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GREEN SYNTHESIS OF SILVER NANOPARTICLES GREEN SYNTHESIS AND ANALYSIS OF SILVER NANOPARTICLES USING LEUCAS ASPERA

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Abstract- The green synthesis of silver nanoparticles using plant leaf extract (*Leucas aspera)* for the reduction of aqueous silver ions. Stable silver nanoparticles were formed by treating aqueous solution of silver nitrate ($AgNO_3$) with the plant leaf extract. The formation of dark brown color confirmed the synthesis of silver nanoparticles. UV- visible spectroscopy was used to monitor the quantitative formation of silver nanoparticles. The size and shape of the nanoparticles was characterized by using SEM (Scanning Electron Microscopy). This environmental friendly method provides simple, easy and cost effective faster synthesis of nanoparticles than chemical methods. Further, the antimicrobial activity of synthesized silver nanoparticles showed effective inhibitory activity against pathogens and non pathogenic bacterium strains viz. *E. coli, Bacillus cereus, L. monocytogenes, M. luteus,S. aureus, Yersenia.* The cytotoxic effect of synthesized silver nanoparticles is studied by using Brine Shrimp Lethality Test (BSLT).

Keywords- Green Synthesis, Silver Nanoparticles, Plant Leaf Extract, SEM, Antimicrobial Activity, Brine Shrimp Lethality Test

I. INTRODUCTION

1.1. Nanoparticles

Nanotechnology is a field of applied science focused on the design, synthesis, characterization and application of materials and devices on the Nano scale, which has vast range of applications, such as in medicine, electronics, biomaterials and energy production. Nanoparticles present a higher surface area to volume ratio with decrease in the size of the particles. Nanoparticles of noble metals, such as silver, gold and platinum are widely applied in products that directly come in contact with the human body, such as shampoos, soaps, detergent, shoes, cosmetic products, and toothpaste, besides medical and pharmaceutical applications.

1.1.1. Silver Nanoparticles:

Silver nanoparticles (Ag-NPs) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts. Ag-NPs have distinctive physico-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and non linear optical behavior. These properties make them of potential value in inks, microelectronics, and medical imaging. Besides, Ag-NPs exhibit broad spectrum bactericidal and fungicidal activity.

1.1.2..Biosynthesis of Silver Nanoparticles:

The chemical method of synthesizing silver nanoparticles is extremely expensive and also involves the use of toxic, hazardous chemicals, which pose potential environmental and biological risks. The method for biosynthesis process is used of plants for the fabrication of nanoparticles is a rapid, low cost, ecofriendly. Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. The microbial enzymes or the plant phytochemicals with anti-oxidant or reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles. The plant mediated nanoparticles synthesis is preferred as it is cost

effective, environmental friendly and safe for human therapeutic use. Silver nanoparticles are attractive especially for antimicrobial activity.

1.2. Leucas aspera:

Leucas aspera commonly known as 'Thumbai' is distributed throughout India from Himalayas to Ceylon and in the plains of Mauritius, java and Philippines. The plant is used traditionally as an antipyretic and insecticide. Medicinally, it has been proven to possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive and cytotoxic activity and as an antidote to snake venom. *Leucas aspera* is typically found in dry, open, sandy soil and is abundant in areas with waste. *Leucas aspera* is an annual plant that can reach heights of 15–60 cm. The leaves of the *Leucas aspera* are linear, obtuse and are petiolate. They can reach up to lengths of 8.0 cm and 1.25 cm broad. The epidermis of leaves contains thick waxy cuticle and is traversed with stomata useful for transpiration.

Potential Benefits of *Leucas aspera*:

- *Leucas aspera* is available abundantly and it is cheap
- It has more medicinal values
- It can be used to produce silver nanoparticles with less pollution

1.3. BRINE SHRIMP LETHALITY TEST:

Brine Shrimp Lethality Test was first introduced by Meyer in 1982. This test was designated as a bench- top assay to rapidly screen the plant extracts for their cytotoxic effect. It is used to determine the value i.e. minimum amount of drug required to kill 50% of the survivability. The applications of the assay are considered useful in preliminary assessment of toxicity and have been used for the detection of fungal toxins in plant extract toxicity, animal and fish feeds**.** The brine shrimp lethality assay represents a rapid, inexpensive and simple, reliable bioassay for assessing the bioactivity of medicinal plants towards brine shrimp and in doing so, predicts the cytotoxic and anti-tumor properties of plant materials.

II. OBJECTIVES

- To use the Leucas aspera plant to synthesize silver nanoparticles
- To compare the yield in different temperatures 80^0C, 90^0C and 95^0C
- To characterize the silver nanoparticles from UV-Vis spectrophotometer and SEM
- To study the cytotoxic effect using BSLT
- To find the anti- microbial activity using different pathogenic organisms

III.MATERIALS AND METHODS

3.1. Materials:

Chemicals used in this process are 1mM $AgNO_3$. The plant *Leucas aspera* was harvested from agricultural university, Bangalore.

3.2. Preparation of aqueous plant extract and synthesis of silver nanoparticles:

Weigh 1g of the leaf powder of *Leucas aspera* and dissolve it in 10ml of distilled water (10%). Keep the sample for incubation in boiling water bath at 60^0C for 20 minutes. Filter the sample with filter paper, collect the filtrate.

Using filtrate, sample is tested against silver nitrate solution (1mM $AgNO_3$). Take 3 storage vials and prepare 2 blanks and 1 sample. In 1st vial add 10ml of 1mM $AgNO_3$ which acts as a blank**.** In 2nd vial add 1ml filtrate and 9ml distilled water (9:1 ratio). It acts as blank and it is used to compare the color change with sample after incubation. In 3rd vial 1ml filtrate and 9ml $AgNO_3$ solution is added**.** Keep the vials for incubation in boiling water bath for 20mins at 60^0C, if the color change is not observed then repeat the incubation for sample vial at 80^0C for 20mins**.** After incubation the silver nanoparticles are synthesized in the sample vial.

3.3. Separation of silver nanoparticles:

The sample obtained after incubation is centrifuged to concentrate the nanoparticles. Transfer the sample liquid to centrifuge vials and centrifuge for 15mins at 7000rpm. After centrifugation discard the supernatant and collect the pellets in distilled water. Repeat the procedure for 90^0 and 95^0C. The synthesized nanoparticles are analyzed using UV-Vis spectrophotometer. The size and shape were analyzed by Scanning Electron Microscopy (SEM).

3.4. Procedure of Brine Shrimp Lethality Test:

Solution of 1.5% sodium chloride (NaCl) with pH adjusted to 8.4 using sodium bicarbonate NaHCO3 was prepared in a beaker (it acts as a buffer). About 500mg of cyst were incubated in this solution in 250ml flask with constant aeration. The hatching of cyst was observed between 24-48hours. The larvae which represent the naupilli (48-72hours) were utilized for the assay. The synthesized silver nanoparticles (AgNP) were used to conduct the assay. 5ml brine solution is added in the test tubes, to that10 naupilli were placed inside the test tube containing salt solution. The number of surviving or dead shrimps was recorded after 1, 2, 3, 4, 5, 6, 12, and 24hours for the effectiveness of silver nanoparticles. Probit analysis was carried out to determine values. The value positive controls of silver nanoparticles were calculated

3.5. Antimicrobial Activity Test:

Antibacterial activities were tested by the disc- diffusion method. Six standard bacteria; *Bacillus cereus, Escherichia coli, Listeria monocytogenes, Micrococcus luteus, Staphylococcus aureus and Yersinia*. Filter paper discs (what man No.1; 5mm diameter) were impregnated with crude plant extract, silver nitrate (1mM/disc), silver nanoparticles (50mg/ml/disc) and distilled water as blank. The discs were overlaid on nutrient agar plates and incubated at 37^0C, 24hours. Inhibition zones were calculated as the difference between disc diameter (5mm) and the diameter of inhibition.

IV. RESULTS AND DISCUSSIONS

4.1. Synthesis of silver nanoparticles:

The synthesis of silver nanoparticles was confirmed by color change from yellowish brown to dark brown. The synthesized silver nanoparticles are separated by using centrifuge.

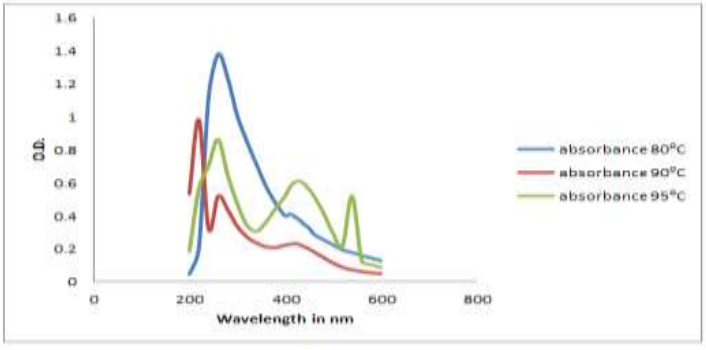
4.2. UV-VIS spectrometer analysis:

The silver nanoparticles were primarily characterized by UV-Vis spectrophotometer which proved to be very useful technique for the analysis of nanoparticles. The silver nanoparticles obtained at 80°C, 90^0C and 95^0C are characterized by this technique. The absorption peaks are 410, 420 and 430nm respectively.

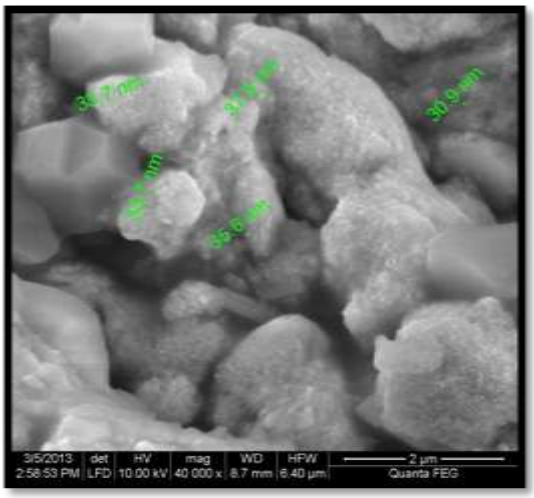
Readings of UV-Vis spectrophotometer at 80^0C, 90^0C and 95^0C is as follows:

Wavelength(mm)	Absorbance	Absorbance	Absorbance
	80^0C	90^0C	95^0C
200	0.043	0.531	0.183
220	0.204	0.983	0.562
240	1.160	0.319	0.702
260	1.380	0.519	0.863
280	1.236	0.436	0.646
300	1.008	0.335	0.474
320	0.851	0.270	0.341
340	0.712	0.233	0.304
360	0.578	0.210	0.360
380	0.476	0.206	0.442
400	0.398	0.220	0.519
410	0.409	0.225	0.573
420	0.394	0.230	0.603
430	0.373	0.222	0.608
440	0.346	0.211	0.592
450	0.324	0.197	0.559
460	0.288	0.180	0.519
480	0.252	0.145	0.417
500	0.223	0.111	0.292
520	0.191	0.086	0.215
540	0.175	0.070	0.159
560	0.157	0.059	0.124
580	0.142	0.052	0.102
600	0.126	0.047	0.085

The Graph of UV-Vis spectrophotometer is given below:



4.3. SEM analysis:



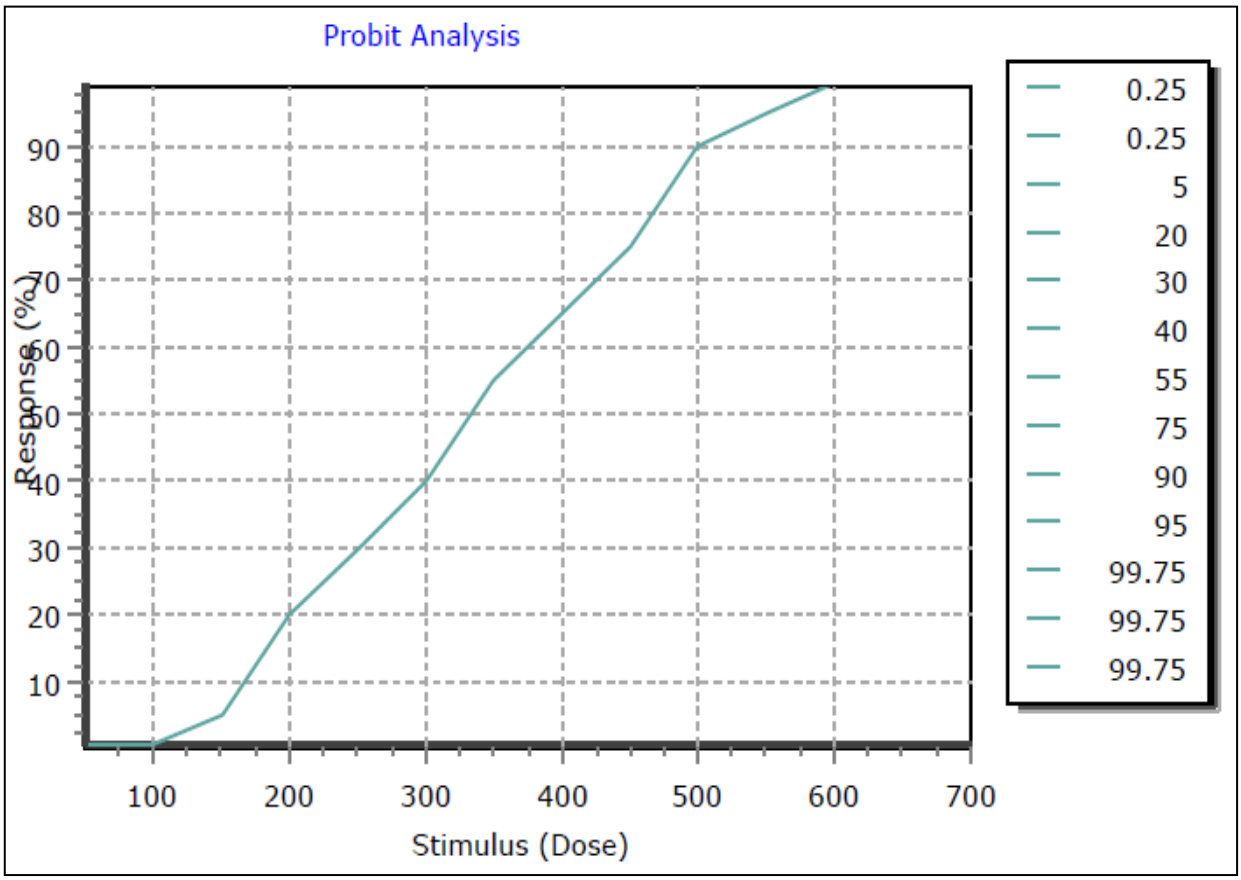
SEM is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons. The size and shape of silver nanoparticles were analyzed by SEM microscope. We obtained silver nanoparticles of uniform size. The average size of the obtained silver nanoparticles is 34.3nm.

4.4. Brine Shrimp Lethality Test:

The BSLT test was conducted on synthesized silver nanoparticles to test the cytotoxicity effect. The value is obtained by Probit analysis.

Concentration(µg/ml)	Effect
0	0
50	0
100	0
150	5
200	20
250	30
300	40
350	55
450	75
500	90
550	95
600	100
650	100
700	100

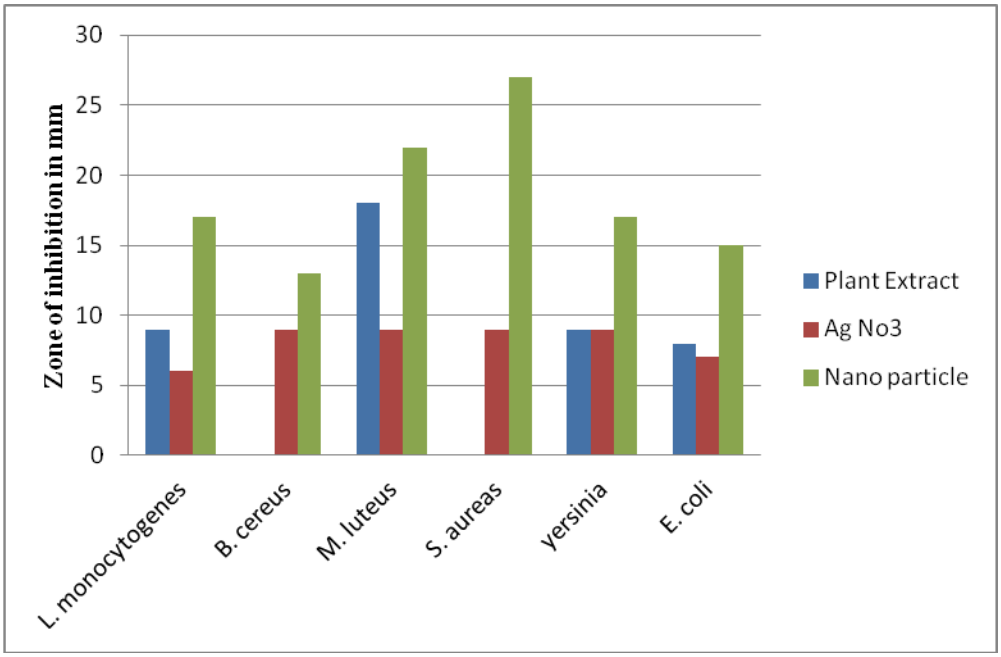
LD_{50} 336.8738



4.5. Antimicrobial Activity:

Bacterial species	**Plant Extract**	**AgNO3**	**Silver Nanoparticle**
Listeria monocytogenes	9	6	17
Bacillus cereus	0	9	13
Micrococcus luteus	18	9	22
Staphylococcus aureus	0	9	27
Yersinia	9	9	17
Escherichia coli	8	8	15

Chart showing zone of inhibitory effect of silver nanoparticles on different pathogens:



Antimicrobial activity showing zone of inhibition

Escherichia coli

Listeria monocytogenes

Micrococcus luteus

Staphylococcus aureus

Bacillus cereus **Yesinia**

V. FUTURE PROSPECTS

- Nanoparticles are widely used to improve the various catalytic reactions due to their novel physico-chemical properties as compared to their bulk size components. Because of it'stheir wide applications, there is need to produce nanoparticles in industries.
- As stated above the physical and chemical methods of nanoparticle synthesis are having many disadvantages. Biological synthesis would be a preferred method because of its environmental friendly approach.
- The use of nanoparticles already established for some medical applications like wound infections, dressing, and treatment of preclinical stages.
- Recent research has revealed exciting new biological properties of NS that could be translated into new therapeutic and pharmacological treatments.
- The antibacterial, antifungal and antiviral properties of silver ions, silver compounds and silver nanoparticles have been extensively studied.

VI. CONCLUSION

Leucas aspera leaf extract can be efficiently used in the synthesis of silver nanoparticles. This plant is easily growing and available in all over regions in India as a medicinal plant so it is selected for the present study. The synthesized silver nanoparticles are confirmed from the yellowish brown color formation and monitored quantitatively by UV-Vis spectroscopy. The SEM result showed the approximate size of nanoparticles about 34.3 nm. This is the first report of synthesizing silver nanoparticles using *Leucas aspera* leaf extract. The production of silver nanoparticles by this plant is used to minimize the environmental pollution problems. The preparation of nanoparticles by using *Leucas aspera* extract as desired quality with low cost and convenient method.

REFERENCES

[1] Mukunthan KS et al; Catharanthus roseus: a natural source for the synthesis of silver nanoparticles, Asian Pacific Journal of Tropical Biomedicine, 270-274, 2011
[2] A.Thirumurugan et al; Biological synthesis of silver nanoparticles by Lantana camara leaf extracts, International Journal of Nanomaterials and Biostructures, 1 (2), 22-24, 2011
[3] Benjamin G, Bharathwaj S, Biological Synthesis of Silver Nanoparticles from Allium Cepa(Onion) &Estimating Its Antibacterial Activity, International Conference on Bioscience, Biochemistry and Bioinformatics, 35-38, 2011
[4] K. Vithiya and S. Sen; Biosynthesis of Nanoparticles, International Journal of Pharmaceutical Sciences and Research, Vol. 2, Issue 11, 2781-2785, 2011
[5] A.Thirumurugan; Biotechnological synthesis of gold nanoparticles of Azadirachta indica leaf extract. International Journal of Biological Technology, 1(1):75-77, 2010
[6] A. Khodabakhshi, Synthesis of Magnetite Nanoparticles and Evaluation of its efficiency for Arsenic Removal From Simulated Industrial Wastewater,, *Vol. 8, No. 3, pp. 189-200, 2010*
[7] Vineet Kumar and Sudesh Kumar Yadav, Plant-mediated synthesis of silver and gold nanoparticles and their applications, J Chem Technol Biotechnol, 84: 151-157, 2009

[8] R. Geethalakshmi et al; Synthesis of plant-mediated silver nanoparticles using Trianthema decandra extract and evaluation of their antimicrobial activities, International Journal of Engineering Science and Technology, Vol. 2(5), 970-975, 2010
[9] R. Srinivasan et al; Leucas aspera - Medicinal Plant: A Review, International Journal of Pharma and Bio Sciences, Vol.1, Issue.2, 153-159, 2011
[10] Abhilash M, Potential applications of Nanoparticles, International Journal of Pharma and Bio Sciences, V1(1), 1-12, 2010
[11] Prathna T C; Biomimetic Synthesis of Nanoparticles, Science, Technology & Applicability, page 1-20.
[12] Arangasamy Leela and Munusamy Vivekanandan; Tapping the unexploited plant resources for the synthesis of silver nanoparticles, African Journal of Biotechnology, Vol. 7 (17), pp. 3162-3165, 2008
[13] Johanna Rautio et al, Effect of synthesis method variables on particle size in the preparation of homogeneous doped nano ZnO material, Microchemical Journal , 91, 272–276, 2009

