INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X
Impact factor: 4.295
(Volume 3, Issue 6)
Available online at www.ijariit.com

Green Synthesis of Silver Nanoparticles using *Barringtonia Acutangula (l.) Gaertn*. and its *invitro* Anticancer Property

S. Prem Kumar
premseenivasagan@yahoo.in
K. S Rangasamy College of Technology
Tiruchengode

S. Rubavathi
rubavathi5@gmail.com
K. S Rangasamy College of Technology
Tiruchengode

Dr. J. Phillip Robinson
philiprobin81@gmail.com
K. S Rangasamy College of Technology
Tiruchengode

ABSTRACT

Green synthesis of silver nanoparticles (AgNPs) is very safe and eco-friendly to the environment as well as human. Plants provide a better platform for nanoparticles synthesis as they are easily available, safe, eco-friendly and free from toxic chemicals as well as provide natural capping agents. Secondary metabolites are the reducing agents in the synthesis of silver nanoparticles. In the present study, the medicinal plant Barringtonia acutangula leaf extract was used as reducing agent. The presence of phytoconstituents of Barringtonia acutangula was analysed. The formation of silver nanoparticles was confirmed by colour change and the characterization was done by UV-Vis, FTIR and SEM. The maximum absorbance of silver nanoparticles was attained at 435 nm wavelength. Protein molecules are act as capping agents of silver nanoparticles was suggested by FTIR. Size of the silver nanoparticles above 100nm which were detected by SEM analysis. The antibacterial activity of silver nanoparticles also reported on two species such as Escherichia coli and Staphylococcus aureusby well diffusion method. Antioxidant analysis of silver nanoparticles was done and the EC_{50} value was determined. The EC_{50} value of reducing power method is 0.813 mg/ml and total antioxidant capacity is 1.95mg/ml. The in vitro anticancer activity of silver nanoparticles was done by MTT assay on two cell lines such as HeLa (Human cervical carcinoma) and MCF-7 (Human breast adenocarcinoma) and the IC_{50} value calculated. For HeLa cell line, the IC_{50} value was obtained as 45.4 µg/ml and the IC_{50} value of MCF-7 is 5.6µg/ml.

Keywords: *Antioxidant, Barringtonia Acutangula, FTIR, SEM, In Vitro anticancer, MTT.*

1. INTRODUCTION

Nanotechnology is an emerging field growing every day in various aspects of our lives. Nanotechnology has recently induced more advancement in the medical field and technology field. Nanoparticles are fundamental building blocks of nanotechnology. Nowadays environmental awareness are increased that pave the way for the researchers to focus on the green chemistry. It is the application of science at molecular level and promising field in emerging new applications in medicine. Nano biotechnology combines with biological approach to generate Nano sized particles having specific agents which can be done with the help of Nanoscience. Compared to biological molecules such as enzymes, nanoparticles are smaller than hundred functionality. For drug delivery applications silver and gold nanoparticles are powerful and promising tool for reducing the infectious disease effectively. The most important and distinct property of nanoparticles is their larger surface area to volume ratio (Leela *et al*., 2008). Nanoparticles can be synthesized using various approaches including chemical, physical, and biological. Physical and chemical methods are more popular for nanoparticle synthesis as large quantity can be produced. But the use of toxic compounds limits their application and also not economically feasible one.

Plants are invaluable sources of pharmaceutical products and plants are also continuously produced secondary metabolites which are used as pharmaceutical effective ingredients (Patel *et al*., 2011). Plants provide a better platform for nanoparticles synthesis as they are easily available, safe, eco-friendly and free from toxic chemicals as well as provide natural capping agents.

Moreover, use of plant extracts also reduces the cost of microorganism's isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms. Silver nanoparticles can be effectively used due to its low toxicity and high biocompatibility. It has more antimicrobial activity against clinical human pathogens like *Salmonella typhi, Klebsiella, Candida sp* etc.The main theme behind this nanoparticle production is the reduction done using phytoconstituents present in the plants. The phytochemicals are directly involved in the reduction of silver nitrate into silver nanoparticles (Sukumaran and Poulose., 2012).Silver nanoparticles find use in many fields, and the major applications include catalysts, optical sensors, and most importantly in the medical field as a bactericidal and as a therapeutic agent. Silver ions are used in the formulation of dental resin composites; as a bactericidal coating in water filters; as an antimicrobial agent, consumer products; as bone cement; and in many wound dressings. In addition to this, they can also be used to reduce toxicity of a therapeutic drug. Nanoparticles are playing the key role to develop therapeutics against cancer (Monalisha*et al*., 2014) due to its easy solubility and deep penetration in organs and tissue.

Barringtonia acutangula is the traditional medicinal plant as popularly used. *Barringtonia acutangula* comes under the Lecythidaceae family. Maninderjit*et al*., (2013) was reported and highlighted the updated information about that the *Barringtonia acutangula* which was commonly used in India by tribal people for the treatment of liver disorders, diarrheal diseases, eye diseases, jaundice, spleenic disorders and worm infection. He also said that all parts of *Barringtonia acutangula* like leaves, fruit, seed, bark and root were used for cure and treatment of many haemolytic diseases, abdominal colic, malarial, diabetes etc.

2. METHODOLOGY

Collection and Identification of the Plant Specimen

The *Barringtonia acutangular* leaves were collected from Kadukkaivalasai village, Ramanathapuram District of Tamil Nadu. The authentication of plant sample was done by Botanical Survey of India (BSI), TNAU at Coimbatore. The plant specimen was submitted and the voucher number is BSI/SRC/5/23/2016/Tech/276. The leaves was shade dried for 5 days at room temperature and grinded using blender into fine powder. The plant were subjected to extraction using different solvents such as petroleum ether, chloroform, benzene, ethyl acetate and methanol based on their polarity.

Phytochemical Tests

The phytochemical screening of *Barringtonia acutangula* was done by the standard methods using various solvents based on their polarity such as Petroleum ether, Chloroform, Benzene, Ethyl Acetate and Methanol which were used to identify the phytoconstituents present in the plant extracts (Solomon *et al*., 2013).

PREPARATION OF 1mM OF SILVER NITRATE ($AgNO_3$) SOLUTION

0.169g of $AgNO_3$was added to the 1L of Double distilled water and stored in brown coloured bottle in dark place.

GREEN SYNTHESIS OF Ag NANOPARTICLES

The plant extract was selected based on the presence of phytoconstituents.10ml of plant extracts were added to the 90ml of 1mM of $AgNO_3$ solution and stored in dark place for 3days. The formation of dark brown colour was observed. Then the solution was centrifuged at 6000rpm for 10mins for further characterization. (Shanmugam *et al.*, 2015)

3. CHARACTERIZATION

UV-Visible Spectrophotometer

The reduction of Ag^+ to Ag nanoparticles was monitored by measuring UV-Visible spectrophotometer (Hitachi- Model U 2900). The wavelength was set from 300 – 600nm for scanning process.

FTIR Analysis

The functional group of *Barringtonia acutangula* mediated synthesis of silver nanoparticles was analysed by FTIR and also gives the information about the vibrational and rotational modes of motion of a molecules. The wavenumber was set from 400 – 4000cm^{-1}. The method of measurement was followed by KBr pelleting method.

SEM Analysis

The morphology and size of synthesized silver nanoparticles were analysed by the Scanning Electron microscope. The electron beam focused on sample and created an image.

ANTIBACTERIAL ACTIVITY

Antibacterial property of synthesized silver nanoparticles using *Barringtonia acutangula* leaf extract was done by agar well diffusion method. In this assay, the *Escherichia coli* and *Staphylococcus aureus* were used for analysis of activity. 100 µl of bacterial cell suspension were spread on Petri plates containing Muller Hinton agar. Tetracycline and Chloramphenicol was used as standard.

Finally the inoculated plates were incubated at 37°C for 24 hours for pathogenic bacteria. Antimicrobial activity was calculated by measuring the diameter of zone of inhibition in mm. The formation of inhibition zone were made as triplicate values.

ANTIOXIDANT ACTIVITY

Antioxidant Property

The antioxidant property of *Barringtonia acutangular* mediated silver nanoparticles was done by Reducing power assay and Phosphomolybdenum assay.

FRAP assay

The reducing power of Silver nanoparticles was analysed by potassium ferricyanide assay. Various concentration of Sample (0-1mg/ml) was added with 1ml of phosphate buffer (0.2M, pH-6.6) and 1ml of potassium ferricyanide (10mg/ml) were mixed together and incubated for 20mins at 50°C. After incubation, 1ml of Trichloroacetic acid (100mg/ml) was to the mixture and then the mixture was allowed to centrifuge for 5mins at 8000rpm. Then, 2ml of upper layer of supernatant was taken and mixed with 2ml of distilled water and 0.2ml of ferric chloride (1mg/ml). Here, the Ascorbic acid was used as reference standard. Finally, the absorbance was measured at 700nm. (Selvakumar *et al.,* 2011)

Phosphomolybdenum Assay

The Total Antioxidant Activity of silver nanoparticles was evaluated by Phosphomolybdenum assay. Molybdate Reagent was prepared by taking1ml each of 0.6 M sulphuric acid, 28mM sodium phosphate and 4mM Ammonium molybdate and mixed with 20 ml of distilled water and made up volume to 50 ml by adding distilled water. Different concentration of methanolic extract were taken. To this, 3ml of molybdate reagent was added and kept it boiling water bath for 90mins at 95°C. After boiling, the mixture was allowed to cool at room temperature. Finally, the absorbance of the mixture was read at 695nm. Ascorbic acid was used as standard**.** (Prieto *et al*., 1999).

Statistical Analysis

The results of antioxidant assays were expressed as mean values and standard deviation on triplicates of samples. The data were evaluated using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using Graph pad Prism software. ($P<0.05$, considered statistically significant)

ANTICANCER ACTIVITY

The in vitro anticancer activity of synthesized silver nanoparticles using *Barringtonia acutangula* leaf extract was done by MTT assay on various cell lines such as Human cervical cancer cell lines (HeLa) and Human breast adenocarcinoma cell lines (MCF-7).(Mosmann *et al.,* 1983)

4. RESULT AND DISCUSSION

Table -1: Phytochemical Analysis of *Barringtonia acutangular* Leaf Extract

Phytoconstituents	Name of test	Leaf extract				
		PE	EAE	BE	CL	ME
1.Alkaloids	Wagner's test	-	-	+	+	+
2.Cardiac glycosides	Keller Kelliani's test	-	-	-	-	+
3.Flavonoids	Alkaline test	+	+	+	+	+
4.Phenols	$FeCl_2$ test	+	+	-	+	+
5.Aminoacids	Ninhydrin test	+	-	-	+	+
6.Saponins	Foam test	+	-	-	+	+
7.Sterols	Tubermannburchard	-	-	-	-	-
8.Tannins	Braymer's test		+	-	+	+
9.Terpenoids	Salkowi test	-	-	-	+	+
10.Quinones	Hcl test	-	-	-	+	+

PE- Petroleum ether; CL-Chloroform; EAE- Ethyl acetate extract; BE- Benzene extract; ME- Methanolic extract based on the results, the methanolic extract was selected for synthesis of Silver nanoparticles. These phytoconstituents induced the synthesis of silver nanoparticles. By that way, these extract was selected for green synthesis. The Phytoconstituents are responsible for the immediate reduction of silver ions (Prabu *et al*., 2012).

GREEN SYNTHESIS OF SILVER NANOPARTICLES

The formation of greenish brown colour which indicates the formation of silver nanoparticles. After 3 days, the green synthesis of silver nanoparticles was formed in dark atmospheric condition and confirmation again done through UV characterization of silver nanoparticles. Silver nanoparticles exhibit absorption under a range of 380-450nm which depends on size and shape of AgNPs (Khan *et al*., 2013).

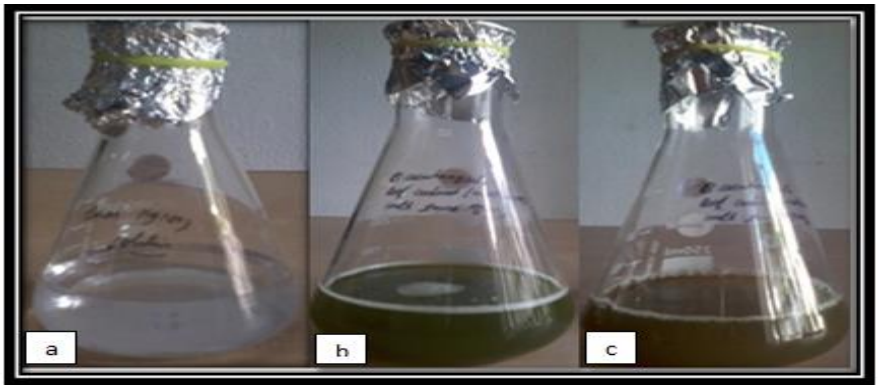


Figure 1.a) 1mM silver nitrate solution b) Plant extract without silver nitrate solution c) Plant extract with silver nitrate solution after incubation

CHARACTERIZATION OF SILVER NANOPARTICLES

UV-Visible Spectrophotometer

The reduction of Ag^+ to Ag nanoparticles synthesized using *Barringtonia acutangula* was characterized by UV-Visible spectrophotometer. Silver nanoparticles appear greenish brown colour in aqueous medium due to the results of vibration in surface Plasmon (Papita*et al.*, 2014). A high peak was obtained from UV-Vis spectrum (figure. 2) as 435 nm which was corresponded to the surface plasmon of Ag nanoparticles, which was similar in seaweed *Ulvalactuca* (Valentin *et al*., 2012)**.**

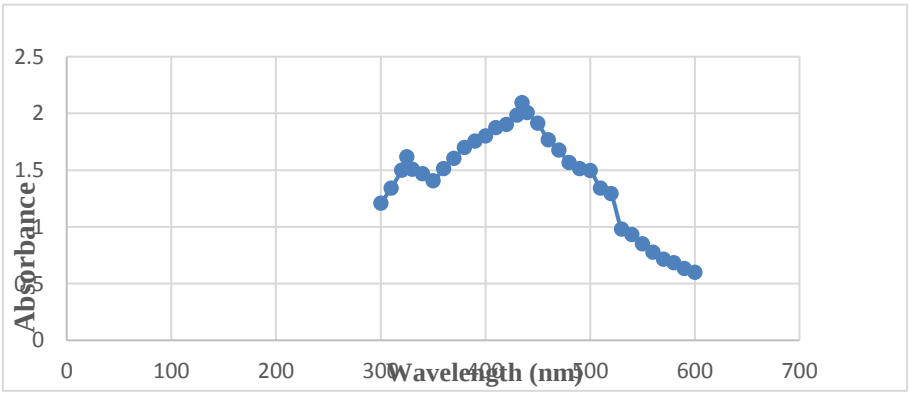


Figure 2. UV-Vis Spectrum of *Barringtonia acutangula* Mediated Ag Nanoparticles

FTIR Characterization

The functional groups of plant secondary metabolites are act as stabilizing agent for Silver nanoparticles formation which was analysed by FTIR spectrum. FTIR is also important characterization for the surface chemistry because the functional groups of plant extract mediated silver nanoparticles can be determined (Monalisha *et al.*, 2014).

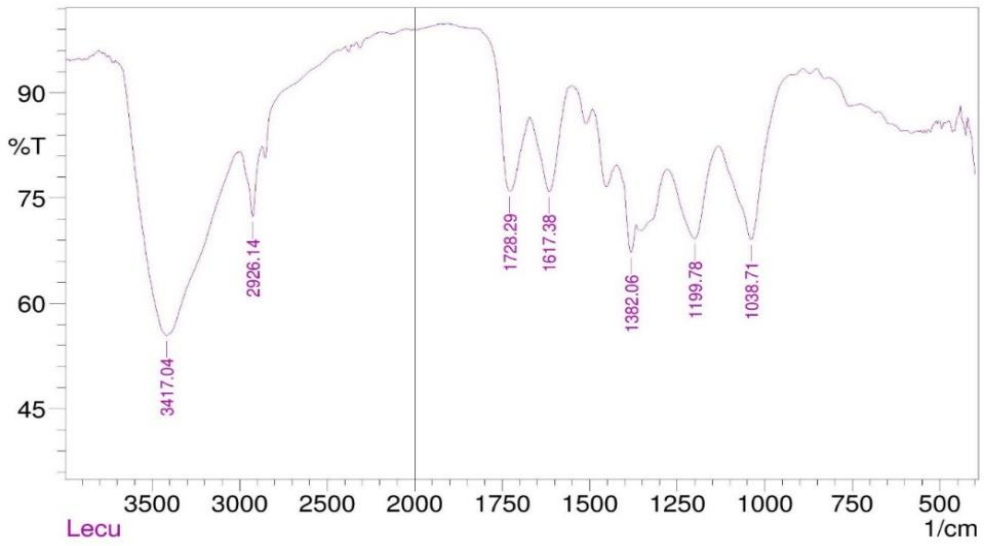


Figure 3. FTIR Spectrum of *Barringtonia acutangula* Mediated silver Nanoparticles

There are 7 peaks was obtained in FTIR spectrum (figure 3) which exhibits the various functional groups. The peaks at 3417.04 (stretching) and 1617.38 (bending) which indicates the N-H (Amine group) while the peaks at 1199.78 shows the C-N (Aliphatic amine group) stretching. The bands at 2926.14 shows the C-H (Alkane group) stretching and the 1382.06 shows the C-H (Alkane group) bending. The peaks around 1728.29 and 1038.71 indicates the C=O (Carbonyl group) Stretching and C-O (Alcohol group) stretching respectively. This results suggests that the function of the biomolecules involved the formation of Silver nanoparticles. From the above results, the Amine groups, Alkane groups, Aldehyde groups and Alcohol groups were act as stabilizing agent for synthesis of Silver nanoparticles. Amine groups are highly exhibited and protein molecules which act as a capping agent for Silver nanoparticles through free amine groups (Abraham *et al*., 2012). The protein molecules are also act as capping agent which was proved by (Valentin *et al.*, 2012) and (Subbaiya *et al.*, 2013).

SEM Analysis

The SEM results provided the morphology and size of the Silver nanoparticles synthesized by *Barringtonia acutangula* leaf extract. There are 3 different magnifications showed in the figure 4.The Silver nanoparticles structure from SEM image were spherical shaped and monodispersed (Annadurai *et al.*, 2014). These size and shapes of silver nanoparticles may be depends on the presence of natural capping agents in *Barringtonia acutangula* leaf extract (Papita *et al.*, 2014). The Size of silver nanoparticles was obtained in SEM image as above 100nm (Scale bar 500nm). The result in the size of silver nanoparticles was greater than the actual size of silver nanoparticles and because they were aggregated with Protein molecules.

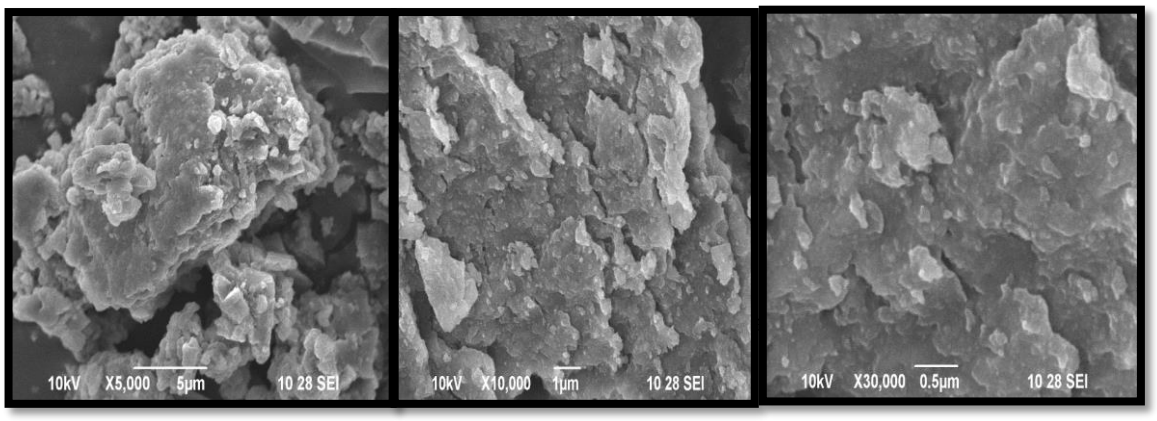


Table- 2: Antibacterial Activity of Silver Nanoparticles and Its Zone Of Inhibition

ANTIBACTERIAL ACTIVITY

The antibacterial activity of Silver nanoparticles was done by agar well diffusion method. From the result of Antibacterial activity, the *Escherichia coli* growth was inhibited such around 2mm distance which was greater than standard (Table 2). But in *Staphylococcus aureus*, the activity silver nanoparticle was decreased when compared to standard drug. The previous study of silver nanoparticles on the same bacterial species also suggested the antimicrobial activity of Silver nanoparticles (Abraham *et al.*, 2012).

Reducing Power Assay

The activity of reducing power of Silver nanoparticles synthesized by *Barringtonia acutangular* leaf extract was shown in figure 5. The presence of antioxidant caused the conversion of Fe^{3+} to ferrous form (Arulpriya *et al.,* 2010). The yellow colour of each sample was turned to greenish blue based on presence of antioxidant compounds. The higher absorbance exhibited the higher activity of reducing power (Prasad *et al*., 2010).

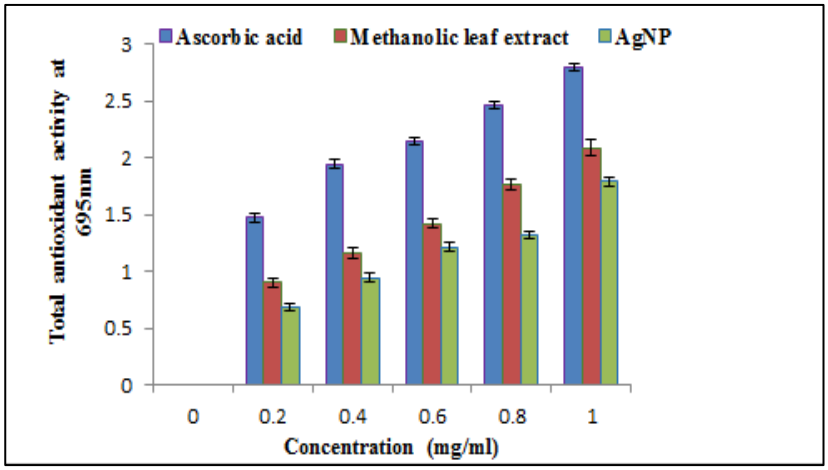


Figure 5. Reducing power of Silver Nanoparticles synthesized by *Barringtonia acutangula* leaf extract

In this case, the reducing power activity of Silver nanoparticles and *Barringtonia acutangula* leaf extract was shown in figure 5. The reducing power activity of Silver nanoparticles shows significant result compared to Standard. Finally, the silver nanoparticles has good reducing power was investigated. Also, the Effective concentration at 50% of Silver nanoparticles was 0.813mg/ml.

Phosphomolybdenum Assay

The total antioxidant capacity of silver nanoparticles synthesized by *Barringtonia acutangula* leaf extract was investigated through phosphomolybdenum assay. The reduction of Mo (VI) to Mo (V) by the silver nanoparticles and plant extract was shown in figure 5.10. The green colour was changed to blue colour (Ravi *et al.,* 2013) by the presence of antioxidant compounds which influenced the formation of Mo (V). The higher absorbance also indicates the higher activity of the samples. From the results of Total antioxidant capacity of silver nanoparticles, the significant results was investigated. Thus, the silver nanoparticles has the good efficient of antioxidant activity. The effective concentration of total antioxidant capacity of silver was achieved as 1.950 mg/ml. Moreover the results also suggested that plant extract itself was responsible for the antioxidant activity as majority and AgNPs was not showed much to the antioxidant activity (Abdel *et al.,* 2014).

Species	**Zone of Inhibition (cm)**		
	Chloramphenicol	**Plant extract (S_1)**	**AgNPs (S_2)**
Escherichia coli	1	1	2
Staphylococcus aureus	2	1.5	1

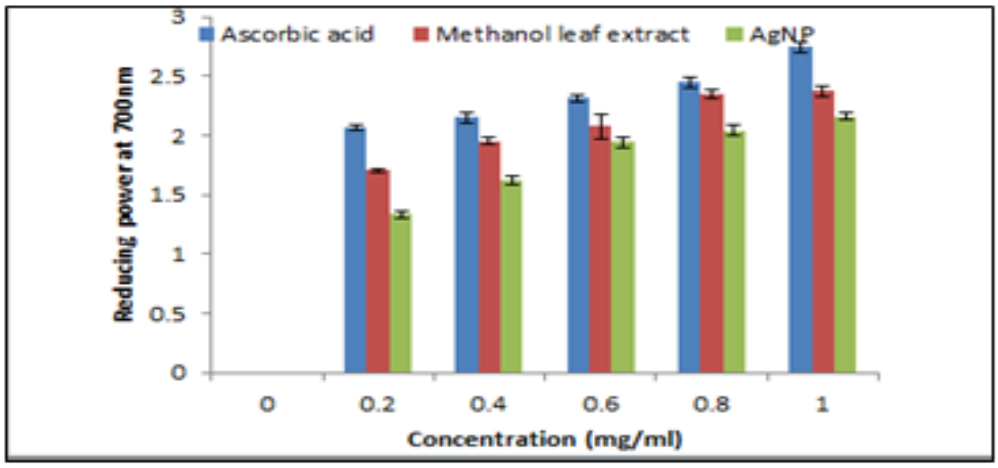


Figure 6. Total antioxidant Activity of Silver Nanoparticles Synthesized by *Barringtonia acutangula* Leaf Extract

Table 3: Effective concentration of Total antioxidant capacity and Reducing power of silver nanoparticles

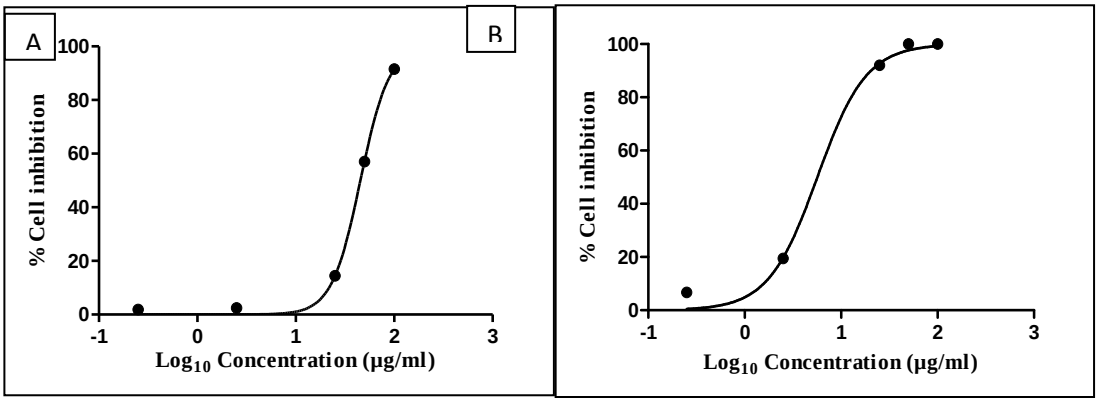
Samples	EC_{50} of Total antioxidant capacity (mg/ml)	EC_{50} of reducing power (mg/ml)
Ascorbic acid	0.839	0.112
Methanol leaf extract	1.592	0.378
AgNP	1.950	0.813

ANTICANCER PROPERTY

The cytotoxic effect of silver nanoparticles was analysed through various cell lines using MTT assay and the IC_{50} values were calculated. In this study, the various concentration (0.25, 2.5, 25, 50, 100µg/ml) of silver nanoparticles were applied on the cell lines. Toxicity of silver nanoparticles based on their concentration-size- shape dependent. Continuously, AgNPs induce the cell growth inhibition and cause cell death (Rabeh *et al.,* 2013).For HeLa (Human cervical cancer) cell lines, the higher cell inhibition was attained at 100(µg/ml) concentration of silver nanoparticles and the cell inhibition was 91.54%. The slope suggested that the increase in drug concentration leads to the higher cell inhibition. The IC_{50} value of cytotoxic property of silver nanoparticles on HeLa cell lines and MCF-7 was achieved as 45.4 µg/ml and 5.6 µg/ml.The cytotoxic effect on MCF-7 (Human breast adenocarcinoma) cell lines showed the significant result when compared to HeLa cell line. Because, the cell population was totally damaged at 50 and 100µg/ml concentration of silver nanoparticles. The lowest IC_{50} value also got in Delavar *et al.,* (2011), in which the nano silver was used against Osteoblast Cancer Cell Line.

Table 4. Cell inhibition in % (HeLa) and MCF -7 and the IC_{50} value of silver nanoparticles

OD at 570nm		% cell inhibition		IC_{50} (µg/ml)	
HeLa	**MCF-7**	**HeLa**	**MCF-7**	**HeLa**	**MCF-7**
0.409	0.369	1.915	6.734	**45.4**	**5.6**
0.407	0.319	2.474	19.444		
0.351	0.031	14.445	92.003		
0.179	0	57.063	100		
0.035	0	91.540	100		
0.417	0.396	-	-		



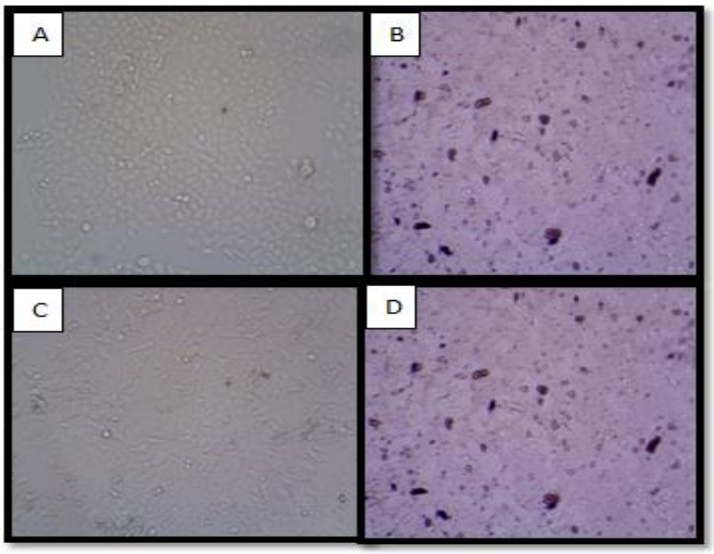


Figure 7. Cytotoxic effect of silver Nanoparticleson cell line
A, B – Control and 100 µg/ml Conc. of HeLa cell line
C, D - Control and 100 µg/ml Conc. of MCF-7cell line

The nanoparticles synthesis using plant have many application in field of medicines, Cancer treatment and Drug delivery. The cytotoxic effect of silver nanoparticles are the result of active physiochemical interaction between silver and functional group present in intra cellular proteins which leads to interact in DNA replication (Ramanathan *et al.*, 2011). The improved cytotoxic effect of silver nanoparticles on cancer cells may be due to the presence of phytoconstituents in plant extract such as flavonoids, tannins etc (Ravikumar *et al.,* 2013). In present study, the IC_{50} value of Silver nanoparticles against HeLa cell line was attained as 45.4µg/ml which was nearest value of *Cynodondactylon* based silver nanoparticles.

5. CONCLUSION

The green synthesis of silver nanoparticles using *Barringtonia acutangula* was demonstrated. The green synthesis of silver nanoparticles is also safe and eco-friendly to the -environment. The toxicity of silver nanoparticles is controllable by reduce or minimally usage of silver nanoparticles. In this present study, the phytochemical analysis was done because it is a better way to select the plant extract for nanoparticles synthesis. Characterization of silver nanoparticles was also done because of the properties of silver nanoparticles well known for the application of Pharmaceutical Biotechnology. The secondary metabolites or the active compounds from plants should able to reduce the ionic compound to single element. These knowledge also very useful to stabilization of silver nanoparticles. Mostly the functional group of the plant active molecules directly involved in the synthesis of silver nanoparticles which was confirmed through FTIR spectrum and the morphological characterization was done by SEM. Naturally the plants has the antioxidant activity but this study suggested that the silver nanoparticles also exhibited antioxidant property. In treatment of cancer, chemotherapy is the best when compared to other therapies. From the present investigation, the anti-cancer property of silver nanoparticles was evaluated and the IC_{50} value was achieved as 5.6 and 45.4µg/ml. Hence, these plant based silver nanoparticles has the ability to control the cancer cells. Final suggestion of this study, the plant based silver nanoparticles is very safe and has more biomedical applications.

6. REFERENCES

1. Abraham J ., Logeswari P., Silambarasan S., Ecofriendly synthesis of silver nanoparticles from commercially available plant powders and their antibacterial properties Scientia Iranica, 2012, 203, 1049-1054.
2. Annadurai G.,Vanaja M., Paulkumar K., Gnanajobitha G., Rajeshkumar S., Malarkodi C., Herbal Plant Synthesis of Antibacterial Silver Nanoparticles by *Solanum trilobatum* and Its Characterization, International Journal of Metals, 2014, Article ID 692461, 1-8
3. Arangasamy Leela and Munusamy Vivekanandan,, Tapping the unexploited plant resources for the synthesis of silver nanoparticles, African.J.Biotechnol. 2008, 7, 3162- 3165.
4. Arul priya P, Lalitha P, Hemalatha S, *Invitro* antioxidant testing of the extracts of *Samanea saman*, Jacq.MerrPelagia Research Library, 2010, 12, 73-79.
5. Arumugam kathirvel and Venugopal sujatha, phytochemical analysis and Antioxidant activity of *Barringtonia acutangula* L gaertn. Leaves, International journal of pharmacy and pharmaceutical sciences, 2012, 4, 277-281.
6. Delavar Shahbazzadeh, Somayyeh Moaddab, Hammed Ahari, Abbas Ali Motallebi, Amir Ali Anvar , Jafar Rahman-Ny, Mohamad Reza Shokrgozar, Toxicity Study of Nanosilver Nanocid on Osteoblast Cancer Cell Line, International nano letters, 2011, 11, 11-16.
7. Florida, M., Aneesh Nair and Sekar, T., Apoptotic induction by leaf extracts of *Barringtonia acutangula* L and *Stereospermum colias* L in colo320 cells, International Journal of Current Research, 2012, 47, 130-133.
8. Maninderjit Kaur, Gurvinder Singh, Chander Mohan, *Barringtonia acutangula*: A Traditional Medicinal Plant, International journal of pharmaceutical sciences Review and Research, 2013, 231, 168-171.
9. Mishra, S., Sahoo, S., Rout, K.K., Nayak, S K., Mishra, S.K., and Panda, P.K., Hepatoprotective effect of *Barringtonia acutangula* Linn.leaves on carbon tetrachloride-induced acute liver damage in rats Indian journal of natural products and resources, 2011, 24, 515-519.
10. Mohamed S. Abdel-Aziz, Mohamed S. Shaheen, Aziza A. El-Nekeety, Mosaad A. Abdel-Wahhab, Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *Chenopodium murale* leaf extract Journal of Saudi Chemical Society, 2014, 18, 356–363.
11. Mohan A., Sasikumar R., Antioxidant, Antimicrobial studies and Investigation of Secondary Metabolites from Stem bark of *Barringtonia acutangula*, International journal of Pharmacognosy and Phytochemical research, 2014, 64, 967-972.
12. Monalisha Rath, Swati S Panda Nabin K Dhal., Synthesis of silver nano particles from plant extract and its application in cancer treatment: a review International Journal of Plant, Animal and Environmental science, 2014, 4 3, 137-145.
13. Mosmann, T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, Journal of Immunological Methods, 1983, 65, 55-63.
14. Khan, M., Khan, M., Adil, S.F., Tahir, M.N., Tremel, W., Alkhathlan, H.Z., Warthan, A.A., Rafiq, M., Siddiqui, H., Green synthesis of silver nanoparticles mediated by *Pulicaria glutinosa* extract, International journal of nanomedicine, 2013, 8, 1507-1516.
15. Nilesh P Babre, Subal Debnath, Manjunath, Y.S., Gajanan Deshmaukh, Hariprasath, K., Kukkala Sharon, Hypolipidemic Effect of Hydro-Alcoholic Extract of *Barringtonia acutangula* Linn Root Extract on Streptozotocin-induced Diabetic Rats Journal of Pharmaceutical Science and Technology 2010, 2, 368-371.
16. Palanivel.V, Sabeel kuttiyil, Senthil kumar, K.L, Evaluation of Antidiabetic activity of *Barringtonia acutangula* L. Gaertn. Leaf extract in Alloxan induced Diabetic rats, International journal of advanced pharmaceutical genuine research, 2013, 12, 1-8.
17. Patel, J.P., Bharat Gami, Kanti Patel and Raksha Solanki, Antibacterial activity of Methanolic and acetone extract of some medicinal plants used in Indian folklore, International Journal of Phytomedicine, 2011, 3, 261-269.
18. Prasad. T, Ankanna S, Elumalai E.K, Savithramma N, Production of biogenic silver nanoparticles using *Boswellia ovalifoliolata* stem bark Digest, Journal of Nanomaterials and Biostructures, 2010, 5, 369-372.
19. Pratap Chandran R., Vysakhi, M.V., Manju, S., Kannan, M., Abdul Kader, S., Sreekumaran Nair A., In vitro Free radical Scavenging activity of Aqueous and Methanolic leaf extracts of *Aegle tamilnadensis* Abdul kader Rutaceae, International Journal of Pharmacy and Pharmaceutical Sciences, 2013, 53, 819-823.
20. Prieto P., Pineda M., and Aguilar M., Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E Analytical Biochemistry, 1999, 337-341.
21. Priya Banerjee, Mantosh Satapathy, Aniruddha Mukhopahayaya Papita Das, Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis Bioresources and Bioprocessing, 2014, 13, 1-10.
22. Rabeh, Abeer K. Alaa M. Shawkey, Mohamed A. Abdulall Ashraf O. Abdellatif, Green nanotechnology: Anticancer Activity of Silver Nanoparticles using *Citrullus colocynthis* aqueous extracts, Advances in Life Science and Technology, 2013, 13, 60-70.
23. Ravikumar, V., Sankara, R., Karthika, A.,Prabua, A., Karthika, S., Subramanian, K.,*Origanum vulgare* mediated biosynthesis of silver nanoparticles for its antibacterial and anticancer activity Colloids and Surfaces B: Biointerfaces, 2013, 108, 80-84.
24. Ramanathan T, Satyavani K, Gurudeeban S, Balasubramanian T, Biomedical potential of silver nanoparticles synthesized from calli cells of *Citrullus colocynthis* L. Schrad, Journal of Nanobiotechnology, 2011, 943, 1-8.

25. Selvakumar K., Madhan R., Srinivasan G., Baskar V., Antioxidant assays in Pharmacological Research, Asian journal of Pharmacological Technology, 2011,14, 99-103.
26. Shanmugam, N., Premanand, G., Kannadasan, N., Sathishkumar, K., Viruthagiri, G., *Nelumbo nucifera* leaf extract mediated synthesis of silver nanoparticles and their antimicrobial properties against some human pathogens Applied Nanoscience, 2015, DOI 10.1007/s, 13204-015-0442-6.
27. Soloman, C.U., Arukwe, U.I., Onuoha, I., Preliminary phytochemical screening of different solvent extracts of stem bark and roots of Dennetia tripetala G. Baker, Asian journal of plant science and research, , 2013, 33, 10-13.
28. Subbaiya, R., Lalitha, A., and Ponmurugan, P., Green synthesis of silver nanoparticles from leaf extract *Azhadirachta indica* and to study its anti-bacterial and antioxidant property, International journal of current microbiology and applied science, 2013, 2, 228-235.
29. Sukumaran, P., Poulose E. K, Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects, International Nano Letters a springer open journal, 2012, 2, 1-10.
30. Vanmathi selvi K., Sivakumar T., Antihelminthic, Anticancer, Antioxidant activity of silver nanoparticles isolated from *F.oxysporum*, International Journal of Current Research in Chemistry and Pharmaceutical Sciences, 2014, 11, 105-111.
31. Valentin Bhimba B., and Saraniya Devi J.,Anticancer Activity of Silver Nanoparticles Synthesized by the Seaweed *Ulva lactuca* Invitro, Open Access Scientific Reports, 2012, 1, 1-5.

