ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 10:1 (2016) 47-57

records of natural products

Cytotoxic Effect on MG-63 Cell Line and Antimicrobial and Antioxidant Properties of Silver Nanoparticles Synthesized with Seed Extracts of *Capsicum* sp.

Nidhi Singh, Ankita Chatterjee, Koustav Chakraborty, Sneha Chatterjee and Jayanthi Abraham*

Microbial Biotechnology Laboratory, School of Biosciences and Technology,

VIT University, Vellore-632014, Tamil Nadu, India

(Received July15, 2014; Revised September 8, 2014; Accepted March 28, 2015)

Abstract: Applying the concept of ethnobotany, plant extract was taken into consideration as an alternative to chemicals synthesis of silver nanoparticle. The extracts from the chilli seeds were used to synthesize silver nanoparticles (AgNPs). In this study two species of chilli, *Capsicum annuum* and *Capsicum frutescens*, have been used to analyse the characteristics of the bio-active compounds found in their seeds. Analysis of the bioactive compound was performed by using Soxhlet extraction with solvents followed by Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and GC-MS. Furthermore, green synthesis of nanoparticles with chilli extracts was carried out using silver nitrate to detect its antimicrobial activity. The characterizations of both the nanoparticles were carried out using UV-Vis Spectroscopy, Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffractometry (XRD), Scanning Electron Microscopy (SEM) and energy Dispersive X-Ray Spectroscopy (EDX). Antimicrobial activity against clinical pathogens and the antioxidant assay using DPPH and FRAP assays were performed. The cytotoxicity effects on osteosarcoma cell lines were also evaluated with the synthesized AgNPs.

Keywords: Ethnobotany; antimicrobial; silver nanoparticles; antioxidant assay; cytotoxicityassay.

1. Introduction

Synthesis of nanoparticles using biological materials has gained in recent years and among them, metallic nanoparticles are most promising as they contain remarkable antimicrobial activity [1]. Environmentally benign methods for biosynthesis of silver nanoparticles (Ag-NPs) using plant extracts has become a much sought after alternative to chemical and physical ones [2]. In comparison to the chemical methods, synthesis of nanoparticles using plant extract is much faster. Amongst the nanomaterials available for the biosynthesis of antimicrobials silver has emerged to be most effective in the field of nanomedicine. Green synthesis of nanoparticles [3] using plant extract is of significant importance as it shows an effective antimicrobial activity in both its metallic and nanoparticles forms against various multi drug resistant pathogens [2]. Phytochemical analysis has revealed the presence of alkaloids, flavanoids, polyphenols and sterols in the bell pepper extracts which could be the reason of antimicrobial capacity of the chillies [4].

* Corresponding author: E-Mail: jayanthi.abraham@gmail.com; Phone: 09843580709

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 08/01/2015 EISSN:1307-6167

Silver nanoparticles confer many advantages over other drugs. In the same concentrations which are low enough to be susceptible to human cells, it is lethal for many bacteria, viruses and other pathogenic microorganisms. Particularly being a potential bactericidal without any side effects on humans, nanosilver particles occur to be promising antimicrobial drugs. The most important application in commercial level in medical industry is as topical ointments to prevent infection in burns and open wounds [5], as it displays effective antimicrobial surface coating.

2. Materials and Methods

2.1. Sample Collection

The seeds of the two chilli varieties *Capsicum annuum* and *Capsicum frutescens* were purchased from Khadhi, Vellore, Tamil Nadu, India. The seeds were stored in air tight container for further processing. The clinical pathogens used in antimicrobial studies were obtained from Microbial Biotechnology Laboratory, School of BioScience and Technology, VIT University, Vellore.

2.2. Preparation of Capsicum annuum and Capsicum frutescens seed extracts

The seeds of both the samples were ground in a mixer grinder and the powdered samples were extracted using Soxhlet apparatus. The samples were extracted with two solvents hexane followed by acetone. Aqueous extract was also prepared using water as the solvent system in the Soxhlet apparatus, for both the test samples. Following extraction filtration was performed using Whatman filter paper No. 1 and the obtained extracts were subjected to concentration vacuum evaporater. The dried extracts were stored at 4℃ for further analysis.

2.3. Antibacterial assay of the seed extracts

The well-diffusion method was performed to test the sensitivity of the clinical pathogens to the hexane, acetone and aqueous extracts by measuring the zone of inhibition of given concentration of the seed extracts. Clinical bacterial isolates i.e, *Escherichia coli*,*Serratiamarcescens*, *Salmonella* sp.,*Shigella*sp.,*Enterobacter*sp., *Staphylococcus aureus*,*Klebsiellapneumoniae*, *Pseudomonas aeruginosa*and *Proteus mirabilis* were swabbed on Mueller Hinton agar plates and wells were cut and loaded with 100µl of seed extracts prepared. Plates were incubated at 37°C for 24-48 h and were observed for zone of inhibition. After incubation period results were recorded in the form of a clear zone of inhibition.

2.4. Thin Layer Chromatography (TLC)

TLC plates were prepared using silica gel slurry on the glass slides. The samples were loaded on the TLC plates and after it was air dried, the plates were placed in the solvent system (hexane:formicacid:acetic acid, 2:1:1) prepared within the chamber. When the solvent front reached three-fourth, the plates were air dried and observed under UV. The distance of the sample run was measured and the Rf value was calculated.

2.5. High Performance Liquid Chromatography (HPLC)

HPLC was done for both the extracts. The mobile phase used was acetone:water (60:40). 20µl of the sample was injected into HPLC unit (Waters 1525 binary HPLC pump, Milford, USA) on a Symmetry C18 column (Waters 5 µm, 4.6 mm × 150 mm). The mobile phase was isocratic consisting of acetonitrile:water in the ratio 60:40 with a flow rate of 1 mL/min. The run time was 20 min and the absorbance was recorded at a wavelength of 220 nm [6].

2.6. Biosynthesis of silver nanoparticles (AgNPs)

20g of dry seed powder from *Capsicum annuum* and *Capsicum frutescens* were boiled individually in 100 mL of de-ionized water for 5 min., cooled and filtered. The extracts were stored at 4℃ for further experiments. The filtrates were used as stabilizing agent for 1 mM of silver nitrate ($AgNO_3$). Further, 10 mL of aqueous extracts were added to 90 mL each of aqueous solution of $AgNO_3$ (1 mM).The Erlenmeyer flasks were incubated at 37℃ for reduction of Ag^+ to Ag^0 nanoparticles for 24-72 h [7]. For AgNPs, the solution turned from yellowish to bright yellow and dark brown. Periodic sampling was carried out at 15min, 30min, 45min, 60min, 90min, 24h, 48h and 72h for further characterization of AgNPs. All the experiments were carried out in dark conditions.

2.7. Characterization of silver nanoparticles

UV-vis spectrophotometer (HITACHI, Model U-2800 spectrophotometer) was used at a wavelength of 300-540nm to determine the time point of maximum production of AgNPs and the de-ionized water served as blank.

Atomic Force Microscopy (Model- Nanosurfeasyscan 2 AFM, made in Switzerland) was used to characterize the samples from the maximum time point of production of AgNPs for its detailed size, morphology and agglomeration of silver. AFM image was taken with silicon cantilevers with force constants 0.02- 0.07 N/m, tip height 10 – 15 nm contact mode.

For XRD measurements the AgNPs were dried in the oven at 60°C [8]. The phase formation and purity were recorded using X-ray Diffractometer (Model-D8 Advance, made in BRUKER Germany). The binding properties of AgNPs synthesized by the seed extracts were investigated using FTIR analysis. Freeze dried and powdered AgNPs were diluted with potassium bromide in the ratio 1:100 and recorded the spectrum in FTIR.

For analysis of nanostructure the samples were coated with gold on sputter coater (JEOL, Model 1600 Ion sputter) and observed in SEM (JEOL, Model 6390) individually. Further, chemical composition of the nanoparticles was characterized by Energy Dispersive X- Ray Spectroscopy (EDX) spectrum of sample at ACC voltage 20.0 kV.

2.8. Antioxidant assay

DPPH (1,1-Diphenyl-2-picryl-hydrazyl) assay was done to calculate the total antioxidant content of the extract. DPPH was purchased from Sigma-Aldrich. Ascorbic acid was taken as the standard for the assay. Stock solution of DPPH was made in methanol to a concentration of 200 mM and 0.1M, pH 5.5 acetate buffer was also prepared freshly. 100µl of the nanoparticle extracts (10mg/mL) were added with 100µl of DPPH reagent and 2.8mL of acetate buffer. The reaction tubes were wrapped in aluminium foil and kept for incubation in dark for 30 min. Later absorbance was read at 517 nm using UV-Vis spectrophotometer [9].

$$\text{Percentage scavenging activity} = \frac{Ao-A}{Ao}\text{[10]}$$

A_O – Absorbance of standard
A – Absorbance of sample

FRAP (Ferric reducing antioxidant power) assay was done in accordance with modified Benzie and Strain [11]. The stock solutions were prepared fresh, acetate buffer, pH 3.6, 10mM TPTZ (2,4,6-tripyridyl-s-triazine) obtained from Sigma-Aldrich in 40mM HCl and 20mM $FeCl_3.6H_2O$. The FRAP reagent was made by mixing these solutions in 10:1:1 ratio respectively. The nanoparticle extracts 10mg/mL (100µL) were allowed to react with 2.9 mL FRAP reagent for 30 min in dark conditions. Ascorbic acid was used as the standard. After incubation absorbance was recorded at 593 nm due to the colored product formation [12]. Further FRAP value of the nanoparticles was calculated as:

$$\text{FRAP value} = \frac{A}{Ao} \times \text{FRAP value of standard*}$$

*FRAP value of ascorbic acid = 2
A_O – Absorbance of standard
A – Absorbance of sample

2.9. Cytotoxicity assay of the extracts

MTT assay was performed to determine the cytotoxic property of the synthesized nanoparticles against MG 63 (osteosarcoma cell line). It was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). The cells were maintained and the cell suspension was diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/mL. Further cell line was seeded into 96-well titre plates and incubated to allow for cell attachment at 37℃, 5% CO_2, 95% air and 100% relative humidity. Different concentrations of the extracts were made and added to the wells already containing 100µl of the culture medium and incubated for 48 h at 37℃. Non-treated cells were taken as control and all the trials were done in triplicates.

After 48 h, 15 µL of MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide) (5 mg/mL) in PBS was added to each well and incubated at 37℃ for 4 h [13].The tetrazolium salt is used to determine cell viability in assays of cell proliferation and cytotoxicity [14]. The medium with MTT was then removed and the formed formazan crystals which were solubilised in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage of cell inhibition was determined using the following formula.

$$\text{Percentage Cell Inhibition} = 100 - \left({}^{A}/_{Ao}\right) \times 100$$

where, A – Absorbance of sample
A_O – absorbance of control

Nonlinear regression graph was plotted between Percentage of Cell inhibition and Log concentration; IC50 was determined using GraphPad Prism software.

3. Results and Discussion

3.1. Isolation of the extract

The extracts were obtained from two species of chilli *Capsicum annuum* (C1) and *Capsicum frutescens* (C2) using the Soxhlet extractor. Two solvents were used in the extraction process, which were hexane and acetone. After extraction and filtration, the samples were dried and the dried sample was used for further analysis.

3.2. Synthesis of silver nanoparticles

The filtered aqueous extract of both the *Capsicum* sp. inoculated with seed extracts and $AgNO_3$ were checked for silver nanoparticle synthesis based on the change in color of the solution. Aqueous silver ions were reduced to AgNPs when added to biomass of the crude chilli seed extracts of both the *Capsicum* sp. It was observed that the color of the solution turned from cream to brown upon synthesis of AgNPs. Higher the concentration of $AgNO_3$, faster is the nanoparticle synthesis

3.3. Thin layer chromatography (TLC)

The samples were separated by using thin layer chromatography. Hexane: formic acid: acetic acid in a ratio of 2:1:1 was used as the mobile phase as formic acid has the strongest elution strength followed by acetic acid and hexane. The Rf value of C1 sample extract in acetone was 0.70 and 0.81 and 0.94 in hexane. The Rf value of C2 sample was 0.82 with acetone and 0.81 with hexane.The Rf values obtained with the combination of solvents used refer to the presence of triterpenes in the sample extract [15].

3.4. High performance liquid chromatography (HPLC)

The C1 sample when dissolved in acetone demonstrated a broad retention peak at 1.806 min. A low intensity peak was observed at 2.622 min and 3.082 min (Figure 1a) while the C1 in hexane demonstrated a retention peak at 3.142 min and 2.703 min. A low intensity peak was observed at 1.839 min (Figure 1b). In HPLC analysis the retention peak was at 1.828 min for C2 sample with acetone (Figure 2a) and 2.758 min and 3.192 min (Figure 2b) for C2 sample with hexane.

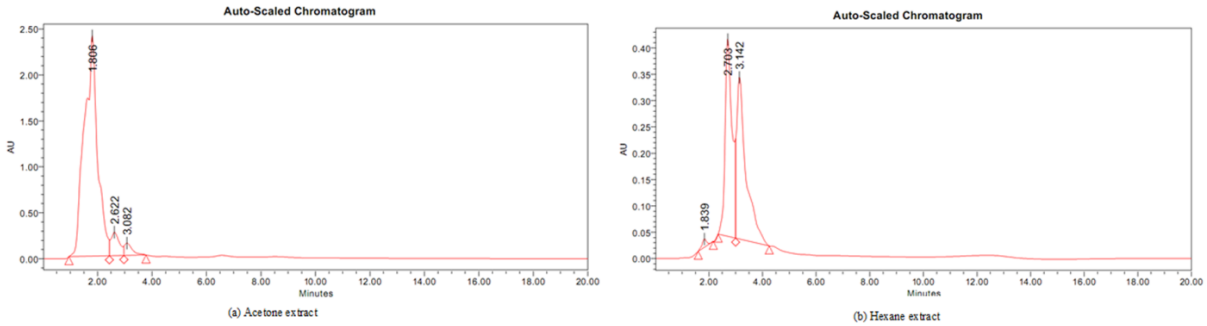

Figure 1.HPLC of *Capsicum annuum* (a) acetone extract (b) hexane extract

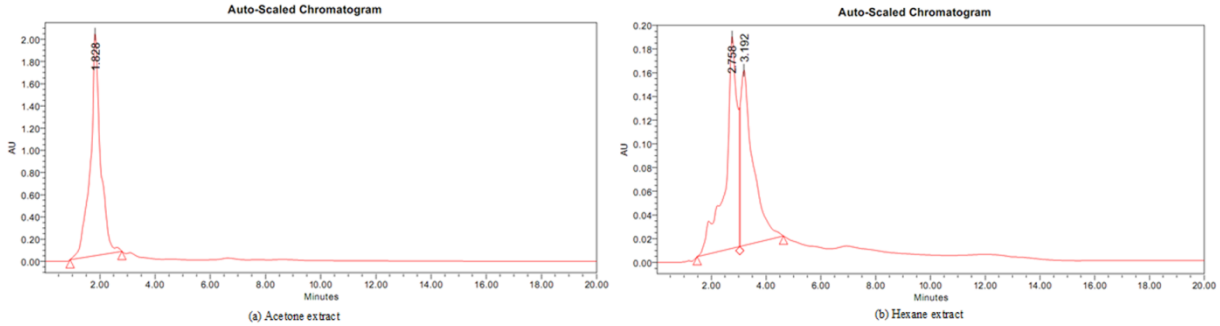

Figure 2. HPLC of *Capsicum frutescens* (a) acetone extract (b) hexane extract

3.5. Biochemical characterization of silver nanoparticles

3.5.1.UV-Vis Spectrophotometer

The characterization was done using UV-Vis spectrophotometer and the formation and stability of the reduced AgNPs in the colloidal solution at various time intervals 24h, 48h and 72h was recorded. The absorbance was read between 200-600 nm and the values were corrected to 10mm path length. The result showed an increase in absorption in the consecutive time intervals. UV-Vis spectra for C1 AgNPs which showed strong peaks at 400-440 nm confirming the presence of the nanoparticles

showing the silver surface plasmon resonance band at 400-440nm as shown in figure 3a. Furthermore, C2 AgNPs also projected peaks between 420-440 nm in the UV-Vis spectra revealing the existence of nanoparticles (Figure 3b).

The UV-Vis absorption spectrum can be considered as a promising method for AgNPs processing. Due to excitation of surface plasmon vibrations in the metal nanoparticles, AgNPs exhibit a yellowish brown colour in water. The results indicate that secondary structures of proteins is not affected by the consequence of reaction involving the Silver (Ag^{+}) ions or while binding with silver nanoparticles. The biological molecules are capable of performing function to form and stabilize AgNPs in aqueous medium. The protein can bind to AgNPs through free amino groups in the proteins. So there is a possibility of stabilization of AgNPs by surface-bound proteins.

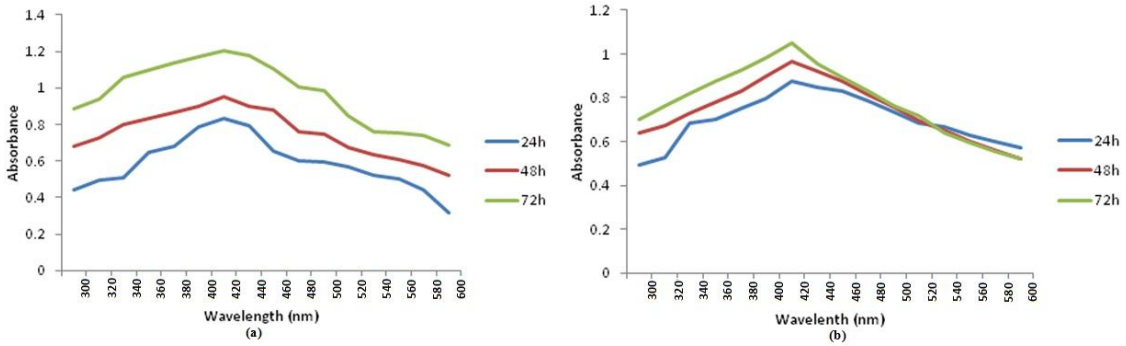

Figure 3.UV-Vis spectrum of (a) *Capsicum annuum* AgNPs (b) *Capsicum frutescens* AgNPs.

3.5.2. Antimicrobial activity of Silver nanoparticles

The zone of inhibition using well diffusion method was performed to evaluate the antimicrobial activity of the crude extract and the AgNPs synthesized against clinical pathogens. Pathogenic strains such as *Staphylococcus aureus, Escherichia coli, Enterobacter* sp., *Serratia marcescens, Shigella* sp., *Salmonella* sp., *Pseudomonas aeruginosa, Proteus mirabilis, and Klebsiella pneumonia*. With the crude extract, the well diffusion method showed a zone of inhibition against *Salmonella* sp. with the C1 (acetone) sample (Table 1). The antimicrobial activity was also monitored for the samples C1 (hexane), C2 (acetone) and C1 (hexane) against *Shigella* sp. (Table 1).

Table 1. Antimicrobial activity of solvent extracts of Capsicum seeds

S. No.	Pathogen	*Capsicum annuum*(Zone of inhibition in mm)		*Capsicum frutescens*(Zone of inhibition in mm)	
		Acetone extract	Hexane extract	Acetone extract	Hexane extract
1	*Staphylococcus aureus*	10	-	-	-
2	*Escherichia coli*	-	-	-	-
3	*Enterobacter* sp.	-	-	-	-
4	*Serratia marcescens*	-	-	10	20
5	*Shigella* sp.	20	-	15	-
6	*Salmonella* sp.	14	-	-	-
7	*Pseudomonas aeruginosa*	-	-	-	-
8	*Proteus mirabilis*	-	-	-	-
9	*Klebsiella pneumoniae*	-	-	-	-

The samples C1 and C2 containing 100 µl AgNPs showed zone of inhibition in case of *Klebsiella pneumoniae* and *Serratia marcescens* at both 48h and 72h (Table 2). The 72 h samples showed more promising results, thus further AFM, XRD, FTIR, SEM, EDX and cytotoxicity assays were performed for these samples.

Table 2. Antimicrobial activity of 100 µl of AgNPs synthesized with *Capsicum* seed extracts

S No.	Pathogen	*Capsicum annuum* AgNPs Zone of inhibition (mm)			*Capsicum frutescens* AgNPs Zone of inhibition (mm)		
		24h	48h	72h	24h	48h	72h
1	*Staphylococcus aureus*	-	-	-	-	-	-
2	*Escherichia coli*	-	-	-	-	-	-
3	*Enterobacter*sp.	-	-	-	-	-	-
4	*Serratiamarcescens*	-	10	14	-	10	13
5	*Shigella*sp.	-	-	-	-	-	-
6	*Salmonella* sp.	-	-	-	-	-	-
7	*Pseudomonas aeruginosa*	-	-	-	-	-	-
8	*Proteus mirabilis*	-	-	-	-	-	-
9	*Klebsiellapneumoniae*	-	11	-	-	13	22

3.5.3. Atomic Force Microscopy (AFM)

The nanoparticles synthesized were observed by AFM and the size of the C1 AgNPs was measured to be 64.8 nm (Figure 4a) and C2 AgNPs was 102 nm (Figure 4b). This confirms the presence of nanoscale sized particles in the samples processed. AFM results confirmed the size to be in nanosclae of 100 nm.

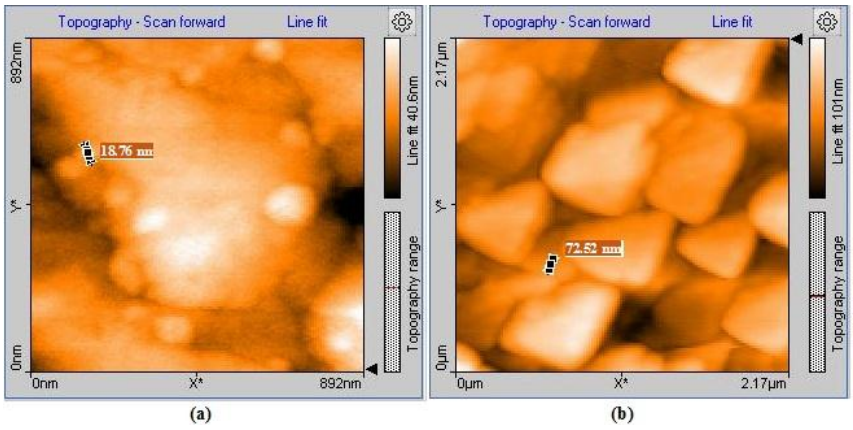

Figure 4. AFM of (a) *Capsicum annuum*AgNPs (b) *Capsicum frutescens*AgNPs.

3.5.4. X- Ray Diffraction (XRD)

XRD graphs of the samples revealed three distinct peaks for *Capsicum annuum* (C1) as well as *Capsicum frutescens*(C2). The observed peaks of C1 sample at 2θ of 32.31°, 46.28° and 77.75° corresponds to (121), (231) and (311) planes respectively (Figure 5a). Similarly for C2 samples the 2θ values of 32.35°, 46.38° and 76.80° falls in (121), (231) and (311) planes respectively (Figure 5b). X-ray diffraction at 2θ values showed similar results at (121), (131) and (311) planes corresponding to the results reported by Mohseniazar et al. [16] and Vivek et al. [17], indicating the peaks corresponding to these planes.

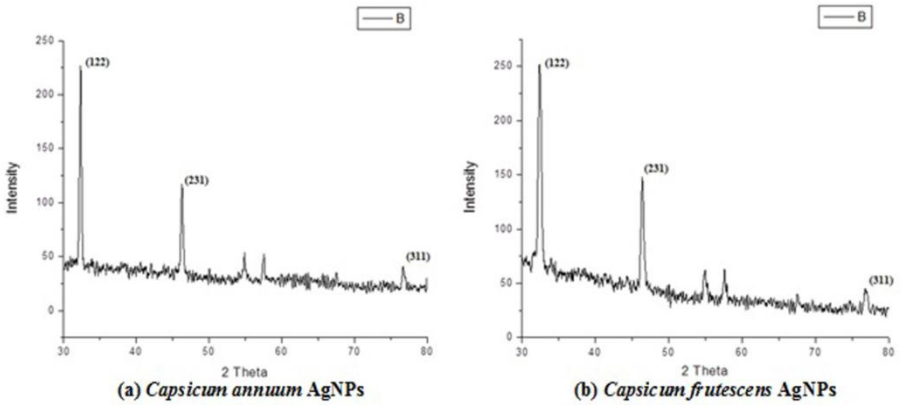

Figure 5.XRD of (a) *Capsicum annuum*AgNPs (b) *Capsicum frutescens*AgNPs.

3.5.5. Fourier Transform Infrared Spectroscopy (FTIR)

Presence of bands by FTIR was showed at 1629 cm^{-1} and 1633cm^{-1} corresponding to C1 and C2 samples (72 h) respectively (Fig. 6a and 6b). The analysis revealed the presence of amide groups corresponding to 3332.99 cm^{-1} in C2 sample, which are found to be relevant with the presence of $AgNO_3$[14] (C=C) stretches are found in both the samples whereas (O-H) is observed in C1 sample indicating the presence of hydroxyl group. The results indicate that secondary structures of proteins is not affected by the consequence of reaction involving the Silver (Ag^+) ions or while binding with silver nanoparticles.

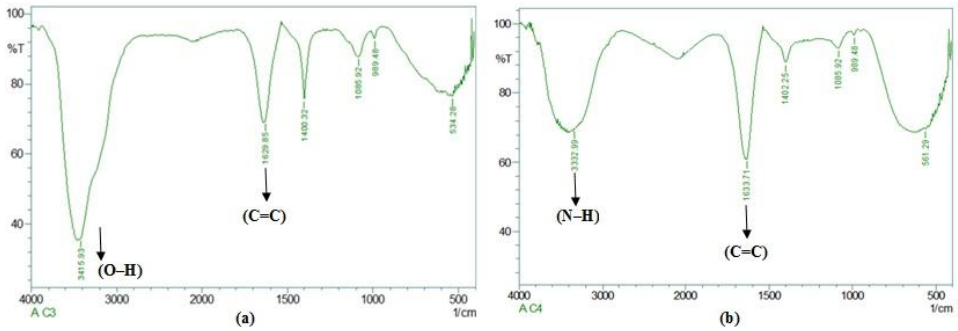

Figure 6.FTIR spectra of (a) *Capsicum annuum*AgNPs (b) *Capsicum frutescens*AgNPs.

3.5.6. Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX)

Figure 7a gives the SEM picture of C1 Ag NPs. It is clearly evident that the size of the particles is in nano range and it is also observed that the nanoparticles produced are aggregated in nature. The EDX result confirms the presence of silver nanoparticles as shown in figure 7b. SEM picture of C2 AgNPs (Figure 8a) clearly indicates the shape to be irregular and of nano range and EDX (Figure 8b) confirms the presence of silver nanoparticles. Through SEM images it can be said that the C1 AgNPs form aggregates in nature and C2 AgNPs are irregularly shaped but both are in nano range.

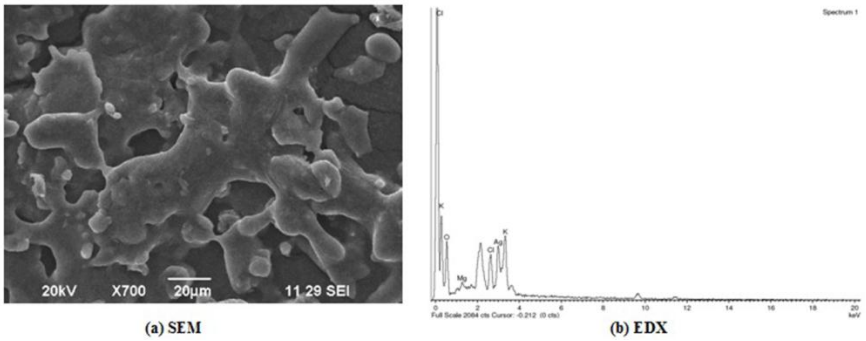

Figure 7.*Capsicum annuum* (a) AgNPs SEM image (b) AgNPs EDX.

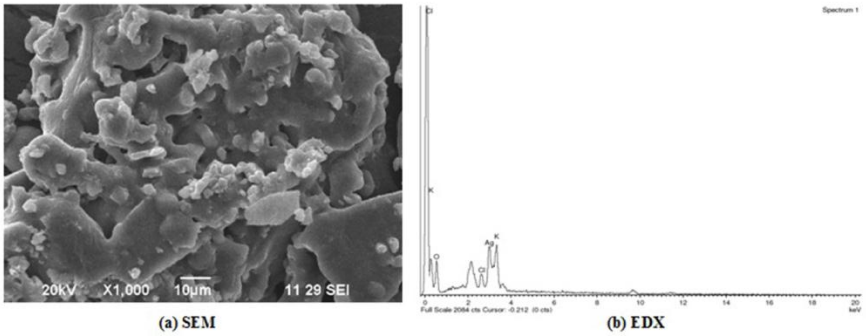

Figure 8.*Capsicum frutescens*(a) AgNPs SEM image (b) AgNPs EDX.

3.6. Antioxidant assay of nanoparticles

DPPH assay has advantages of being stable, sensitive, simple and more feasible. The DPPH assay deals with the ability of the extract to donate hydrogen to the DPPH radical resulting in bleaching of the DPPH solution. The greater the bleaching action by the nanoparticles indicates higher the antioxidant property [18]. The changes in the free radical scavenging activity of the crude nanoparticle extracts vary according to the percent inhibition. The ferric reducing antioxidant potential (FRAP) assay is another simple and inexpensive procedure that tally with the total antioxidant levels. According to the FRAP assay, it is evident that C2 shows greater FRAP values than C1. The higher the FRAP value, the greater is the antioxidant property.

In both the DPPH radical scavenging assay as well as FRAP assay, the activity is observed as change in the color of the solution after the incubation time is over. It is clearly observed that C2 has better antioxidant properties than C1. As concentration of extract increases, the antioxidant potential also shows a positive response. Similar kind of study has been reported in *Cyphomandrabetacea* fruit by Hasan and Bakar[19] and *Nicotianatobacam* L. by Nacoulma et al. [20], belonging to same family of Solanacea.

3.7. Cytotoxicity assay of the extracts

The *in vitro* screening of the silver nanoparticles of both the chilli varieties showed potential cytotoxic activity against the osteosarcoma cell lines. The result revealed that MG 63 cells proliferation were inhibited significantly at IC_{50} value of 64.99 µg/mL for C1 sample extract and 60.42 µg/mL for C2 sample extract. The assay was performed from 12.5µg/mL to 200µg/mL extracts in triplicates and it showed promising anticancer effects of both the extracts, C2 being more effective, revealing the fact that it can be used as an anticancer drug (Fig. 14). There are reports of silver nanoparticles possessing antitumor potential against THP-1 cancer cell lines [21], Hep2 cells, MCF7 cells and HT29 cells [16], etc., synthesized from different sources. To the best of our knowledge this is the first report on the cytotoxic effects of silver nanoparticles from two different *Capsicum* sp. against osteosarcoma cell

lines (MG63). Moreover, Faedmaleki et al. [22] illustrated that nano-silver had 44 times more inhibition effect on the growth of cancerous cells (HepG2 cell line) as compared to the normal cells (primary liver cells of mice), which might further be justified that AgNPs form a potential candidate for cancer treatment.

4. Conclusion

Silver nanoparticles were synthesized using two *Capsicum* sp. with $AgNO_3$ as a source of Ag^+ ions. The method presented was very simple and resulted in enhanced antibacterial activity of the extracts. The UV result was obtained between 400-1000 nm and all the reactions were carried out under dark conditions. Present investigation has an additional advantage as antioxidant activities of the extracts was also confirmed. Further, good response was seen against osteosarcoma MG63 celllines, which might be due to AgNPs as well as the other metabolites of the extract. Thus, it is suitable for clinical applications. There are no conflicts of interests to declare.

References

[1] M. Rai, A. Yadav and A. Gade (2009).Silver nanoparticles as a new generation of antimicrobials,*Biotechnol. Adv*. **27**, 76-83.

[2] A. Nanda, A. Akila and B. Nayak (2011).Role of bioactive compounds in enhancing the effect of silver nanoparticles, *IEEE*.doi: 978-1-4673-0074-2/11/$26.00.

[3] M. Thilagam, A. Tamilselvi, B. Chandrasekaran and C. Rose (2013).Photosynthesis of silver nanoparticles using medical and dye yielding plant of *Bixaorellana* L. leaf extract.*JPSI*.**2**, 9-13.

[4] R. Koffi-Nevry, K. Kouassi, Z. Nanga and G. Loukou (2012). Antibacterial activities of two bell pepper extracts: *Capsicum annuum* L. and *Capsicum frutescens*, *Int. J. Food Prop*. **15**, 961-971.

[5] K. Arunachalam and S. Annamalai (2012). *Chrysopogonzizanoides*aqueous extract mediated synthesis, characterization of crystalline silver and gold nanoparticles for biomedical application, *Int. J. Nanomedicine*. 8, 2375-84.

[6] R. K. Shakhidoyatov and B. T. Sagdullaev (2001). Capsaicine in *Capsicum annuum* condensed extract determined by HPLC, *Chemistry of Natural Products***37**, 575-576.

[7] M. Thirunavoukkarasu, U. Balaji, S. Behera, P. K. Panda and B. K. Mishra (2013). Biosynthesis of silver nanoparticles from leaf extract of *Desmoniumgangeticum* (L.) DC and its biomedical potential, *Spectrochim.Acta A*.**116**, 424-427.

[8] C. Ramteke, T. Chakrabarti, B. Sarangi and R. Pandey (2013). Synthesis of silver nanoparticles from the aqueous extract of leaves of *Ocimum sanctum* for enhanced antibacterial activity, J. Chem. Article ID 278925.

[9] O. P. Sharma and T. K. Bhat (2009). DPPH antioxidant assay revisited, *Food Chem*. **113**, 1202-1205.

[10] C. Priyanka, D. A. Kadam, A. S. Kadam, Y. A. Ghule and V. T. Aparadh (2013). Free radical scavenging (DPPH) and Ferric reducing ability (FRAP) of some Gymnosperm species, *Int. J. Res. Botany.* **3**, 34-36.

[11] I. F. F. Benzie and J. J. Strain (1996). The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power": The FRAP Assay, *Anal. Biochem*.**239**, 70–76.

[12] K. Thaipong, U. Boonprakob, K. Crosby, L. C. Zevallos and D. H. Byrne (2006). Comparison of ABTS, DPPH, FRAP and ORAC assays of estimating antioxidant activity from guava fruit extract, *J. Food Comp. Anal*. **19**, 669-675.

[13] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull and D. Vistica et al. (1991). Feasibility of high flux anticancer drug screen using a diverse panel of cultured human tumour cell lines, *Natl. Cancer Inst*. **83**, 757-766.

[14] J. S. Devi and B. V. Bhimba (2012). Anticancer activity of silver nanoparticles synthesized by the seaweed *Ulvalactuca* in vitro, *Open access scientific reports***1**, 242.

[15] A. Mohammad, S. A. Bhawani and S. Sharma (2010). Analysis of herbal products by thin-layer chromatography: a review. *IJPBS*.**1**, 1-50.

[16] M. Mohseniazar, M. Barin, H. Zarredar, S. Alizadeh and D. Shanehbandi (2011). Potential of microalgae and lactobacilli in biosynthesis of silver nanoparticles, *BioImpacts***1**, 149-152.

[17] M. Vivek, P. S. Kumar, S. Steffi and S. Sudha (2011). Biogenic silver nanoparticles by *Gelidiellaacerosa* extract and their antifungal effects, Avicenna J. Med. Biotechnol. **3**, 143-148.

[18] J. M. Oke and M. O. Hamburger (2002). Screening of some Nigerian medicinal plants for antioxidant activity using 2, 2- diphenyl- picryl- hydrazyl radical, *Afr. J. Biomed.Res*. **5**, 77- 79.

[19] S. H. A. Hassan and M. F. A. Bakar (2013). Antioxidant and Anticholinesterase activity of *Cyphomandrabetacea* fruit.*Scientific World Journal*doi: 10.1155/2013/278071.

[20] A. P. Nacoulma, M. Compaoré, M. Lorenzi, M. Kiendrebeogo and O. G. Nacoulma (2012). *In vitro* antioxidant and anti-inflammatory activities of extracts from *Nicotianatabacum* L. (Solanaceae) leafy galls induced by *Rhodococcusfascians*, *J. Phytopathology***160**, 617-621.

[21] R. Thombre, S. Mehta, J. Mohite and P. Jaisinghania (2013). Synthesis of silver nanoparticles and its cytotoxic effect against THP-1 cancer cell lines, Int. J. Pharm. Bio. Sci. **4**, 184-192.

[22] F. Faedmaleki, F. H. Shirazi, H. Rastegar and A. Salarian (2012). Toxicity effect of silver nanoparticles on HepG2 cell line and mice liver primary cell culture, *Res. Pharma.Sci*. **7**, 181.