing power of the aqueous extract also shows higher absorbance at 700 nm indicating its high reducing property.

Visual Characterisation- As the broccoli extract was mixed with aqueous solution of 1mM silver nitrate it started to change colour from colourless to reddish brown due to reduction of silver ions; which indicate formation of silver nanoparticles (Fig. -1b).

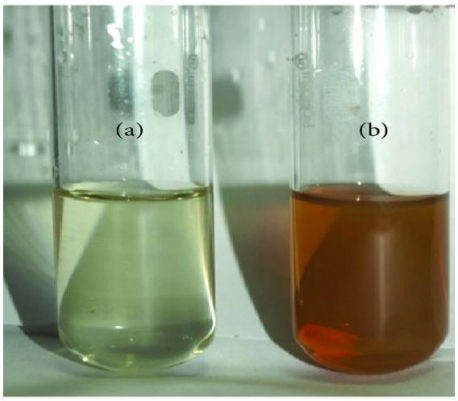

Figure1: (a) Broccoli florets and (b) Broccoli extract and synthesized Silver nanoparticles

Different parameters were optimized viz. Ratio of volume of extract and $AgNO_3$, 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.

Ratio of volume of extract: $AgNO_3$- The time taken for the formation of AgNPs depends on the ratio of volume of extract to $AgNO_3$ and the results are given in (Table-2). The time taken for the formation of silver nanoparticles was found to be less for 5ml of extract and 95ml of 1mM $AgNO_3$ solution. This ratio was found to be ideal because biosynthesized nanoparticles showed maximum absorption at 425nm which is in agreement with reported values[24]. [Fig-2a].

Table 2: Rate of formation of Silver Nanoparticles for different compositions

Compositions	Ratio	Time Hr
5ml extract + 15 ml $AgNO_3$	1:3	02
5 ml extract + 25 ml $AgNO_3$	1:5	02
5 ml extract + 50 ml $AgNO_3$	1:10	1.5
5 ml extract + 75 ml $AgNO_3$	1:15	01
5 ml extract + 95 ml $AgNO_3$	1:19	0.5

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 composition5 mL of the extract and 95 mL of $AgNO_3$. The AgNPs were formed within 45minutes. at 27°C however, at 34°C the AgNPs are formed at 30minutes and under waterbath conditions it was formed within 10minutes [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 2min.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.

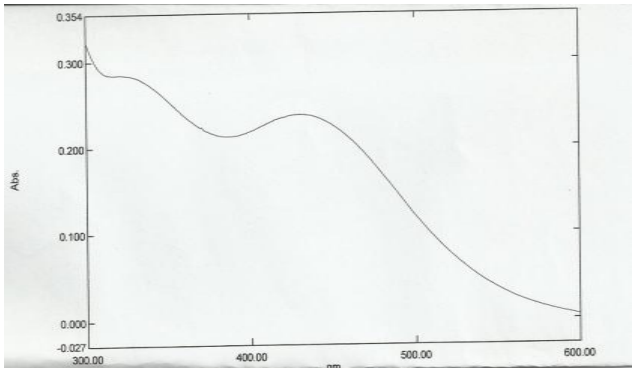

Figure2a : UV-VIS spectrum of Ag nanoparticles under optimum conditions

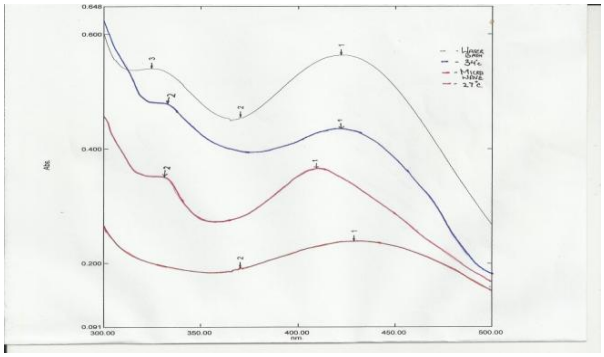

Figure 2b: UV-VIS absorption of Ag Nanoparticles synthesized at different and under different heating conditions

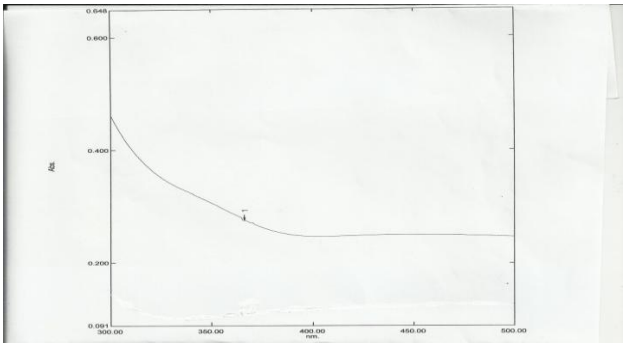

Figure 3: UV-VIS Spectrum of Ag Nanoparticles under autoclave condition

Stability of AgNPs -The UV visible spectrum for the biosynthesised AgNPs using 5 mL of extract and 95 mL of $AgNO_3$ was recorded over a period of time for 15 days. There was no change in the UV visible spectrum. The biosynthesised nanoparticles were found to be stable for 15 days at p^H 6-7 without any sign of precipitation and without any change in λmax value as indicated in the Fig-4.

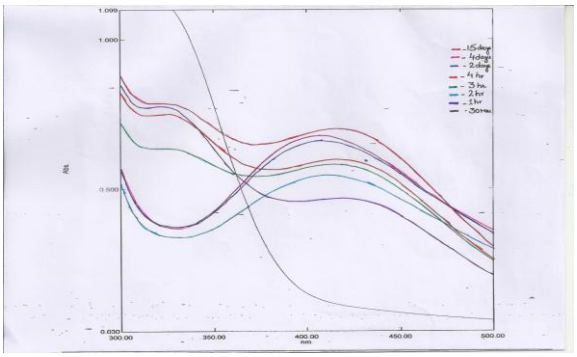

Figure 4: UV- VIS spectra of Silver Nanoparticles showing stability .

Characterisation of Biosynthesised 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 [30]. For an ellipsoidal particle there are two peaks whereas for spherical particle there is only one peak centered at 420nm, in the UV –VIS spectrum. The absorption spectrum of AgNPs formed in the reaction has an absorption peak at 425nm which indicates particles are spherical in shape. The absorption peak maximum is attributed to the Mie scattering by silver metal [31]. The appearance of yellow colour indicated the formation of AgNPs in the reaction mixture, as it is well - known that AgNPs exhibits striking colours(light yellow –brown) due to the excitation of surface plasmon vibrations in the particles[32]. In the present study there is only one peak at 425nm indicating that the AgNPs are spherical in shape.

FT-IR -FT-IR spectrum of Broccoli extract was taken before and after the synthesis of AgNPs is shown in Fig.(5a and 5b).The spectrum was recorded in the wavelength region between 400cm^{-1} to 4000cm^{-1}.The spectrum shows peaks at 3424cm^{-1}, 3433cm^{-1} (strong O-H bonding) which indicates the presence of –O-H stretching of carboxyl group and N-H stretching of secondary amides. These peaks indicate the presence of bonded hydroxyl groups. Further, the peaks observed at 2924cm^{-1}, 2856cm^{-1}, 2333cm^{-1} represents the C-H stretching bonds of alkanes. The peak observed at 1625cm^{-1} and 1383cm^{-1} represent the N-H deformation, and C=C aromatic conjugates. The sharp peak at 1020cm^{-1} is assigned to C-N stretching vibrations of proteins [21].The C-S stretching appears as a weak band in the 700-600cm^{-1}. The position of these bands are close to that reported for native proteins [33].The Fig-5a indicates the presence of many fundamental groups involved in the conversion of silver ions to silver nanoparticles. The IR spectrum of the AgNPs indicates the absence of many fundamental groups and peaks of lower intensity. This disappearance of the bands and decrease in intensity is attributed to reduction of silver ions.

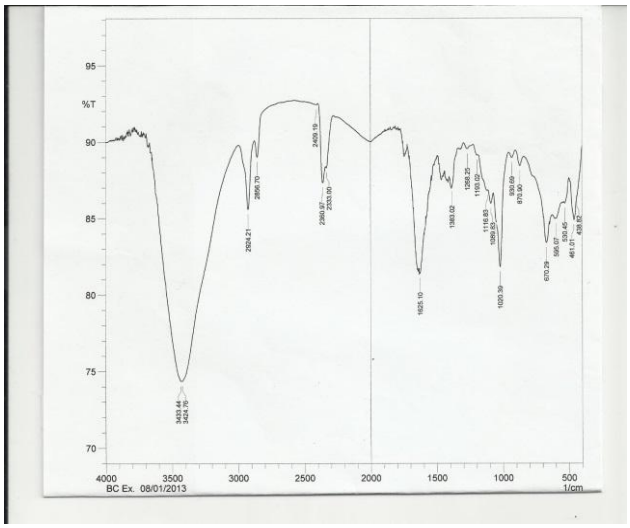

Figure 5a: FT-IR spectrum of Broccoli extract

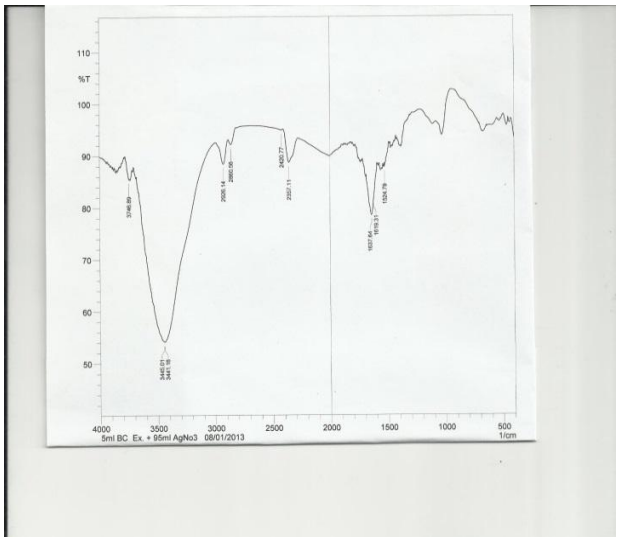

Figure 5(b): Biosynthesized Silver Nanoparticles.

XRD - XRD patterns taken using powder X-ray diffractometer instrument (SEIFERT JSO DEBYEFLEX 2002) in the angle range 10°-70° of the AgNPs at 2θ, scan axis 2:1sym 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- centred cubic silver. The peaks match with the joint Committee on powder Diffraction Standards (file No. 04-0783), which further proves the formation of crystal AgNPs[34]. Furthermore, the average diameter of the AgNPs is calculated as 46nm by Scherrer formula using FWHM obtained from the diffraction peaks:

$$D = 0.89\lambda/\beta\cos\theta$$

Where D is the mean grain size, λ is the wavelength of Cu target, β is the FWHM of the diffraction peaks and θ is the diffraction angle. Thus XRD is commonly used to determine the chemical composition and crystal structure of a material [35].

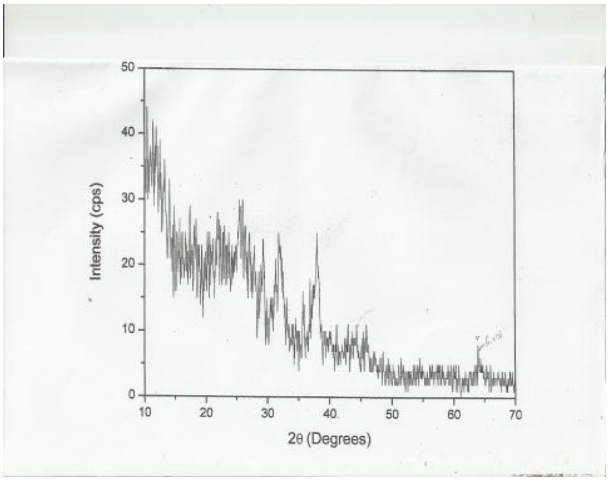

Figure 6 :XRD pattern of Biosynthesized Silver Nanoparticles.

SEM Studies-Scaning electron microscopy provided further insight into the morphology and size details of the AgNPs. Experimental results showed that the diameter of the prepared nanoparticle was about 40-70nm and the shape was spherical as shown in (Fig-7). Similar phenomenon has been reported [36, 37].

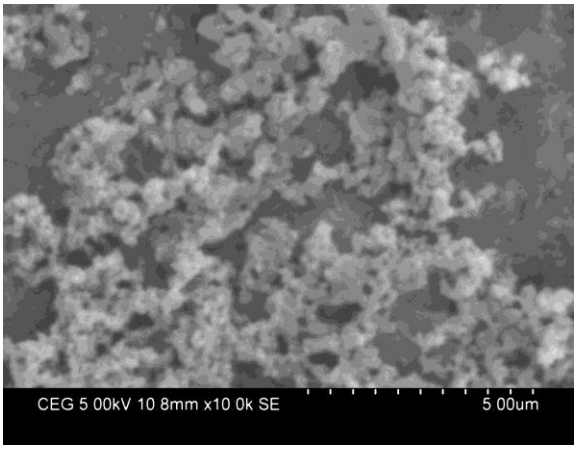

Figure 7: SEM micrograph of Silver Nanoparticles.

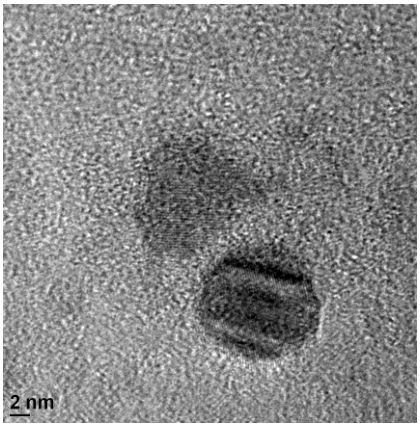

Figure 8a: TEM images of Silver Nanoparticlesa

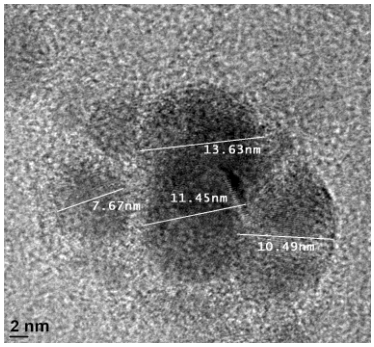

Figure 8b: Nanoparticles showing capping

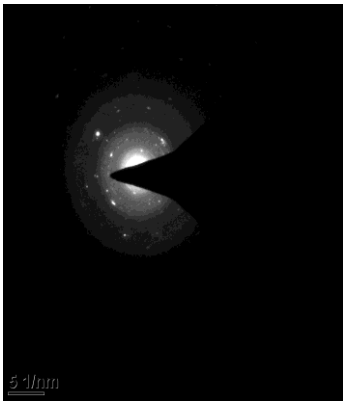

Figure 8c: Selected area Electron Diffraction Pattern (SAED)

TEM Studies -TEM analysis reveals that the AgNPs are predominantly spherical (Fig .8). The overall morphology of the silver nanoparticles produced by reduction of Ag^+ ion with 1mM $AgNO_3$ is composed of almost uniform nanoparticles. Further the capping ability of Broccoli floret extract was observed (Fig.-8).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 corresponds to the different crystallographic planes of face centered cubic (fcc) structure of elemental silver as seen in (Fig-8 c).

EDAX-The EDAX pattern clearly shows that silver nanoparticles formed by the reduction of silver ions using fresh Broccoli extract are crystalline in nature (Fig.-9) The EDS spectrum was recorded in the spot- profile mode. The optical absorption peak is observed at 3KeV, which is typical for the absorption of metallic AgNPs. Strong signals from the Ag atoms are observed, while weaker signals from C, Cu and Si atoms were also recorded. From the EDS signals, it is clear that AgNPs reduced by Broccoli extract have the weight percentage of elemental Ag as 73.76%.

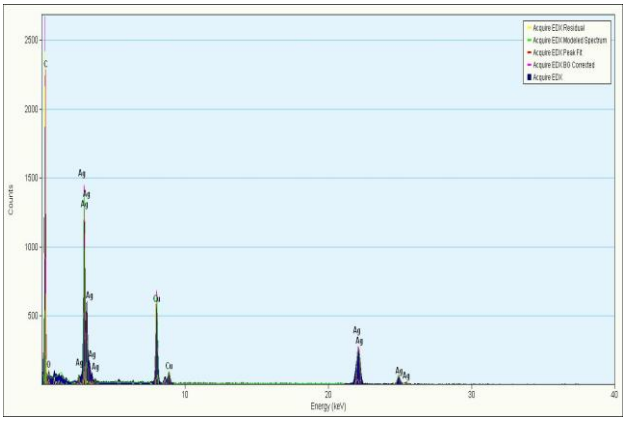

Figure 9: EDAX spectra of Silver Nanoparticles

Pharmocognostic Evaluation of Silver Nanoparticles-The free radical scavenging property as measured by DPPH method showed that the percentage of inhibition increases with increases in concentration of synthesized silver nanoparticles as indicated in Table -3. This confirms the antioxidant activity of biosynthesized AgNPs [Fig-10]

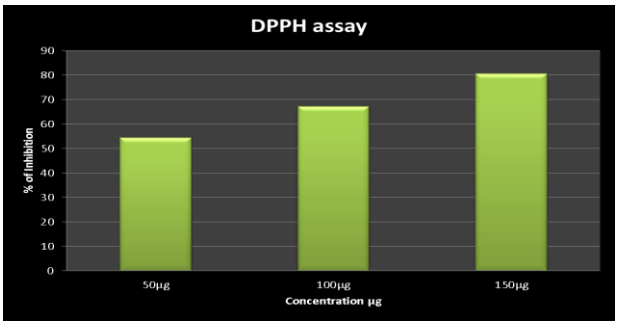

Figure 10: DPPH scavenging activity of Silver Nanoparticles

Antimicrobial activity- Antimicrobial activity of biosynthesized silver nanoparticles was examined carried out on four human pathogens, such as K.Pneumoniae, E.Coli, S. Saprophyticus and B.Cereus . E.Coli, K. Pneumoniae are gram -ve and B.Cereus, S. Saprophyticus are gram +ve bacteria [Fig-12]. Biosynthesized silver nanoparticle showed clear zone of inhibition as indicated in Table -4 against K. Pneumonia, E.Coli, and S.Saprophyticus. It is reported that Ag nanoparticles attach to the surface of the cell membrane, disturbs its function and penetrates directly with the bacterial outer membrane and release Ag ions .Ciprofloxacin 25µg/ml was used as +ve control. AgNPs may show antimicrobial activity against B.Cereus at higher concentration.

a) E.Coli,

b) S. Saprophyticus

c) B. Cereus

d) K.Pneumoniae

Figure-11: Zone of inhibition of green synthesis of AgNPs against Bacterial Pathogens. Solid - AgNPs, Liquid - Control

Table 4: Showing zone of inhibition against Bacterial Pathogens

Organism	Zone of Inhibition (mm)
Klebsiella Pneumonia	12
Escheria Coli	10
Staphyococcus Saprophyticus	9
Bacillus Cereus	R

Cytotoxicity of Silver Nanoparticles- The invitro cytotoxicity of the AgNPs was evaluated on breast cancer MCF-7 cell line at different concentrations [Table-5]. Cytotoxicity analysis of the sample shows a direct dose relationship; cytotoxicity increased at higher concentrations. The sample demonstrated a considerable cytotoxicity against MCF-7 cell lines. The result showed that MCF-7 cells proliferation was significantly inhibited by AgNPs with an IC_{50} value 121.56µg/ml of the concentration. Cyclophosphaamide is used as standard control. The % toxicity increases with increase in concentration of the silver nanoparticles suggest that bio synthesised silver nanoparticles could be of immense use in medical field to certain extent as anticancer agent [Fig-12].

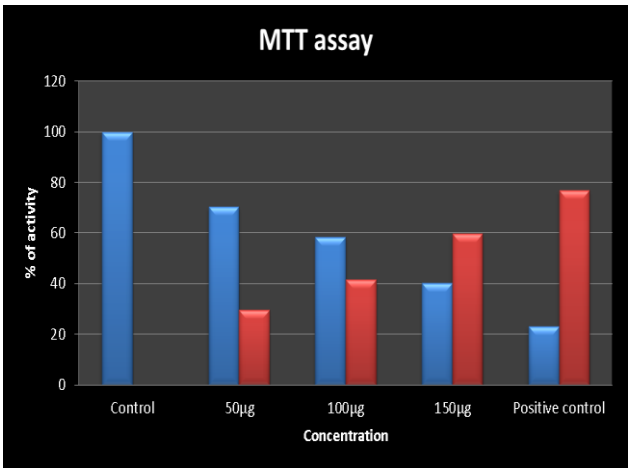

Figure 12: Cytotoxic effect of Silver Nanoparticles

CONCLUSION

The development of reliable and eco-friendly 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 extract of the floret of Broccoli as the reducing ,stabilising and capping agent. The Biosynthesized 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 in the range 40nm- 50 nm. The AgNPs have antibacterial activity and cytotoxic effects .The biosynthesized silver nanoparticles proved to be potential candidates for medical applications where antioxidant, antimicrobial and, cytotoxic activities are highly essential. Hence the synthesized nanoparticles are more efficient in the drug delivery process.

Acknowledgement

The authors are thankful to the Instrumentation centre of Ethiraj College for Women (Autonomous), Chennai, for recording UV-VIS and FT-IR spectra. We also thank the University of Madras for recording XRD pattern, IIT Madras for TEM, EDX analysis and Anna University for SEM analysis.

REFERENCES-

1. Du Li, Jiang H ,Liu X and Wang E. Biosynthesis of gold nanoparticles assigned by E.coli DH5ά and its applications on direct electrochemistry communication ,(2007),91165-1170.
2. John De, Paul J, Born A .Drug delivery and nanoparticles; Application and hazards (2008). Journal nanomedicines 3(2) 133-149.
3. Kairemo K,Ebra .P, Bergstron .K, Pavwels .E.K.J. Nanoparticles in cancer (2008) Current radiopharma 30-36.
4. Adini A.R, Redlich .M,Teene.R ; Medical application of inorganic fullerene like nanoparticles. Journal of material chemistry. (2011) doi: 10.1039/CIJM11799H.
5. Elechiguena .J.L,Burt .J.L,Morones .J.R,Bragado .A.C, GaX GaO, LARA H.H Interaction of Ag nanoparticleswith HIV -1(2005).Nanotechnology 3:1-10.
6. Morones.J.R,Elechiguena.J.L,Canacho.A,Holt.K,Kouri.J.B,Ramfrez.J.T,Yacaman.M.J,(2005).The bactericidal effect of silver nanoparticles, Nanotechnology 16:2346-2353.
7. Parashar.V, Parashar.R, Sharma.B, Pandey.A.C.Digest of jouran of nano materials and bio structure-4(1), 45-50(2009)
8. Kowshik.M, Ashtaputre.S, Kharazzi.S Vogel.W ,Urban.J ,Kulkarni.S.K, Panikar K.M (2003). Extracellular synthesis of silver tolrent strain MKY3. Nanotechnology 14: 95-100.

9. Nabikha.K, Kathiersan, Raj.A, Alikunhi.M, Nabeel, (2009). Synthesis of antimicrobial silver nanoparticles by callus and leaf extract from salt marsh plants, Sesuvium portulacastrum L (2009). Colliods and Surface:Biointerfaces 79: 488- 493
10. Shankar, S.S., Rai, A., Ankamwar, B., Singh, A., Ahmad, A., Sastry, M., 2004. Nat. Mater. 3, 482–488.
11. Wiley, B.J., Im, S.H., McLellan, J., Siekkinen, A., Xia, Y., 2006. J. Phys. Chem. B 110, 156–166.
12. Nadworny, P.L., Wang, J., Tredget, E.E., Burrell, R.E., 2008. Nanomedicine: Nanotech. Biol. Med. 4, 241–251.
13. Rogers, J.V., Parkinson, C.V., Choi, Y.W., Speshock, J.L., Hussain, S.M., 2008. Nanoscale Res. Lett. 3, 129–133.
14. Gurunathan, S., Lee, K., Kalishwaralal, K., Sheikpranbabu, S., Vaidyanathan, R., Eom, S., 2009. Biomater. 30, 6341–6350.
15. Jain D, Kumar Daima H, Kachhwaha S, Kothari SL: Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti-microbial activities. Dig J Nanomat Bios 2009; 4:557–563.
16. Harekrishna B, Dipak KB, Gobinda PS, Priyanka S, Sankar PD, Ajay Misra: Green synthesis of silver nanoparticles using latex of Jatropha curcas. Coll Surf A: Physicochem Eng Aspects 2009; 339:134–139.
17. Padmanaban Sivakumar, Chandran Nethradevi , Sahadevan Ranganathan : Synthesis of silver nanoparticles using lantana camara fruit extract and its effect on pathogens.Asian J Pharm37 Clin Res2012; 5(3):97-101.
18. Sathyavathi R, Balamurali Krishna M, Venugopal Rao S, Saritha R, Narayana Rao D: Biosynthesis of Silver Nanoparticles Using Coriandrum Sativum Leaf Extract and Their Application in Nonlinear Optics. Adv Sci Lett 2010; 3:138–143.
19. Sridhara V, Pratima K, Krishnamurthy G, Sreenath B. Vegetable assisted synthesis of silver nanoparticles and its antimicrobial activity against two human pathogens. Asian J Pharm Clin Res ,Vol 6,Issue 2,2013,53-57.
20. Jannathul Firdhouse M, Lalitha p. Green synthesis of silver nanoparticles using aqueous extract of Portulaca Oleracea (L) Asian J Pharm Clin Res, Vol 6, Issue 1, 2012
21. Krishnaraj, C., Jagan, E.G., Rajasekar, S., Selvakumar, P., Kalaichel- van, P.T., Mohan, N., 2010. Colloids and Surfaces B: Biointerfaces 76, 50–56.
22. Sofowara.A1993 Medicinal plants and traditional medicines in Africa Spectrum books ltd, Ibadan, Nigeria, pp.191-289.
23. Horwitz.W "Official method of analysis of the official analytical chemists," 13th ed, Association of official analytical chemists, Washington DC 476(1980).
24. Savitree M,Isara.P, Nittaya S.L, and Worapan.S(2004),Radical scavenging activityand total phenolic content of medicinal plants used in primary health care, Journal of pharma. Sci,Vol. 9(1)Page No. 32-35.
25. Prieto-Simon B, Cortina M, Campass M, Calas –Blanchord (2008) Electrochemical biosensors a tool for antioxidant capacity assessment sens. Actuators B: CHEM 129:456-460.
26. Oyaizu M.1986. Studies on product of browning reaction prepared from glucose amine. Japanese Journal of nutrition 44:307-315.
27. Harbone, J.B and Baxter, H (1995), Phytochemical Dictionary, A Handbook of Bioactive compounds from plants, London: Taylor and Francis.
28. 28.Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J ClinPathol 1966; 45: 493-6.
29. Mossman, T. 1983. Rapid colorimetric assay for cellular growth and survival – application to proliferation and cytotoxicity assays. J.Immunol. Methods 65: 55-63.
30. Creighton J A,Eadont D G, Ultra violet –visible absorption spectra of the colloidal metallic elements. J. Chem Soc., Faraday Trans. 1991; 87:3881-3891.
31. Aoki K, Chen J, Yang N, Nagasava H. Nanochemistry. Langmuir.2003; 19:9904.
32. Kapoor S. Preparation, characterization, and surface modification of silver particles. Langmuir, 1998; 14:1021-1025.
33. Macdonald IDG,Smith WE, Orientation of cytochrome C adsorped on a citrate- reduced silver colloid surface.Langmuir.1996;12:706-713.
34. Swanson, Tatge, Natl. Bur. Stand. (U.S.), Circ Vol. 539, No.1, 1953, 23.
35. Udayasoorian C, Vinoth Kumar R, Jayabalakrishnan M. Extracellular synthesis of silver nanoparticles using leaf extract of Cassia auriculata. Dig J Nanomater Bios. 2011; 6(1) 279-283.
36. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. Synthesis of Gold Nanotriangles and Silver Nanoparticles Using Aloevera Plant Extract. Biotechnol Prog., 2006; 22:577.
37. J. Saraniya devi, B. Valentin Bhimba, Krupa Ratnam. Invitro anticancer activity of silver nanoparticles synthesized using the extract of gelidiella sp. Int J Pharm Pharm Sci, Vol 4, Suppl 4, 2012, 710-715.
38. Bhosle SM, Huilgol NG and Mishra KP. Enhancement ofradiation induced oxidative stress and cytotoxicity in tumor cells by ellagic acid. Clinica Chimica Acta. 2005; 359: 89-100.