SYNTHESIS OF NANOPARTICLES FROM CLERODENDRUM PHLOMIDIS L. AND ITS ANTIMICROBIAL ACTIVITY

Dhulgande Govind Santoba[1], Ghogare Datta Subhashrao[2]
[1]Department of Botany, Sir Parashurambhau College, Pune, (M.S.) India.
[2]Department of Botany, Matsyodari Shikshan Sanstha's, Arts, Science and Commerce College, Ambad Dist. Jalna (M.S.) India.

Abstract:
In recent years, green synthesis of silver nanoparticles (AgNPs) has gained much interest from chemists and researchers. In this concern, Indian flora has yet to divulge innumerable sources of cost-effective non-hazardous reducing and stabilizing compounds utilized in preparing AgNPs. This study investigates an efficient and sustainable route of AgNP preparation from 0.1 M aqueous AgNO3 using leaf extracts of *Clerodendrum phlomidis* L. well adorned for its wide availability and medicinal property. Plants are not only beautiful but majestic because they are rich sources of various medicinally important substances. They explore the huge diversity which can be utilized towards rapid and single step protocol preparatory method for various nanoparticles keeping intact 'the green principles' over the conventional ones and proving their dominance for medicinal importance. Nanobiotechnology is presently one of the most dynamic disciplines of research in contemporary material science whereby plants and different plant products are finding an imperative use in the synthesis of nanoparticles (NPs).The beauty of the synthesis is: no involvement of any surfactant, catalyst or template. The aqueous silver ions are reduced to silver nanoparticles when exposed to leaves extract. Among all the nanoparticles synthesized; silver nanoparticles have attained special place in the area of nanotechnology because of their antimicrobial and biomedical applications. In totality, the AgNPs prepared are safe to be discharged in the environment and possibly utilized in processes of pollution remediation. Also antimicrobial activity by Agar well diffusion method was tested against strains of Gram negative as well as Gram positive bacteraia. The approach was cost effective, very efficient, eco-friendly and easy alternative to conventional methods.

Keywords: Leaf extract, Silver nanoparticles, Green synthesis, *Clerodendrum*, antimicrobial activity.

Introduction:

Indian greeneries are the chief and cheap source of medicinal plants and plant products. From centuries till date, these medicinal plants have been extensively utilized in Ayurveda. Recently, many such plants have been gaining importance due to their unique constituents and their versatile applicability in various developing fields of research and development. Plants are not only beautiful but majestic because they are rich sources of various medicinally important substances. They explore the huge diversity which can be utilized towards rapid and single step protocol preparatory method for various nanoparticles

keeping intact 'the green principles' over the conventional ones and proving their dominance for medicinal importance. Nanobiotechnology is presently one of the most dynamic disciplines of research in contemporary material science whereby plants and different plant products are finding an imperative use in the synthesis of nanoparticles (NPs). The beauty of the synthesis is: no involvement of any surfactant, catalyst or template. The aqueous silver ions are reduced to silver nanoparticles when exposed to leaves extract. The bio-reduction and stabilization of so formed silver nanoparticles was monitored by UV-Vis spectrophotometry. Therefore; a simple, cost effective bio-reduction on the principle of 'green synthesis' of silver nanoparticles using the *clerodendrum phlomidis* L. plant extract is reported. The name *Clerodendrum* is from the Greek *kleros*, chance, and *dendron*, a tree, that the plants possessed medicinal properties, which were a possible cure for certain ailments. Commonly planted in the gardens for making hedges, the roots are aromatic and astringent and are used in gonorrhea. By considering this medicinal property the leaf extract of *C. phlomidis* L. was used for antimicrobial activity against the few strains of Gram positive and Gram negative bacteria.

Materials and Methods: Experimental:

Silver nitrate was purchased from Merck Chemicals. All glassware are sterilized with nitric acid and further with distilled water and dried in oven before use. *Clerodendrum phlomidis*
L. leaves were collected from Pune region in the month of January.

Preparation of leaf extract:

The fresh leaves were washed several times with running tap water and after that with distilled water. Around 10 g of leaves were weighed and boiled for 1h in 100 mL double distilled water at 60°C and then the extracts were filtered through whatman filter paper. Then the filtered extract was stored in refrigerator at 4°C for further use in synthesis of silver nanoparticles.

Green synthesis of silver nanoparticles:

100 mL (10^{-3}M) aqueous solution of silver nitrate was prepared in Erlenmeyer flask. Then 1.0, 2.0, 3.0, and 4.0 ml of leaf extract were added separately to 10mL aqueous silver
nitrate solution kept in separate beakers at room temperature (their notation shown in Table 1). The solution was kept in dark chamber until solution color changes to yellow to dark yellow. After, 15 min, the solution turns yellow to yellow-red or dark brown indicating the formation of silver nanoparticles. The bioreduction of silver ions was monitored by periodic sampling by the UV spectrophotometer.

Characterization of Silver nanoparticles: UV-Vis Spectrophotometer analysis:

The UV- Vis spectrum was recorded for the plant leaf extract of *C. phlomidis* L.

Antimicrobial activity:

Antimicrobial activity of *C. phlomidis* L. assited with silver nanoparticles were carried out by well diffusion method against strains of Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram negative bacteria (*Pseudomonas aeruginosa* and *E. coli*). The bacterial cultures were feshly prepared. After 24 hours of incubation at room temperature the zone of inhibition was recorded. Experiments were repeated for three times.

Results and Discussion:

Table: 1. Notation of silver nanoparticles synthesized using *Clerodendrum phlomidis* L. leaf extract.

Sr. No.	Sample	Plant extract (ml)	AgNo3 Solution (ml)	Absorbance
1	A	10	--	1.2
2	A1	1	10	1.9
3	A2	2	10	2.7
4	A3	3	10	4.1
5	A4	4	10	5.6

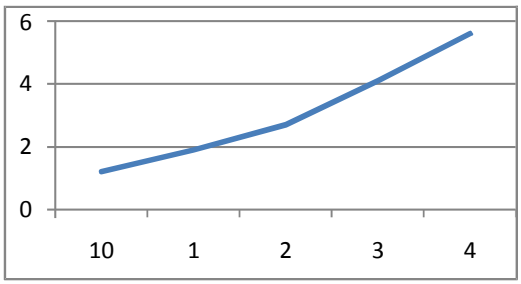


Figure: 1. UV-Vis absorption spectrum of Plant extrac

Visual characterization:

The bioreduction of silver nanoparticles using plant leaf extract was observed on the basis of the change in color. The solution was heated upto 60 ^{0}C temperature for 20 minutes in water bath and the change in color yellow to dark brown was visualised. The color change is due to reduction of silver metal ions into silver nanoparticles. The intensity of the color change increased in direct proportion to the increase in concentration of plant leaf extracts. Similar results were recorded by Muthukumar, et. al.,; 2014 in *Euphorbia tirucalli*. This was further confirmed by UV-Vis spectrophotometer analysis.

UV-Vis spectrophotometer analysis:

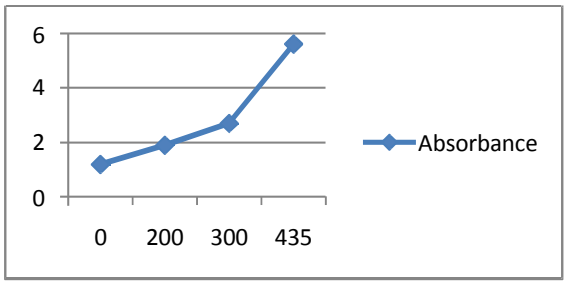
It is used to confirm the formation of silver nanoparticles. The bioreduction of silver ions was monitored by periodic sampling of the reaction mixture that UV-Vis spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous solutions. A color change from pale yellow to dark brown. It may be due to the excitation of surface plasma resonance (SPR) for the synthesized nanoparticles. The periodic scans of the optical absorbance between 200 nm to 600 nm with a Shimadzu UV-Visible spectrophotometer were used to investigate the reduction of silver ions by leaf extract. The reaction mixture exhibits the strong peak at 435 nm for sample A4 (Fig. 1 and 2). Silver nanoparticles exhibit unique and tunable optical properties on account of their surface plasmon resonance; dependent on shape and dispersion of the

Figure: 2. UV-Vis absorption spectra against

nanoparticles. The variation in the values of absorbance confirms to the changes in the particle size. Similar results were recorded by Shakeel Ahmed and Saika Ikram; 2015 using *Terminalia arjuna* leaf extract. The increase of the intensity of the spectra or the increase of the absorbance measured at different time intervals indicates the increase of the silver nanoparticles formed as a consequences present in the *Euphorbia confinalis* stem extract (Netai, et. al.,; 2017).

Antimicrobial activities:

The biologically synthesized silver nanoparticles showed excellent antimicrobial activity against both types of bacteria viz. Gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, Gram negative bacteria *Pseudomonas aeruginosa* and *E. coli* (Fig.3).



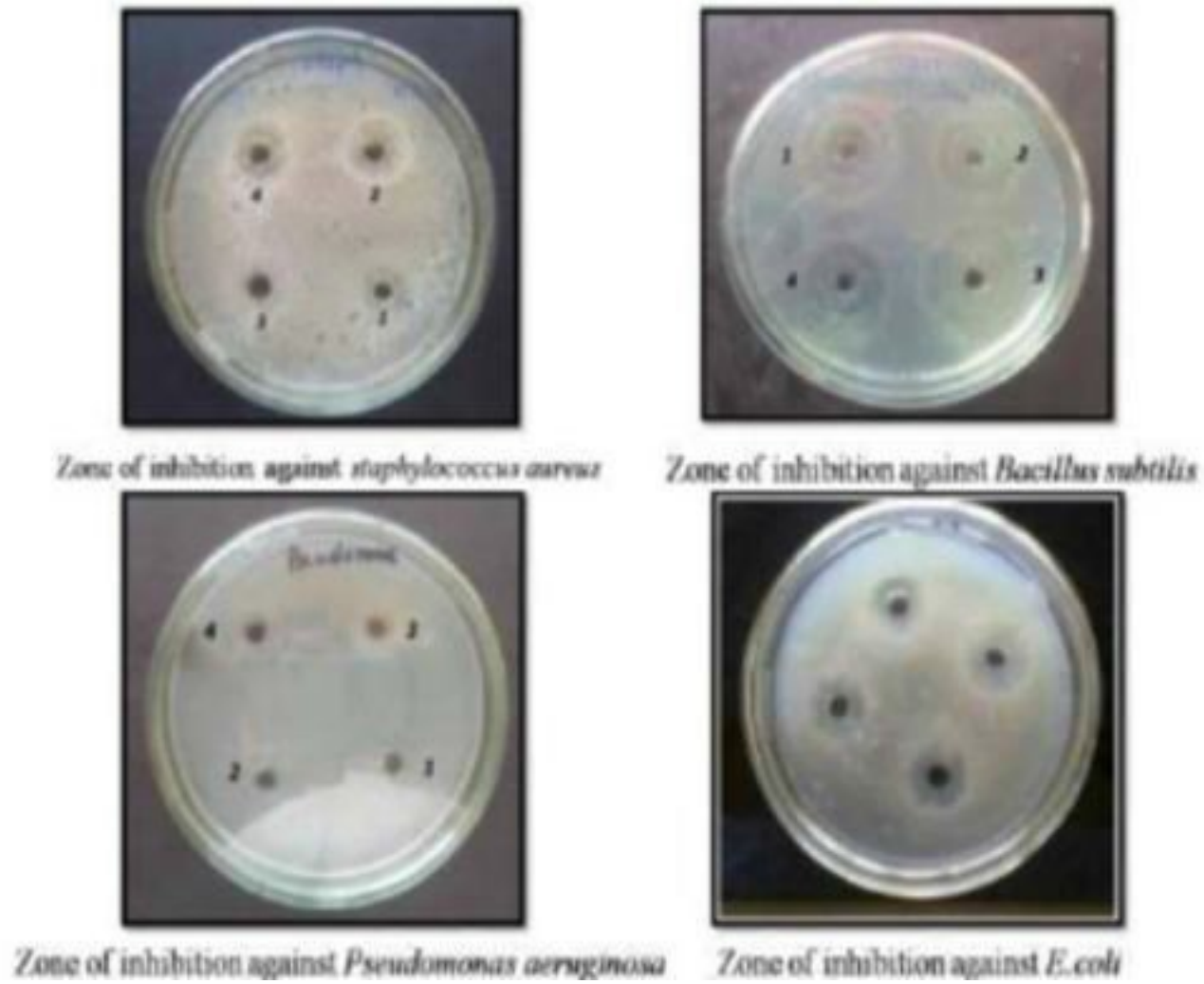


Figure: 3. Zone of inhibition against strains of bacteria.

The zone of inhibition of synthesized silver nanoparticles was found more effective than that of plant extract. In present investigation, Gram positive bacteria showed maximum zone of inhibition than Gram negative bacteria. Siddhartha, et. al., ; 2007 recorded that the Gram negative bacterium E. coli showed maximum zone of inhibition which may due to the cell wall of Gram positive bacteria composed of a thick peptidoglycan layer, which is consisting of linear polysaccharide chains of cross linked by short peptides thus forming more rigid structure leading to difficult penetration of the silver nanoparticle compared to the Gram negative bacteria where the cell wall possesses thinner peptidoglycan layer. Also similar results were given by several workers like Russell AD and Hugo WB,; 1994, Mubarak Alia L. et. al.; 2011 and Suriya J. et al.,; 2012.

Conclusion:

Green synthesis of silver nanoparticles is an easy approach compared to other synthesis of silver nanoparticles because they do not produce toxic wastes in their synthesis. The synthesis of nanoparticles using plant extract is safe and eco-friendly. The present study reveals that the biomolecules are responsible for the bioreduction of silver ions. Also the silver nanoparticles are capable for control the growth of bacteria. It proved to be active against both types of bacteria. The synthesis of nanoparticles from plant extract will be proving of can be used in various

medicinal formulations.

References:

Mubarak Aliya L., Thajuddina N., Jeganathanb K., Gunasekaran M.: Colloids and Surfaces B: Biointerfaces; 85: 360-365; 2011.

Muthukumar R., Chidambaram R. and Ramesh V.: Biosynthesis of Silver nanoparticles from *E. tirucalli* and to check its Antimicrobial activity. Research Journal of Pharmaceutical Biological and Chemical Sciences 5: 589; 2014.

Netai Mukaratirwa-Muchanyereyi, Tinotena Muchenje, Stephen Nyoni, Munyaradzi Shumba, Mathew Mupa, Luke Gwatidzo and Ateek Rahman: Green Synthesis of Silver nanoparticles using *Euphorbia confinalis* stem extract, characterization and evaluation of antimicrobial activity. Journal of Nanomater Mol Nanotechnology, 1-6:3: 2017.

Russell AD and Hugo WB: J Nanosci Nanotechnol; 31: 351-370: 1994.

Shakeel Ahmed and Saiqa Ikram: Silver Nanoparticles: One pot green synthesis using *Terminalia arjuna* extract for biological Application. : J Nanomed Nanotechnol, 6:4; 1-6; 2015.

Siddhartha S, Tanmay B, Arnab R, Gajendra S, Ramchandrarao P. and Debabrata Da.: J Nanosci Nanotechnol; 18: 103-225; 2007.

Suriya J, Bharathi Raja S, Sekar V and Rajasekaran R.: African J Biotechnol; 11: 12192-12198; 2012.