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SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *ANDROGRAPHIS PANICULATA* (LINN.) AND IT'S CYTOTOXICITY AGAINST SHEEP'S BONE MARROW CELLS

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ABSTRACT

Synthesis and characterization of nanoparticles is under exploration due to its wide medical applications and various research interest in nanotechnology. In the current study the plant extract of A*ndrographis paniculata* (Family : Acanthaceae) is used for the synthesis of silver nanoparticles. The plant extract is mixed with AgNO3 and incubated and studied for its synthesis using UV–Vis spectroscopy and it was characterized by FTIR & TEM. The silver nanoparticles synthesized were generally found to be spherical in shape with 1-100 nm. The results showed that the leaf extract is optimum for the synthesis of silver nanoparticles and it is also known to have the ability to inhibit the growth of various pathogenic microorganisms. The cytotoxic ability of silver nanoparticles was tested against sheep bone marrow cells which confirms that the plant mediated synthesis of silver nanoparticles have a significant cytotoxicity against sheep's bone marrow cell lines.

Key Words: Silver Nanoparticle, Cytotoxity, Bone Marrow Cell, ELISA, FTIR, TEM.

INTRODUCTION

The field of Nano science has blossomed over the last twenty years and the need for nanotechnology will only increase as miniaturization becomes more important in areas such as computing, sensors, and biomedical applications. Advances in this field largely depend on the ability to synthesize nanoparticles of various nano materials, based on their sizes, and shapes, as well as their efficiency to assemble them into complex architectures (David D *et al*., 2005). Nanotechnology provides the ability to engineer the properties of materials by controlling their size, and this has driven research toward a multitude of potential uses for Nanomaterial (Saifuddin N *et al*., 2009). Nanoparticle synthesis and study of their size and properties of fundamental importance in the advancement of recent research. It is found that the optical, electronic, magnetic, and catalytic properties of metal nanoparticles also depend on their size, shape and chemical surroundings (Das R *et al*., 2009).

Silver nanoparticles are nanoparticles of silver, i.e. silver particles of between 1 nm and 100 nm in size and have attracted intensive research interest. It is generally recognized that silver nanoparticles may attach to the cell wall, thus disturbing cell-wall permeability and cellular respiration. While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface-to-bulk silver atoms (Prashant S *et al*., 2011). Silver nanoparticles are the most prominent one. The silver nanoparticles may also penetrate inside the cell causing damage by interacting with phosphorus and sulfur containing compounds such as DNA and protein. Generally, silver does not adversely affect viable cells and does not easily provoke microbial resistance. Hence silver containing materials were also

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employed in textile fabrics, as food additives, and in package and plastics to eliminate microorganisms. Because of such a wide range of applications, numerous methods concerning the fabrication of silver nanoparticles, as well as various silver-based compounds containing metallic silver have been developed (Thirunavukkarasu S *et al*., 2011).

Andrographis paniculata is an herbaceous plant of Acanthaceae family which is cultivated all over in India. It is used to treat some infectious diseases, and very often used before antibiotics. Mostly the leaves and roots were used for medicinal purposes. *Andrographis paniculata* is used in traditional Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications (X. Zhang2004). The therapeutic value of *Andrographis paniculata* is due to its mode of action which is perhaps by enzyme induction. The plants extract exhibits anti-typhoid and antifungal activities. The plant is bitter, acrid, cooling, laxative, vulnerary, antipyretic, antiperiodic, anti-inflammatory, expectorant, depurative, soporific, antihelmintic, digestive and useful in hyperdipsia, burning sensation, wounds, ulcers, chronic fever, malarial and intermittent fevers, inflammations, cough, bronchitis, skin diseases, leprosy, colic, flatulence, diarrhea, dysentery, hemorrhoids (Mishra S K *et al*., 2004) etc.

Andrographis paniculata is also reported to possess anti-hepatotoxic, antibiotic, antimalarial, anti-hepatitic, anti-thrombogenic, anti-inflammatory, anti-snake venom, and antipyretic properties to mention a few, besides its general use as an immune stimulant agent (Rahman NNNA *et al*., 1991). Hence the present study has been designed to investigate and characterize the synthesis of silver nanoparticles by using *Andrographis paniculata* and to analyze its cytotoxicity against sheep‘s bone marrow cells.

MATERIALS AND METHODS

Preparation of Plant Extract

The leaves of *Andrographis paniculata* were purchased from the Herbal shop of Chennai, Tamilnadu. The dried plant material was powdered using mortar & pestle to get a uniform size for the extraction of active constituents of the plant.

Assessing the Biosynthesis of Silver nanoparticles

Preparation of Crude Extract

5gram, Leaf powder of *Andrographis paniculata* was taken in a sterile conical flask. 50ml of ethanol was added to it and the mixer was kept in incubator for 48hours at room temperature. After incubation, the solution is subjected to centrifuge at 6000rpm for 10 minutes to obtain the supernatant which was collected from the tube and it was kept for evaporation (to sediment the particles) until it get fully evaporated.

Synthesis of Silver Nanoparticles

After effective evaporation, the settled powder was taken as 25mg, 50mg, 75mg, and 100mg concentration and mixed with 6 ml of Ethanol, for each test tube 44ml of Triple distilled Water is taken in four sterile conical flasks.

To the above mixture 1mM Silver nitrate solution is taken and introduced into the each conical flask and mixed well. The conical flask containing silver nitrate solution is kept in Magnetic stirrer. 6ml of various concentrated sample solution is taken and made to add drop by drop into the silver nitrate solution. This process continues until the colour of the solution changes from green to brown. Then the obtained solution is kept for evaporation at room temperature to obtain as powder. Then the powder is washed twice with distilled water. Then the obtained solution is used for the further processes.

Chemical Preparation of Silver Nanoparticles

To 100 ml, 1mM silver nitrate is taken and it is heated to boiling temperature using hot plate with magnetic stirrer. After attaining boiling temperature 10ml of tri sodium citrate is added drop by drop until it attains pale yellowish colour. Then the solution is kept for evaporation at room temperature to obtain as powder. Then the powder is washed twice with distilled water. Then the obtained solution is used for the further processes.

Characterization of Silver Nanoparticles

Synthesis of silver nanoparticles by reducing the silver ions solutions with *Andrographis paniculata* leaves extract to characterize by using UV-Visible Spectroscopy technique, Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Microscope (TEM) analysis.

Screening of Antibacterial Property in Synthesized Silver Nanoparticles

Antibacterial activity was analyzed with synthesized silver nanoparticles by Well diffusion method against *Escherichia coli,Bacillus* sp, *Salmonella* sp, *Staphylococcus* sp, *Proteus* sp microorganisms. The pathogenic cultures were bringing into broth culture for antibacterial assay. Approximately 5-mm diameter of well was made on Blood Agar Base medium plate with the help of gel puncture. The cultures were swabbed on test media with sterile cotton swab. 20µl of synthesized particles were inoculated to the well, and then the plates were incubated in incubator for 37 ◦C for 24 h, the zones of inhibition was discussed.

Cytotoxicity Assay

The Sheep’s Bone marrow cells was collected from Slaughter house and maintained in the Laboratory. The bone marrow was flushed with Dulbecco’s Modified Eagles Medium (DMEM). The flushed out medium with cells, was centrifuged at 1,500rpm for 7-10min. After

centrifugation the settled pellet was taken and again centrifuged for clarification at 1,500rpm for 10-15min. Then Supernatant was discarded. The pellet was re-suspended in medium and then cultured in culture flask.

The cultured cells were taken in Trypsinization. Again the cells were re-suspended in the DMEM medium. With use of sterile micropipette the 100µl of medium along with cell suspension was added in each of the cells of sterile micro titter plate (96 wells). The inoculated plates were kept for incubation about 24H at 37ºC in presence of 5% CO_2.

After incubation, the sample (i.e Silver nanoparticles and the plant extract) were added to the wells at 100µl respectively. serial dilution were made to successive wells in a pattern. Each well contains random number of cells. After addition, the micro tittre plate containing sample was allowed to incubate for 48H. Then the sample of each well was loaded with 50µl of MTT dye and it is allowed to incubate for 1H. After incubation the wells were read at 630nm using ELISA reader.

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

The Ethanolic extract of *Andrographis paniculata* leaves were used to produce silver nanoparticles and the reduction of silver ions into silver particles during exposure to the plant extract is followed by colour change. It is well known that silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasma vibrations in silver nanoparticles. As the ethanolic extract of *Andrographis paniculata* leaf was mixed in the aqueous solution of silver ion complex, it started to change the colour from green to yellowish brown due to reduction of silver ion which may be the indication of formation of silver nanoparticles (fig 1).

UV-Visible Spectroscopy Analysis of Silver Nanoparticles

Formation of silver nanoparticles from 1mM solution of silver nitrate and reduction of silver ions using ethanol extract of *Andrographis paniculata* was confirmed using UV-Visible spectral analysis. Surface Plasmon Resonance of Spectra for ethanol extract of *Andrographis paniculata* are obtained at 357nm and absorbance value is 0.289 (Fig.2). The Surface Plasmon Resonance of Spectra for synthesized silver nanoparticles was obtained at 362nm and absorbance value is 0.295 (Fig.3). The absorption peak at shorter wavelengths is due to the presence of several organic compounds which are known to interact with silver ions. The concentration of the extract also plays a major role as it is responsible for the synthesis of symmetrical nanoparticles. As metal nanoparticles can be synthesized by reducing metal ions using some chemical molecules, in green synthesis, it is believed that there is a generation of metal nanoparticles.

Transmission Electron Microscope analysis

Synthesis of silver nanoparticles using ethanol extract of *Andrographis paniculata* was characterized by using Transmission Electron Microscope (Philips TECHNAI 10). Samples were prepared using dropping a very small amount of sample on the carbon coated copper grid and micrograph of prepared silver nanoparticles (Fig.4) aggregated nature of silver nanoparticles (Fig.5). This clearly shows that silver nanoparticles are minimum 11nm to maximum 22nm in size and seems to be spherical in shape and the scale corresponds to 200nm.

Fourier Transform Infrared Spectroscopy Analysis

FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and capping of the bio-reduced silver nanoparticles by plant extract. The surface chemistry of the prepared biogenic silver nanoparticles under this condition was studied using Fourier Transform Infrared Spectroscopy (FTIR) and the IR bands of plant leaf ethanol extract appears at 3402, 2930, 1750, 1643, 1449, 1347, 1205, 1080, 1036, 892, 635 cm^{-1} and the peak centered at 2930 cm^{-1} and 1750 cm^{-1} (Fig.6). The FTIR spectrum of the leaf extract synthesized by silver nanoparticles with the absorption bands at 3402, 2919, 2849, 1747, 1644, 1515, 1449, 1376, 1348, 1161, 1081, 1036, 924, 892, 855, 829, 716, 701, 574 cm^{-1} and the peak centered at 3402 cm^{-1} and 2930 cm^{-1} (Fig.7).

Antibacterial Activity

Antibacterial activity of various concentrations of the synthesized silver nanoparticles and plant extract was treated against various pathogenic bacteria like *Escherichia coli*, *Bacillus* sp, *Salmonella* sp, *Staphylococcus* sp, *Proteus* sp and the zone of inhibition of inhibition was tabulated (Table1 & 2).

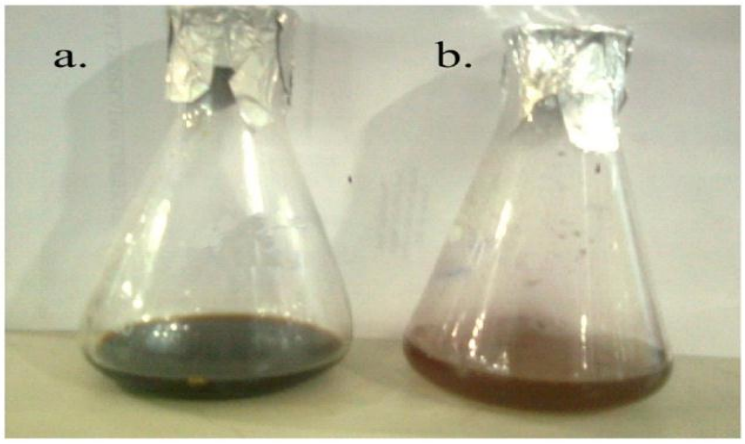
Table 1. Zone of inhibition of nanoparticles against various bacterial strain

S.No	Organisms	Inhibition Zone (mm)						
		Silver nanoparticles (1mg)	Silver nanoparticles (5mg)	Silver nanoparticles (10mg)	Plant extract (1mg)	Plant extract (5mg)	Plant extract (10mg)	Chemical preparation
1	Escherichia coli	14	12	13	12	14	15	9
2	Bacillus	32	15	16	19	30	22	12
3	Salmonella	13	12	14	10	13	14	13
4	Staphylococcus	17	15	16	14	16	17	13
5	Proteus	15	15	16	10	15	16	10

Table 2. Cytotoxicity of silver nanoparticles

S.No	Samples	Readings
1	Silver nanoparticles (1mg)	1.44±0.177
2	Silver nanoparticles (5mg)	1.77±0.109
3	Silver nanoparticles (10mg)	1.16±0.098
4	Ethanol extract of *Andrographis paniculata*	1.193±0.163

Fig 1. Synthesis of silver nanoparticles



a.Green colour shows Ethanol extract of *Andrographis paniculata*
b.Brown colour shows synthesised Silver nanoparticles

UV- Visible Spectroscopy of Ethanol extract of *Andographis paniculata* and Silver Nanoparticle

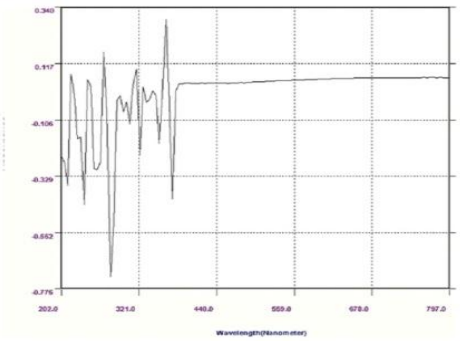


Fig.2 UV- Vis spectra of ethanol extract of *Andrographis paniculata*

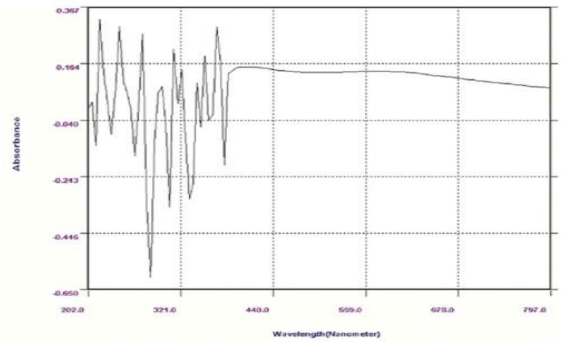


Fig.3 UV-Vis spectra of synthesised silver Nanoparticles

TEM Micrograph of Silver nanoparticles at 200nM scale

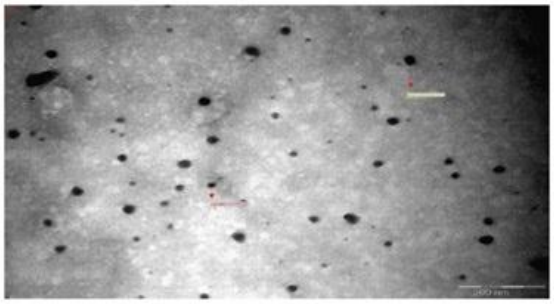


Fig.4. Transmission Electron Micrograph of silver nanoparticle from *Andrographis paniculata*

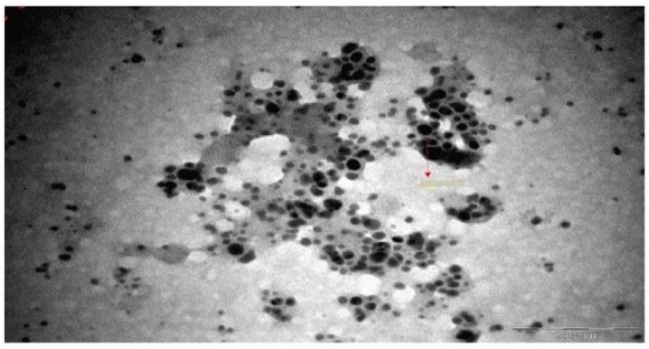


Fig.5. Transmission Electron Micrograph of silver nanoparticle from *Andrographis paniculata* with Aggregated nature

Fig.6. FTIR Spectra of Ethanol extract of ***Andrographis paniculata***

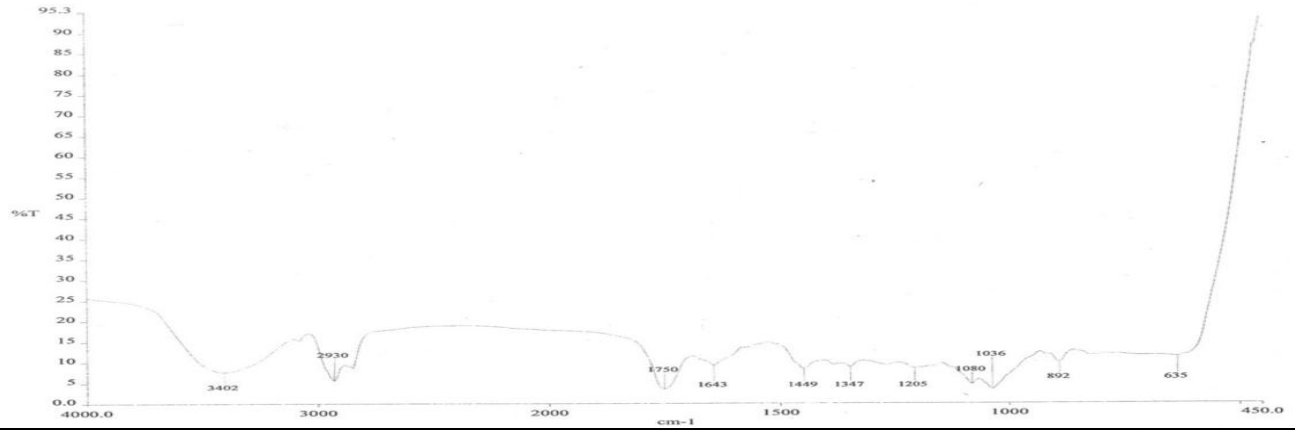
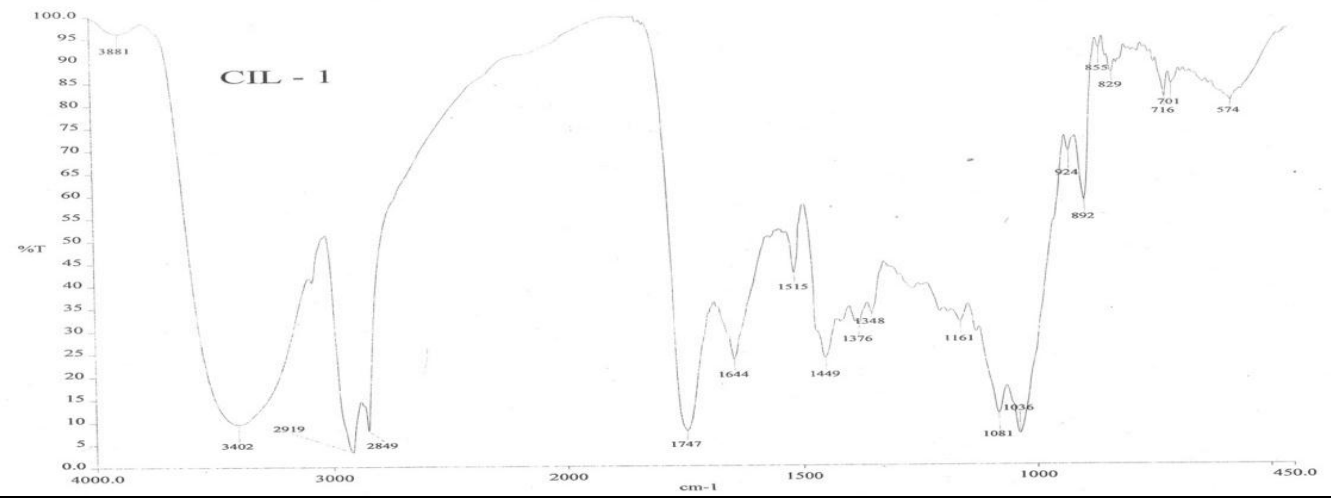


Fig.7. FTIR Spectra of Synthesized Silver Nanoparticles



DISCUSSION

As the ethanol extract of plant leafs was mixed in the aqueous solution of silver nitrate solution, it started to change the colour from green to brown due to reduction of silver ions which indicates the formation of silver nanoparticles (Sathyavani K *et al.,* 2011; Geethalakshmi R *et al.,* 2010; Singh A *et al*., 2010).

The Formation of silver nanoparticles by reduction of $AgNO_3$ (silver nitrate) by ethanol extract of *Andrographis paniculata* were characterized and the weak surface plasmon resonance of leaf extract centered at 357nm and peak of silver nanoparticles appears in 362nm. The similar results were obtained from previously studied in various medicinal plants (Savithramma N *et al*., 2011). Whereas, some other plant extracts which are already studied that shows resulted peak at 420nm that are due to the synthesis of silver nanoparticles by *Citrullus colocynthis* (Sathyavani K *et al*., 2011) and from *Phyllanthus amarus* (Annamalai A *et al*., 2011) and from *Allium* cepa (Antariksh S *et al*., 2010).About 11nm to 22nm spherical shaped silver nanoparticles were synthesized and analyzed by transmission electron microscope. Silver nanoparticles aggregates with extracts were also detected. Mostly dispersed particles are spherical in shape. Most of the isolated particles in the sample prepared in ethanol are spherical. It is resembles to study conducted at silver nanoparticles from fungus *Trichoderma harzianum* (Prashanth S *et al*., 2011) and polychrome silver nanoparticles from ethanol solution (Rong H *et al*., 2002).

Whereas other silver sources such as silver nitrate and silver sulfadiazine releases Ag^+ only. It is believed that silver nanoparticles after penetration into the bacteria have inactivated their enzymes, generating hydrogen peroxide and caused bacterial cell death. The current study used various pathogenic bacteria like *Escherichia coli, Bacillus, Salmonella, Staphylococcus, Proteus*. Among them various concentrations of silver nanoparticles shows good inhibition against *Bacillus, Staphylococcus* (Sadeghi B *et al*., 2010; Shirley A *et al*., 2010). It clearly describes the silver nanoparticles synthesized from ethanol extract of *Andrographis paniculata* have antibacterial activity against several pathogenic bacteria. Reports on inhibitory action of silver ions on microorganisms show that upon silver ion

treatment, DNA loses its replication ability and expression of ribosomal subunit proteins as well as some other cellular proteins and enzymes which are essential for ATP production becomes inactivated (Nithya R *et al.,* 2009).

The cytotoxicity of silver nanoparticles against sheep bone marrow cells *in-vivo* tested at various concentrations. Toxicity effects of silver nanoparticles reduced by various plants and chemical reductions are investigated against various cells. After further investigation this is the first study about toxicity of silver nanoparticles against sheep's bone marrow cell lines. Silver nanoparticles synthesized by using ethanol extract of *Andrographis paniculata* shows significant toxicity against those cells. The results confirms higher concentration of silver nanoparticles (10mg) have ability to reduce the cell number. Finally decided that silver nanoparticles synthesized from ethanol extract of *Andrographis paniculata* also having toxicity against sheep bone marrow cell lines. In earlier cytotoxicity of silver nanoparticles from Geraniol against Fibrosarcoma-wehi 164 were investigated and results shows only 2.6µg/ml of silver nanoparticles was necessary to decrease cell proliferation by 50% and Toxicity of silver nanoparticles from *Suaeda monoica* against Hep-2 cell line which shows Silver nanoparticles at 500nM decreased the viability of Hep-2 cells to 50% (Mona Safaepour *et al*., 2009).

CONCLUSION

The current study investigates the synthezing ability of silver nanoparticles by using leaf extract of *Andrographis paniculata*. The reduction of the metal ions through leaf extract leading to the formation of silver nanoparticles is fairly well-defined dimensions. Further characterization of synthesized silver nanoparticles is done using UV-Vis spectroscopy, FTIR spectroscopy and TEM analysis. Formation of silver nanoparticles from plant extract was confirmed by result of UV-Visible spectroscopy. The surface plasmon resonance of both plant extract and silver nanoparticles obtained at 360nm, 350nm respectively. Transmission Electron Micrograph of Silver nanoparticles finds the shape and size of the nanoparticles. Result of TEM micrograph of silver nanoparticles at 200nm shows that synthesized silver nanoparticles present in size between 11-22nm and spherical in shape. From FTIR analysis it is confirm that the biomolecules are responsible for reduction of silver ions and capping agents in the plant extract. It is also confirms that synthesized silver nanoparticles added with various chemical groups like alcohols, acetones, ketones, etc. Further antimicrobial activity of plant extract and synthesized silver nanoparticles were investigated in well diffusion method. From the results it is clear to know that the silver nanoparticles from *Andrographis paniculata* extract also have the ability to inhibit the growth of various pathogenic microorganisms. Finally, the cytotoxic ability of silver nanoparticles is tested against sheep bone marrow cells which confirms the plant mediated synthesis of silver nanoparticles having significant cytotoxic against those cells.

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