WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 6, 1205-1215. Research Article ISSN 2277– 7105

EVALUATION OF RADICAL SCAVENGING, CYTOTOXIC EFFECT AND ANTIBACTERIAL ACTIVITY OF BIOLOGICALLY SYNTHESIZED SILVER NANOPARTICLES USING PITTOSPORUM F.DRYAND

S.Gopalakrishnan[1], S. Yamini Sudha Lakshmi[2*] and P.Arumugam[3]

[1,3]Department of Applied Sciences, PNG University of Technology, LAE, Papua New Guinea.

[2*]Deptartment of Medical Biochemistry, University of Madras, Chennai, India, Armats Biotek Pvt Ltd, Guidy Chennai, India.

Article Received on
27 March 2015,

Revised on 19 April 2015,
Accepted on 11 May 2015

***Correspondence for Author**
Dr. S. Yamini Sudha Lakshmi
Deptartment of Medical Biochemistry, University of Madras, Chennai, India, Armats Biotek Pvt Ltd, Guidy Chennai, India.

ABSTRACT

Crystalline Silver Nano particles have found numerous applications in the medicinal field include diagnostic applications and therapeutic applications. In this study, the silver nanoparticles was synthesized biologically using Pittosporum Dryand leaf extract as reducing agent and evaluation of radical scavenging and toxicity effect and antimicrobial action were carried out. The formation of silver nanoparticles was indicated by the colour change from colourless to reddish brown and the particles were characterized by UV-vis, FTIR, ERD, SEM, and TEM analysis. These nanoparticles were found to have sufficient antimicrobial activity, signified radical scavenging capacity. These nano particles showed potent cytotoxic activity against MCF7 liver cell line also found that increase in concentration of the sample, the viability of MCF7 cells decreased. Hence silver nanoparticles synthesized from Pittosporum Dryand possess potential applications in medicine and pharmaceutical fields.

KEYWORDS: Biosynthesis, FTIR, XRD, TEM, Radical scavenger, Pittosporum f.Dryand.

1. INTRODUCTION

Infectious dermatological environment is a common cause in rural area and among the tribal population. Application of herbal remedies, tattooing with traditional medicines are usual

practices and are playing a vital role in providing health care to large populations, especially in developing countries. Biosynthesised, nanoparticles are taking vital role in the recent developing nanotechnology. Nanotechnology involves with the synthesis of different sizes, shapes and controlled dispersity[1] of nanoparticles. Metallic nanoparticles like gold and silver nanoparticles have various applications in the field of medicine[2-4], defense[7,8], drug synthesis and, optics[5] due to their physio chemical properties. It is well known fact that silver ions and silver based compounds are highly toxic to microorganisms. This property of silver makes it an excellent choice for multiple roles in medication.

There are several methods to develop silver nanoparticles from plant extract. The biosynthesis is more advantageous over chemical and physical methods as it is environment friendly.[9,10] In the study, biosynthesis of silver nanoparticles were carried out using aqueous leaf extract of Pittosporum Dryand and characterized using UV-visible spectra, scanning and transmission electron microscopy and, X-ray diffraction techniques.The antimicrobial activity have been investigated against Gram negative and Gram positive bacteria.Keeping in view the antioxidant properties of silver nanoparticles and their possible interaction with metallic surface, we investigated the radical scavenging activity using DPPH assay.The unique physical and chemical properties of silver nanoparticles make them excellent candidate for many daily activities. Though silver nanoparticles are rampantly used in many medical procedures and devices as well as various biological fields, they have their drawbacks due to nanotoxicity, thus, the evalvation of cytotoxicity on MCF7 cell lines also studied. To our knowledge and available sources, the biosynthesis of silver nano particles from Pittosporum Dryand plant leaf extract, radical scavenging acticity and cytotoxicity are not reported so far.

2. MATERIALS AND METHODS

The fresh leaves were collected and checked for disease attack, if any. Then, the selected healthy leaves were washed with sterile distilled water and were cut into small pieces. 10g of sample was weighed and ground by using mortar and pestle by adding 50ml of sterile distilled water. Then the mixture was centrifuged at 5000rpm for 15minutes and the supernatant was collected by filtering and kept for further use.

2.1. Synthesis of silver nanoparticles under Sunlight irradiation

1 mM $AgNO_3$ solution was prepared and Plant extract (3ml) was taken in conical flask and to this 10 ml of 1 mM $AgNO_3$ solution was added with constant stirring and exposed under sunlight radiation and observed the colour change. The colour change of the solution was

checked periodically, Bioreduction of silver ions in the solution was monitored by measuring using UV-Vis spectra then the conical flask was incubated at room temperature for 48 hours. The colour change of the leaf extract from colouless to reddish brown indicated the silver nanoparticles were synthesized from the Plant extract. The content was centrifuged at 10,000 rpm for 15 minutes. The supernatant was used for the characterisation of the silver nanoparticles.

2.2. Bulk productionof silver nanoparticles under optimized condition

100ml $AgNO_3$ (1mM) with 30ml extract was prepared and exposed to optimized conditions for the optimal time and placed at room temperature for 24 h for the particles to settle. To the particles now settled at the bottom of the container, about 1ml acetone was added for the removal of the moisture content from the nanoparticles. The nanoparticle suspension were transferred to a watch glass, air dried, collected, weighed and stored in a sterile container for further characterisation.

2.3. Characterisation of Silver nanopaticles

The reduction of pure silver ions were observed by measuring the UV-Vis spectrum of the reaction at different time intervals taking 1ml of the samples, compared with 1 ml of distilled water used as blank. UV-Vis spectral analysis has been done by using An Elico spectrophotometer at a resolution of 1 nm from 200to 1100 nm using a one-centimetre quartz cuvette. The purified suspension was oven dried and the powder was subjected to FTIR spectroscopy analysis(Perkin-Elmer spectrometer FTIR Spectrum one in the range4000–400 cm−1 at a resolution of 4 cm^{-1} in KBr pellet). The size and shape of synthesized AgNPs was characterized by Scanning electron microscope and transmission electron microscope. SEM observations were carried out on a ZEISS EVO 40 EP. The TEM observations were made in JEOL JEM 100SX Transmission Electron Microscope at an accelerating voltage of 80kv. The determination of the formation of silver nanoparticle and confirmation of the presence of elemental silver observed by an X' Pert Pro X-ray diffractometer operated at a voltage of 40kv and a current of30mAnwith Cu Kα radiation.

2.4. Antibacterial activity using Disc Diffusion Method

The paper disc method of testing bacterial sensitivity to chemotherapeutic agents is generally accepted as the most effective practical means for clinical work. The Difco "Dispens-o-disc" places 15 filter paper discs impregnated with different anti-bacterial agents onto an agar plate inoculated with the test organism. After incubation at 37^0C for 24h, the sensitivity of the

microorganism to antibiotics is reported on the basis of the presence or absence of a zone of inhibition.

2.5. Estimation of Radical scavenging Activity Using DPPH Assay

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.[12] 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; % Radical scavenging activity(%RSA) = [(Aof control – A of sample) / (A of control)] x 100

A of control is the absorbance of BHT radical + ethanol; A of sample is the absorbance of DPPH radical + AgNPs / standard.

2.6. Determination of Invitro Assay of Cytotoxic Activities

Silver nanoparticles also proved to be toxic to invitro mouse germ line stem cells as they impaired mitochondrial function and caused leakage through the cell membranes. Nanosilver aggregates are said to be more cytotoxic than asbestos.[13] There is evidence that shows that silver ions cause changes in the permeability of the cell membrane to potassium and sodium ions at concentrations that do not even limit sodium, potassium, ATP, or mitochondrial activity.[14] Nanosilver is also known to show severe toxic effects on the male reproductive system. Research shows that nanosilver can cross the blood-testes barrier and be deposited in the testes where they adversely affect the sperm cells.[15]

In the present study cytotoxic effect was determined against MCF7 cell line.[16]

The invitro cytotoxicity of the AgNPs was evaluated against MCF7 liver cell line at different concentrations. Cytotoxicity analysis of the sample shows a direct dose relationship; cytotoxicity increased at higher concentrations .Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine serum, at 37^0C 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% = (At/Ac) x100 Cytotoxicity % = 100 - % Viability

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

3. RESULTS AND DISCUSSION

3.1. UV-visible analysis

Fig 1 represents the UV-Vis spectrographs of the silver nanoparticles synthesised from the crude extract Maximum absorbance was seen at 455nm, indicating that the formation of spherical silver nanoparticles in majority or anisotropic particles whose appearance and ratio increases with time.

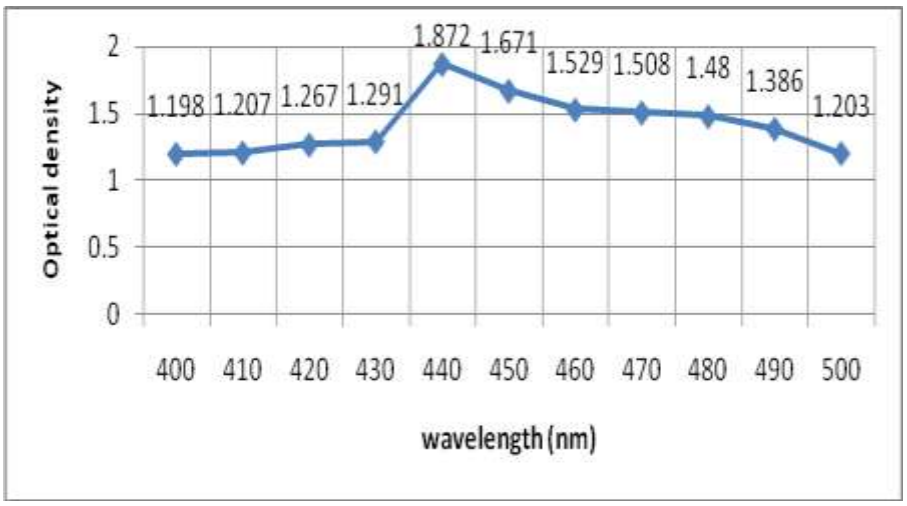

Fig1: UV-Vis Absorption Spectrum of nanoparticle synthesized from crude extract of Dysoxylum mulleri at different time Intervals.

3.2. FTIR analysis: The characteristic peaks at absorption (cm^{-1}) values 3434.32, 2923.17 and 1626.02 shows the presence of amine N-H, alkyl C-H and amide C=O groups. In the FTIR data of silver nanoparticles synthesised using *P. ferrugineum* shows peaks at absorption values of 3325.34, 2917.38, 1663.99 and 1541.15 are characteristics of Alcohol/Phenol O-H, Alkyl C-H and Aromatic C=C groups.

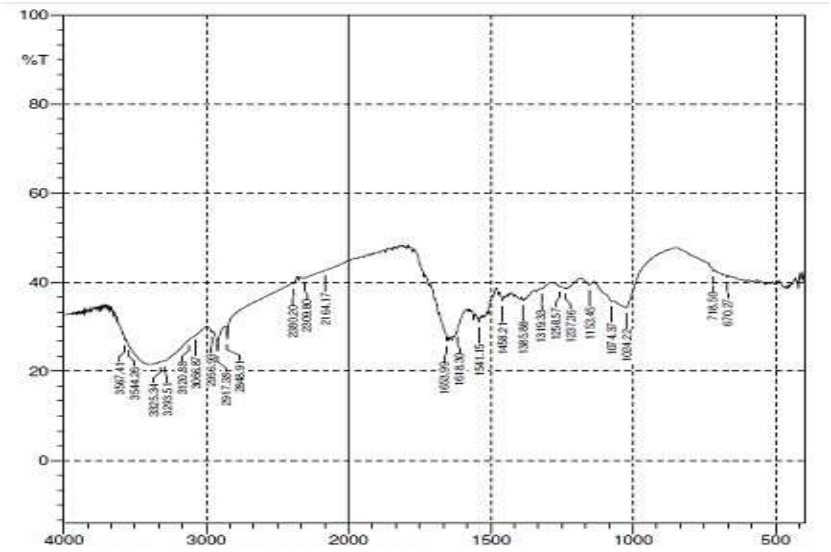

Fig 2: FTIR spectrum of silver nanoparticles synthesized using *P. ferrugineum*

3.3. SEM analysis: It is clear from the SEM picture that the nanoparticles synthesized using *P. ferrugineum* were of size 0.40µm – 0.50µm. However the particle size reduction using physical methods could be considered in order to increase their efficiency.

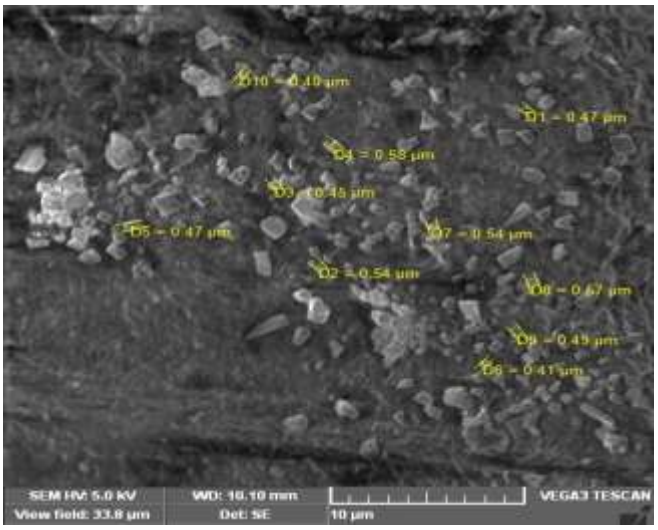

Fig 3: SEM image of silver nanoparticles synthesized using *P. ferrugineum*

3.4. TEM analysis: The AgNP synthesized when scanned using TEM from which we coclude that the average mean size of AgNPs was in between 5-22nm and seemsto be spherical in morphology shown in (Fig 4). Thus the transmission electron microscopy gave a detailed descriptive image of AgNPs synthesized with their structural details and their size.

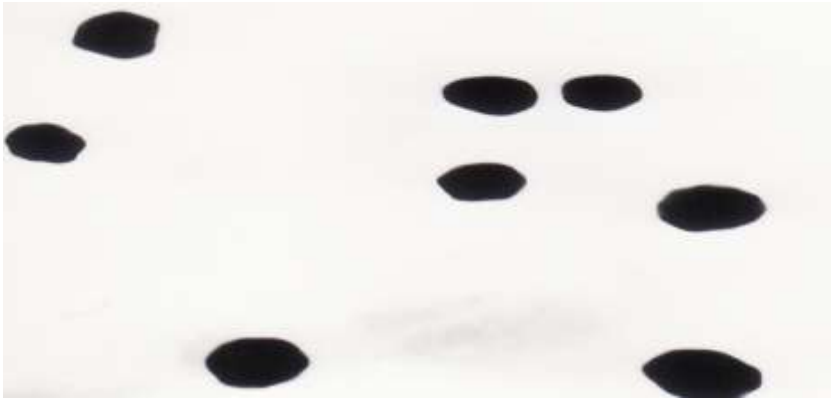

Fig 4: Tem analysis and particle size distribution

3.5. XRD Analysis

XRD analysis of nanoparticles synthesized using *P. ferrugineum* shows 4 major peaks at 29.18^{o}, 32.18^{o}, 38.45^{o} 47.18^{o} and can be indexed the angle values of (111), (200), (220), (240) signifies crystalline particles. This analysis revealed that nanoparticles are orthorhombic crystals.

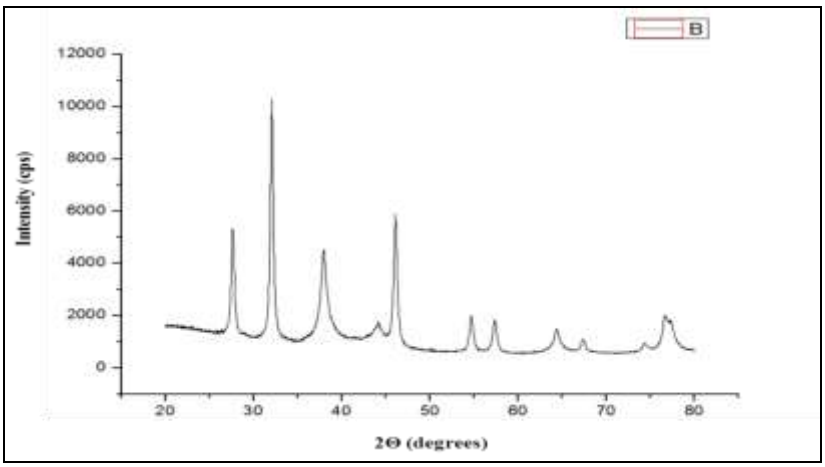

Fig5: XRD of silver nanoparticles synthesized using *P. ferrugineum*

3.6. Antimicrobial activity

The antibacterial activity of biosynthesized AgNPs was examined and carried out on fifteen Gram +ve and Gram –ve bacteria, such as Bacillus cereus, Bacillus subtilis, Citrobacter freundii, Escherischia coli, Klebsiella pneumonia, Neisseria gonorrhea, Salmonella typhimurium, Staphylococcus aureus, Streptococcus pneumonia, M.lutea. Biosynthesized silver nanoparticle showed clear zone of inhibition as indicated in Table -1. It is reported that AgNPs attach to the surface of the cell membrane, disturbs its function and penetrates

directly with the bacterial outer membrane and release Ag ions. Chloramphenicol 10 µg and Tetracycline 10 µg are used as standard.

Table 1: Bioassay of 3mM concentration of Silver Nanoparticles synthesized from Pittosporum ferrugineum.

Name of Microbes	Inhibition zone (mm)	Standard: Chloramphenicol 10 µg (mm)	Standard: Tetracycline 10 µg (mm)
B. cereus	12	20	26-27
B. subtilis	14	27	30
C. freundii	12	25-26	20
E.coli	12	-	-
K. pneumonia	12	19-20	16
M. lutea	16	18	12
N. gonorrhoea	12-13	25-26	20
S. typhimurium	10-12	10	17-18
S. aureus	15-16	19	12
S. pneumonia	13	14-15	13

3.7. Evaluation of Radical scavenging activity of silver nanoparticles (DPPH assay)

Radical scavenging activity (RSA) of silver nanoparticles synthesized from *Pittosporum ferrugineum* leaves were found to vary between 9 and 54%. The data was considered significant when compared with the standard used. The IC_{50} value was calculated as 180µg/ml.The RSA values are shown in Table-2 and in the fig 6.

Table 2: Radical Scavenging Activity of silver nanoparticles synthesized from *P. ferrugineum*

S.No	Concentration (µg)	% RSA P.Ferrugineum
1	20	9.98
2	40	13.5
3	60	19.55
4	80	23.89
5	100	27.22
6	120	32.7
7	140	38.56
8	160	46.88
9	**180**	**50.42**
10	200	54.93

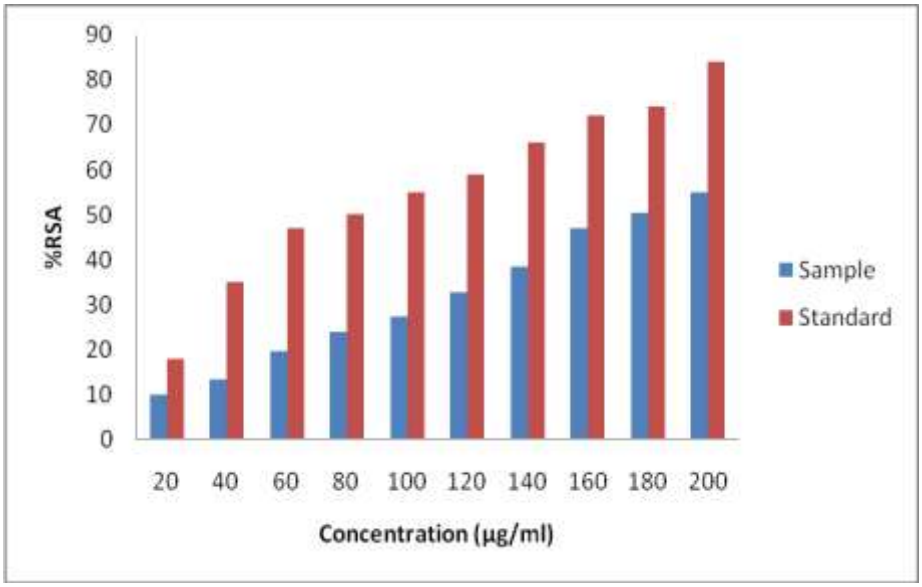

Fig 6: RSA of silver nanoparticles synthesized from *P. ferrugineum*

3.8. Evaluation of Cytotoxicity of AgNPs on MCF7 cell lines

The invitro cytotoxicity of the AgNPs was evaluated against MCF7 liver cell line at different concentrations [Table-3]. 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-7].

The silver nanoparticles showed (Table 3 & Fig7) potent cytotoxic activity against MCF7 liver cell line. The IC_{50} value was studied as 100µg/ml at which the cell viability was recorded as 53.33 %. It was also studied that with increasing concentration of the sample, the viability of MCF7 cells decreased.

Table 3: Evaluation of Cytotoxicity of AgNPs on MCF7 cell lines

S.No	Concentration (µg)	Cell viability (%)
		P.Ferrugineum
1	1	95.17
2	10	87.47
3	20	75.89
4	50	60.23
5	100	53.33

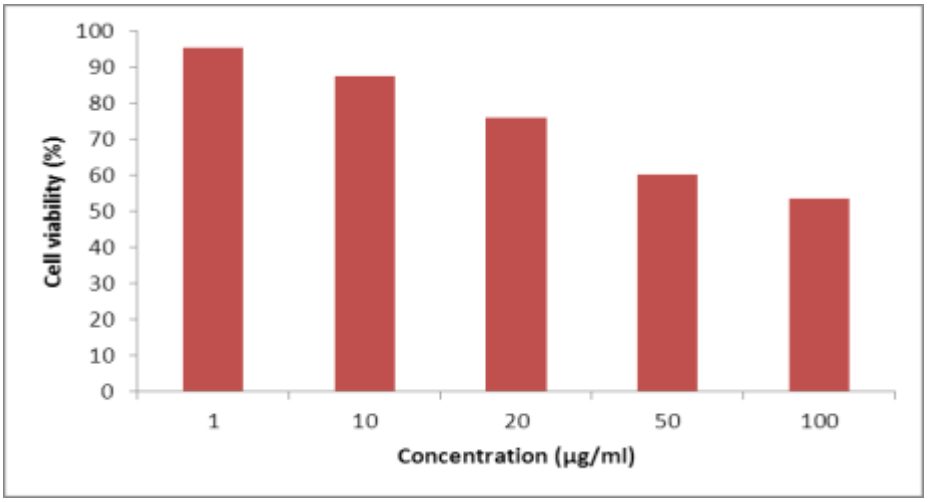

Fig 7: Evaluation of Cytotoxicity of AgNPs on MCF7 cell lines

4. 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, radical scavenging 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

I wish to express my deep sense of gratitude and thanks to the Research Committee, Department of Applied Science, PNG University of Technology for providing funds, chemicals, other requirements and guidance with respect to research work and to make success of this research study.

REFERENCE

1. Manish Dubey,Seema Bhadauria, B.S. Kushwah, Digest Journal of Nanomaterials and Biostructures, 2009; 4(3): 537-543.

2. Jose A. Rojas-Chapana and Michael Giersig, J.of Nanoscience and Nanotechnology., 2006; 6: 316.
3. Tadanori Yamada et al. Nature Bionanotechnology., 2003; 21: 885.
4. Robert A.,Freitas Jr.,JD., Nanomedicine: Nanotechnology, Biology and Medicine., 2005 1: 2.
5. A.B. Landsdown, A. Williams., J Wound Care., 2007; 16: 15.
6. Mukherjee, A. Ahmad, D. Mandal,S. Senapati,S. R. Sainkar, M.I. Khan,R. arishcha,P. V. Ajaykumar, M. Alam, R. Kumar, M. Sastry, 2001; Nano Lett. 1: 515.
7. Han, M., Gao,X.,Su,J. Z. & Nie, S., 2005; Nature Biotechnol.19: 631.
8. Joseph Wang.,Analytica Chimica Acta., 2003; 500: 247.
9. Kowshik.M, Ashtaputre.S, Kharazzi.S Vogel.W ,Urban.J ,Kulkarni.S.K, Panikar K.M. Extracellular synthesis of silver tolrent strain MKY3. Nanotechnology., 2003; 14: 95-100.
10. Nabikha.K, Kathiersan, Raj.A, Alikunhi.M, Nabeel. Synthesis of antimicrobial silver nanoparticles by callus and leaf extract from salt marsh plants, Sesuvium portulacastrum L. Colliods and Surface:Biointerfaces., 2009; 79: 488- 493
11. Thaipong K. Boonprakob U. Crosby K. Cisneros-Zevallos L. Comparison of ABTS, DPPH.FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J.Food .Compost Anal, 2006; 19669-675 DOI: 101016/jjfca 2006.01.003.
12. Harbone, J.B and Baxter, H (1995), Phytochemical Dictionary, A Handbook of Bioactive compounds from plants, London: Taylor and Francis.
13. Soto, KF, Murr, LE, Garza, KM: Cytotoxic responses and potential respiratory health effects of carbon and carbonaceous nanoparticulates in the Paso del Norte airshed environment. Int. J. Environ. Res. Public Health., 2008; 5: 12–25.
14. Kone, BC, Kaleta, M, Gullans, SR: Silver ion (Ag+)-induced increases in cell membrane K+ and Na+ permeability in the renal proximal tubule: reversal by thiol reagents. J. Membr. Biol., 1988; 102: 11–19.
15. McAuliffe, ME, Perry, MJ: Are nanoparticles potential male reproductive toxicants? A literature review. Nanotoxicol., 2007; 1: 204–210.
16. Mossman, T. Rapid colorimetric assay for cellular growth and survival – application to proliferation and cytotoxicity assays. J.Immunol. Methods., 1983; 65: 55-63.