"ASSESSMENT OF ANTIBACTERIAL ACTIVITIES OF SILVER NANOPARTICLES SYNTHESIZED FROM THE LEAVES OF *MUNTINGIA CALABURA*"

Sruthy Mohan * and Dr.M. Thangavel

Research scholar, Dpt. Of Microbiology, Sree Narayana Guru College, Coimbatore.

Professor and Head, Dpt. Of Microbiology, Sree Narayana Guru College, Coimbatore.

ABSTRACT

The emergence of nanotechnology has provided a vast research area in recent years by intersecting with various other branches of science and forming impact on all forms of life. Silver nanopartilces synthesized from the leaves of *Muntingia calabura* , it was evident that they shows significant antibacterial activity against *Pseudomonas* sp. In this study silver nanoparticles were synthesized by using aqueus extract of *Muntingia calabura* leaves. The colour change of the solution indicate the presence of silver nanoparticles. The antimicrobial activities of silver nanoparticles have been investigated by a number of researchers day by day.

INTRODUCTION

Nanotechnology is a field of science which deals with production, manipulation and use of materials ranging in nanometers. The emergence of nanotechnology has provided a vast research area in recent years by intersecting with various other branches of science and forming impact on all forms of life. In nanotechnology nanoparticles research is an important aspect due to its innumerable applications. Nanoparticles have expressed significant advances owing to wide range of applications in the field of bio-medical, sensors, antimicrobials, catalysts, electronics, agricultural, bio-labeling and in other areas. As opposition with the conventional methods to produce nanoparticles, the term herbal nanotechnology has become of more interest as it makes use of nanoparticles which are made from herbal extracts and are less hazardous when interacted with human as it uses less toxic chemicals.

Nanotechnology is a fast expanding area of science. This area of research is anticipated to lead to the development of novel, multifunctional applications which can recognize cancer cells, deliver drug to target tissue, aid in reporting outcome of therapy. One of the important role is monitor intracellular changes to help prevent precancerous cells from becoming malignant. The safety of Nano medicine is not fully defined. The use of nanotechnology in medicine needs adequate evaluation of its risk and safety factors. Anyway it is

possible that Nano medicine in future would play an important role in treatment of human disease and also in enhancement of normal human physiology. Nano biotechnology have a great impact on the economy and society in the early 21st century, comparable to that of cellular biology, molecular biology and information technology.it is sure that nanotechnology will be the next medicinal and industrial revolution.(Ventra *et al.*, 2004).

Nanoparticles are partilces between 1 and 100 nanometers (nm) in size with a surrounding interfacial layer. In nanotechnology a particle is defined as a small object that behaves as a whole unit with respect to its properties and transport. Particles are further classified according to diameter. Researchers are developing nanoparticles designed to pass through the brain barrier and target tumors of a type of brain cancer called glioblastoma delivering to chemotherapy drugs to the tumor. The polymer coated iron oxide nanoparticles are highly effective for the treatment of chronic bacterial infections . The surface change of protein filled nanoparticles has been shown to affect the ability of the nanoparticle to stimulate immune responses. Researchers are thinking that these nanoparticles may be used in inhalable vaccines. Cerium oxide nanoparticles act as an antioxidant to remove oxygen free radicals that are present in a patient blood stream following a traumatic injury. Researchers developing different ways to use carbon nanoparticles called nano diamonds in medical applications. Magnetic nanoparticles widely used in medicine for magnetic separation techniques as contrast agents in magnetic resonance imaging (Stechell *et al.,* 1985; Olsvik *et al*.,1994; safarikova *et al*., 2001; Deponte *et al.,* 2004;).Iron oxide nanoparticles are widely used in drug targeting systems. These particles can be attracted by an external magnetic field (Muller *et al*., 2001).

Green synthesis of nanoparticles is a promising area, because of its applications in the field of physics, chemistry , biology and medicine. The synthesized nanomaterials are used in medicinal and technological aspects. There are several methods used for the synthesis of silver nanoparticles. It includes both physiochemical and biological methods. Examples , solution reduction methods (Goia *et al*., 1998), photo chemical reaction (Taleb *et al.,* 1997), thermal decomposition of silver compounds (Esumi *et al.,* 1990), sonochemical methods (Zhu *et al.,* 2000), radiation methods (Henglein *et al* ., 2001) , microwave assisted methods (Pastoriza – Santos *et al*., 2002) and biological methods or green synthesis include production of nanoparticle using bacteria (Saifuddin *et al*., 2009), fungi (Bhainsa *et al*., 2006), and using enzymes (Willner *et al*.,2007). Recently via green chemistry methods (Begum *et al*., 2009 ; Song J.Y.*et al.*,2009). The use of environmentally benign materials like plant leaf extract (Parashar *et al.,* 2009; Abboudy *et al*., 2013) for the synthesis of silver nanoparticles offers number of benefits in both pharmaceuticals and biomedical fields.

Biological synthesis also called green synthesis of nanoparticles where biological enzymes from plant extracts, fungi, bacteria are used. This method is eco-friendly and reduces toxicity and waste production. Silver Nano particles synthesized by green chemistry methods offer a novel and potential alternative to

chemically synthesized nano particles. Evaporation- condensation and laser ablation are the two important physical methods used for the production of silver nanaoparticle. The absence of solvent contamination in the prepared thin films and the uniformity of nano particle are the main advantages of physical synthesis methods. Chemical reduction method is one of the most important method used for the chemical synthesis of silver nano particles. Disodium citrate, sodium borohydride, ethanol, ethyl alcohol..etc are the reductants used in chemical synthesis. The reduction of silver nitrate in aqueous solution by reducing agent , ethanol in the presence of a stabilizer sodium citrate is done. The particle delivered can be dried and suspended out for use.

The presence of some toxic chemicals adsorbed on the surface of nanoparticles that are synthesized by chemical methods may have several adverse effect on the biomedical fields. The green synthesis is a suitable method compared to physio chemical methods. Green synthesis is effective, eco- friendly, helps for the large scale production of nanoparticles. There is no need of high pressure, temperature, energy and toxic impurities. The inhibitory effect of silver nanoparticles on microbes like bacteria and fungi was detected in the past , and is widely used in industrial and medical processes (Jose *et al*., 2005 ; Lok C *et al*., 2007). The most important application of silver nanoparticles is in medical industries and is widely used in topical ointments to prevent infection against burns and open wounds (Ip , M, Lui *et al.*, 2006).

The present study focus on the synthesis of silver nanoparticle by an aqueous leaf extract of *Muntingia calabura* . These silver nanopartilces were found to be extremely effective against different bacterial and fungal pathogens. The common name for *Muntingia calabura* is calabur tree , panama berry, ornamental cherry, jam fruit tree, Singapore cherry, west indian cherry. It belongs to the family muntingiaceae. It is a shrub or tree up to 12 meter tall with spreading branches (Lim *et al* .,2012)., the leaves of the *Muntingia calabura* are alternate oblong or lanceolate, 4-15cm long and 1-6 cm wide , with toothed margin and covered in short hairs The flowers are small with 3 cm wide , solitary or inflorescence of 2 or 3 flowers with five sepals hairy, five white petals , many stamens with yellow anthers and a smooth ovoid ovary. (Lim *et al*., 2012; Smith Jr *et al*., 1965; Nelson , 1910) .

The fruit of the *Muntingia calabura* is an edible berry , is red at maturity with 1.5 cm wide (Lim *et al.*, 2012; Smith *et al.*, 1965). *M.calabura* is native to Southern Mexico , the Caribbean, Central America and Westernsouth America and Argentina (Lim *et al* ., 2012; Boning, Charles 2006). The *M. calabura* specieses colonize disturbed habitats in tropical low land areas. (Vazquez – Yanes *et al.*, 1999). And the *M. calabura* thrives in poor soil, able to tolerate acidic and alkaline conditions and drought but it doesn't grow on saline condition (Lim, Dr.T.K.2012). The seeds of the M. calabura are dispersed by birds and fruits bats. *M. calabura* is planted as a source of timber and fuel. The fruits of *M.calabura* used for the preparation of jams and the leaves can be used for making tea. *M.calabura* is also a traditional medicinal plant, and it leaves widely used for the treatment of head aches, prostate problems and gastric ulcers. The bark of this plant is

used as antiseptic, and the flowers are widely used as antiseptic, anti spasamodic and fruits are used as anti diarrheic and respiratory problems (Mahmood *et al.*, 2014).

MATERIALS AND METHODS

Collection of leaf samples from *M. calabura*

Leaves that appeared healthy were collected from different branches of *Mutingia calabura* from Sree Narayana Guru College, Coimbatore, Tamil nadu. The plant leaves were collected and brought to the laboratory in a sterile container.

Preparation of plant extract

The leaves was taken and subjected to aqueous extract preparation, 25g of green tender leaves were thoroughly washed with tap water followed by double distilled water twice. The leaves are cut in to small pieces and were boiled in 100ml of distilled water. After 15 minutes the aqueous extract was filtered through What man No.1 filter paper. The aqueous extract of the plant used as reducing agent for the synthesis of silver nano particles.

Preparation of silver nitrate solution

A concentration of 1M $AgNO_3$ solution was prepared by dissolving 0.169 $AgNO_3$ in 1000ml of double distilled water and used for the green synthesis of silver nano particles (AgNPs).

Green synthesis of AgNPs

The filtered aqueous extract of *M.calabura* leaves was added individually to 90 ml of 1M $AgNO_3$ in a 250 ml Erlenmeyer flask. Then kept in room temperature for 48hrs at dark . The process was continued till the change of colour occurred from yellow to dark brown indicating the completion of silver nanoparticle synthesis.

UV- visible spectra analysis

The reduction of pure silver ions was observed by UV – visible spectroscopy. The absorption maxima were measured by using UV spectrophotometer between 300-800nm wavelength.

Characterization of silver nanoparticle using electron microscope (SEM)

SEM analysis was carried out to determine the particle morphology. The biologically synthesized silver nanoparticle sample was centrifuged. The pellet was collected and dried. The fine sample was used for SEM analysis. The characterization of silver nanoparticle by SEM was done at the Department of Nano sciences & Technology, Bharathiar University, Coimbatore.

FTIR analysis

The Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out to know the different functional groups that act as bioreductors into reduce Ag^+ ions to Ag_0. The leaf extract and nanoparticle sample were subjected to FTIR in which the samples were irradiated by a broad spectrum of infra-red light and the level of absorbance at a particular frequency was plotted after fourier transformation of the data. Compounds contained in the extracts were identified according to standard infrared chart.

Phytochemical analysis (Qualitative approach)

The phytochemical analysis was performed to identify the presence or absence of different secondary metabolites like alkaloids, tannins, flavonoids, sterols.. etc.

A: Test for alkaloids

Mayer's test: To 2 ml of plant extract 3 ml of ammonia was added and allowed to stand for few minutes. 3ml of chloroform was added to it and allowed to stand in a water bath till chloroform evaporates. Add Mayer's reagent, cream colour indicate the presence of alkaloids.

B: Test for steroids and triterpenoids

To 2 ml Plant extract few drops of acetic anhydride added. Boiled and then cooled. Concentrated H_2SO_4 was added from the sides of the test tube and then observed for the formation of brown ring at the junction of two layers.

C: Test for phlobatannin

To 1 ml of plant extract few drops of concentrated HCl was added. It allowed to stand in a water bath for few minutes. Red colour precipitate indicate the presence of phlobatannins.

D :Test for reducing sugar

To 2 ml of plant extract was taken in a test tube and 1 ml ethanol and Fehlings solution A and B was added and boiled for two minutes. Red colouration indicate the positive result.

E : Test for flavonoids

To 5 ml of ammonia and few drops of concentrated sulphuric acid was added to 2 ml of plant sample. Yellow colour indicate the presence of flavonoids.

F : Test for saponins

To 1 ml extract was treated with 1% lead acetate solution. Formation of white precipitates indicates the presence of saponins.

Antibacterial activity

Antibacterial activity of sample was determined by well diffusion method on Muller Hinton Agar (MHA) medium.The Muller Hinton Agar medium was weighed as 3.8 gms and dissolved in 100 ml of distilled water and add 1 gm of agar. Then the medium is kept for sterilization. After sterilization the media was poured in to sterile petriplates and were allowed to solidify. After the medium was solidified, the inoculums were swabbed on the MHA plates with sterile swab moistened with the bacterial suspension. Wells were made by using cork borer. Different concentration of samples (20µl, 40µl and 60µl) and positive control (streptomycin 1mg/ml-10µl) were loaded in respective wells. These plates were incubated for 24 hours at 37^0C . Then the microbial growth was determined by measuring the diameter of zone of inhibition.

RESULT

Phytochemical analysis

Phytochemicals are naturally found in plants, they are biologically active and function to protect plants against invasion, disease and infection. Phytochemicals produced by plants through primary and secondary metabolism, and play an important role in plant growth. In the present study is based on the synthesis of silver nanoparticles using aqueous extract of *Muntingia calabura* leaves.

The plant extract showed the positive result for phytochemical analysis. From the qualitative phytochemical test it was revealed that the *Muntingia calabura* leaf extract shows the maximum presence of phytochemicals (Table 5,Fig : 2 & 3).

Biological Synthesis Of Silver Nanoparticles

Silver nano particles were syntheised by using aqueous extract of *Muntingia calabura* leaves. The plant extract were pale yellow in colour before the addition of silver nitrate solution .The synthesis of silver nano particles exhibited as the colour change from yellow to brown. The samples were observed periodically for the change of colour from pale yellow to different shades of brown (Fig : 4,5 and 6).

- **Characterisation of Green Synthesised Silver Nanoparticle**

UV spectrum analysis of biologically synthesised nanoparticle

The absorption spectrum showed maximum peak at 430nm for the nanoparticle synthesized using aqueous extract of *Muntingia calabura* leaves .

Fourier Transform Infrared Spectroscopy

The FTIR analysis of M. calabura leaf extract shows peaks at 2862.36 indicates the presence of C-H stretch. The peak at 2098.55 shows the presence of C=C stretch (preferably belonging to a benzene ring). The peak at 1990.54 shows the presence of C-N groups while the peak at 1080 indicate the presence of C-F stretch.

The FTIR analysis of nanoparticle sample (aqueous leaf extract of *M.calabura*) shows peak at 2769.78 indicate the presence of OH group (indicating the presence of alcohol). The peak at 2152 shows the presence of C-N group. The peak at 1072.42 indicate the presence of C-F group (alkyl halides).

SEM analysis

SEM images shows similar appearance for the presence of silver nanoparticles synthesized from *M.calabura*. The SEM images shows cluster of nanoparticles in different size. The SEM analysis confirmed the presence of nanoparticles (fig: 10).

Antibacterial activity

The inhibitory effect of silver nanoparticles synthesized from the aqueous leaf extract of *M.calabura* were tested by using well diffusion method on MHA (Muller Hinton Agar) plates against 3 human pathogens such as *Staphylococcus* sp., *Escherichia coli*., and *Pseudomonas* sp. and the results obtained are given in Table 6.

On the basis of antimicrobial activities of silver nano particle synthesized from the leafs of *Muntingia calabura*, it was evident that they shows significant antibacterial activity against Pseudomonas sp., but it does not showed any activity against *Staphylococcus* sp. and *Escherichia coli* (fig:11).

Table 1: Phytochemical Evaluation Of *Muntingia calabura* Leaf Extract

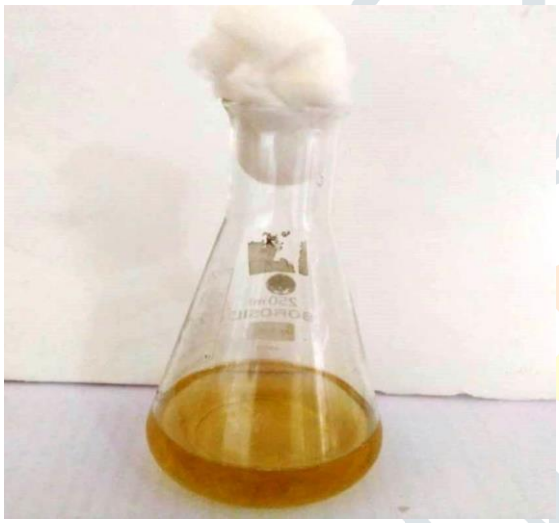
Serial No.	Phytochemicals	Aqueous extract
1	Alkaloids	++
2	Reducing sugar	++
3	Terpenoids	+
4	Steroids	+
5	Flavanoids	++
6	Saponins	-

Presence (+); High level (++) ; Absence(-);

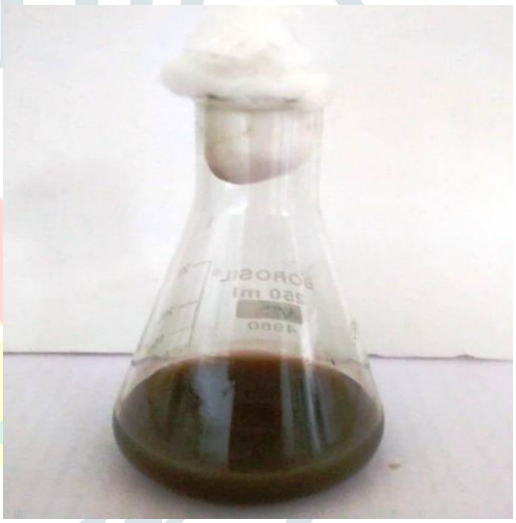
Table 2:Zone Of Inhibition Produced By Silver Nanoparticles From The Leaves OF *Muntingia calabura* Against Bacterial Pathogens

Sl No.	Bacterial pathogens	Zone of inhibition in mm			
		20µl	40µl	60µl	Streptomycin (10µl)
1	*Staphylococcus* sp.	-	-	-	15
2	*Escherichia coli*	-	-	-	16
3	*Pseudomonas* sp..	12	17	15	16

***Muntingia calabura* plant**

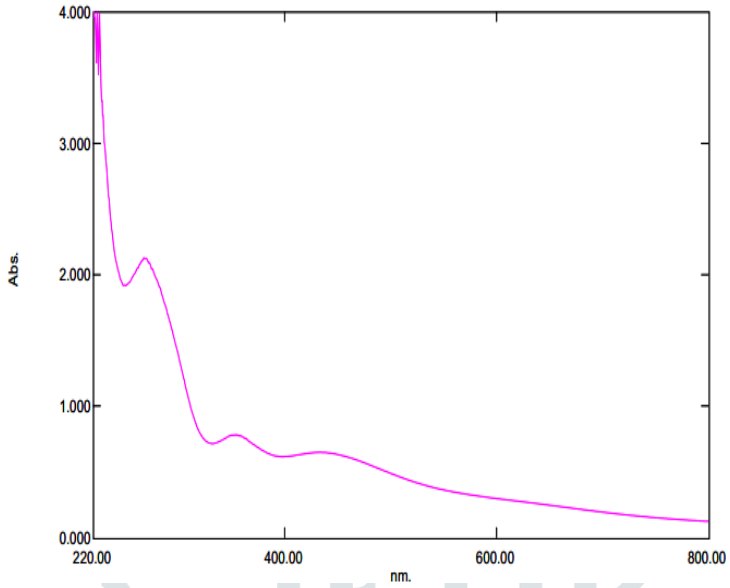


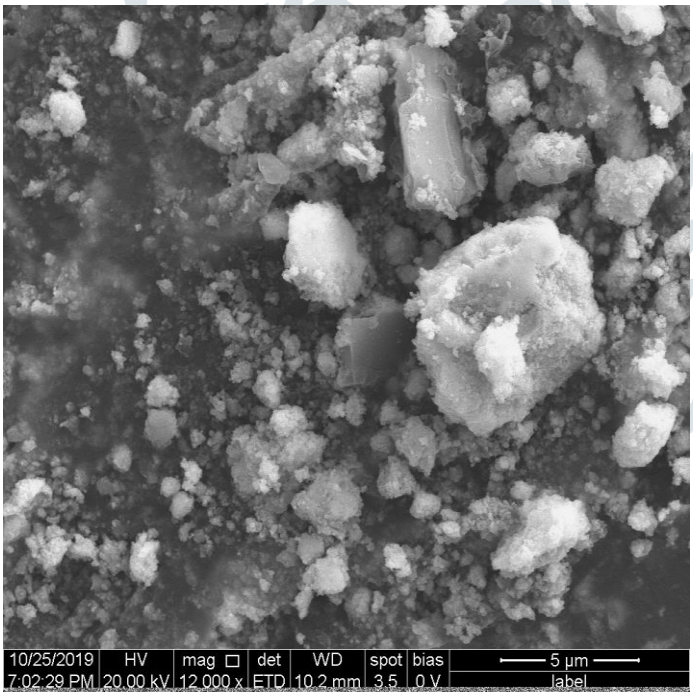
Aqueous leaf extract with $AgNO_3$ solution



After the formation of AgNPs

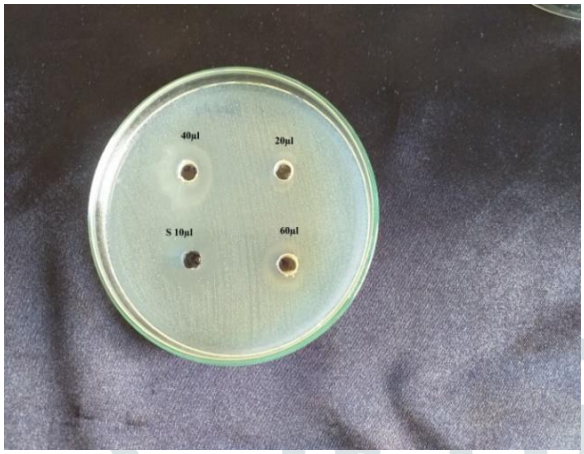
UV-Visible spectrum of silver nanoparticles using the aqueous leaf extract of *Muntingia calabura*.





SEM analysis of silver nanoparticles using the aqueous leaf extract of *Muntingia calabura*

FIG :11 ANTIBACTERIAL ACTIVITY OF SILVER NANO PARTICLES FROM THE LEAVES OF *Muntingia calabura* AGAINST BACTERIAL PATHOGENS



***Pseudomonas* sp.**

***Staphylococcus* sp.**

Escherichia coli

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