Synthesis of Eco- Friendly Plant mediated Nanoparticles from *Leucas aspera* (Thumbai) and its Antimicrobial Properties in Food Packaging during Disaster Risk Management

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Abstract

Silver nanoparticles due to their unique properties, find use in day-to-day applications in human life. The major advantage of using plant extracts for silver nanoparticle synthesis are easily available, safe, and nontoxic. The main mechanism of the process is plant-assisted reduction due to phytochemicals and are quicker than microbes in synthesis. In this present work, we have worked on environment friendly technique on green synthesis of silver nanoparticles from 1mM $AgNO_3$ solution through the extract of *Leucas aspera* which possess both the reducing as well as capping agent. The particle size, Zeta potential and Antimicrobial property showed an average size of 23 nm with stability solution fifty percentage. Further, these green synthesized nanoparticles were found to be highly toxic against different human pathogens which could be an added advantage as an antimicrobial agent in food packaging.

Keywords: Plant extracts, silver nanoparticles, green synthesis, antimicrobial coating

Introduction

Nanotechnology is an upcoming interdisciplinary science. Nanoparticles are clusters of atoms with the size range of 1-100nm. Nanotechnology is one of the most promising areas in modern nanoscience and technology. Nanoparticles are classified solely based on their size and may or may not exhibit size-related properties that differ significantly from those observed in bulkmaterials (ASTM, 2006 & Buzeauetal, 2007). Nanosilver is not a new discovery which has been known for over 100 years (USFDA, 2010). The unique property of nanosilver are mainly attributed to high surface area to volume ratio, leading to many industrial sectors to incorporate nanomaterials into their products. Nanocrystalline silver

particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics (Schultz *et al.*, 2000), antimicrobials, therapeutics (Rai &Yadav, 2009;Elechiguerra *etal.*,2005), catalysis(Crooks 2007) and micro- electronics(Gittins *et al.*, 2000).

Silver is a naturally occurring precious metal, often as a mineral ore in association with other elements. It has an atomic weight of 107.8. Two other natural isotopes 106.90 Ag and 108.90 Ag with abundance of 52 and 48 percentage of silver metal (Panyala *et al,* 2008).Silver metals and silver dressings when used in reasonable amounts has no negative effect on the human body and has potent natural and inhibitory antimicrobial activity (Margaret *et al*, 2006; Sarkar *et al*, 2007) against many microbial pathogens such as viruses, fungi ,yeast etc (Morones *et al*,2005 and Zhang and Sun , 2007). Silver nanoparticles size measure from 5 to 50 nm. Due to small particle size, the total surface area of silver exposed in solution is maximized, resulting in the highest particle effect per unit of silver (Alt *et al*; 2004). The use of environmentally benign materials like plant leaf extract (Parashar *et al*, 2009), bacteria (Saifuddin *et al*, 2009), fungi (Bhainsa and Souza, 2006) and enzymes (Willner *et al*, 2007) for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness because they do not use toxic chemicals for the synthesis protocol, since the chemical synthesis method may cause toxicity adsorbed on the surface. Therefore, green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly. In this method, there is no need to use high pressure, energy, temperature and toxic chemicals.

Disasters undermine development progress, constrain economic growth and threaten food production. Systematic disaster risk management – as an integrated component of development – plays a critical role in assuring future agricultural production and access to food and water by the world's most vulnerable people. By using innovative approaches, which is easy to handle disasters:

1.1.Use of Natural Polymer Matrices

Active Nano - particles from the medicinal plants is better than synthetic polymer since they:

- Improves the performance of food packaging materials.
- Impart an additional functionality (antimicrobial, anti-oxidant, scavenging properties), thus prolonging the Shelf-life of the packaged product.
- Keep out oxygen and retain carbon dioxide, which can rival traditional active packaging technologies.
- involve biologically safe substances which do not have any harmful side-effects

2. Materials And Methods

2.1 Preparation of the plant powder and extract

The Fresh Medicinal plants of Thumbai (*Leucas aspera)* (plate I) were obtained from *Annaikatti* Hills, Coimbatore, Tamil Nadu, India. These Plant leaves were washed to remove impurities and dried under sunshade completely to remove the moisture. It was then powdered in a mixer and then sieved using a series of sieves (of successively decreasing pore size) to get the minimum possible and uniform size range. The final sieved powder was used for all the analyses. For the production of extract, 10g of the plant powder was boiled for 5 mins with 100ml distilled water. The boiled extract is filtered, centrifuged and treated with 1mM$AgNO_3$ to prepare silver nanoparticles.

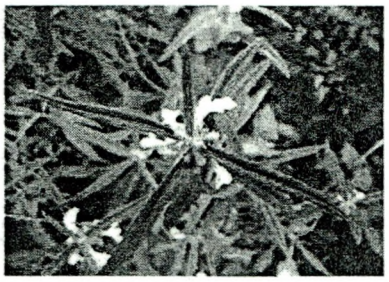

Plate I – Leucas aspera

2.3 Characterization of Silver Nanoparticles

The objective of this experiment is to analyze the characterization of silver nanoparticles was carried out using Particle analyzer, Zeta Potential and Antimicrobial Assays of the biosynthesized silver nanoparticles against the test organisms

2.3.1. Particle size measurement:

Particle sizing experiments were carried out by means of laser diffractometry, using Malvern Zetasizer ZS90

2.3.2. Zeta Potential

The stability and surface charge of synthesized silver nanoparticles was analyzed by using Malvern Zetasizer ZS90.

2.3.3. Antimicrobial Potential

The test microbes included in this study was *E.coli* (MTCC40), *Salmonella enterica* (MTCC3219) and *Shigella dysenteriae* (PSGIMS &R). The organisms were obtained from MTCC Chandigarh and PSG IMS&R, India. The pure cultures of bacteria were maintained on Nutrient agar slants. The cultures were maintained in refrigerator for use. Periodical transfers were made aseptically and regularly checked for contamination.

Screening for antimicrobial activity was done by the agar diffusion method. For the agar diffusion method, the log phase cultures were spread over the Nutrient agar medium plates using a sterile cotton swab in order to get a uniform microbial growth on test plates. Then approximately four wells of uniform sizes (0.65cm) were made with a cork-borer in each organism's plate, and 50µl of the encapsulated silver colloids were pipetted directly into the well. The plates were incubated in upright position, the bacterial culture plates were incubated at 37°C for 24-48 hours.

2.3.4. Results and Discussion

It is evident that the reduction of silver ion into silver particles in contact to the leaf extracts was identified by colour change from green to dark brown in aqueous solution due to excitation of surface plasmon resonance phenomenon (Plate II). As the *Leucas aspera* extract was mixed in the aqueous solution, silver ion complex started to change the colour from green to dark brown due to reduction of silver ion which indicated the formation of silver nanoparticles.

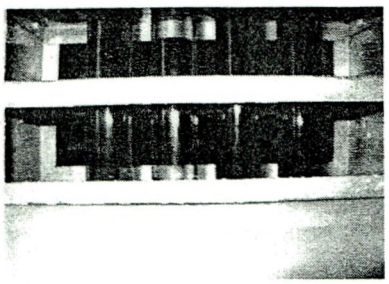

Plate II. Leucas aspera solution

A. ***Leucas aspera*** nanoparticle solution with 1mM AgNo3 after 4 hrs of incubation

B. Leucas aspera leaf extract before adding 1mM AgNo3

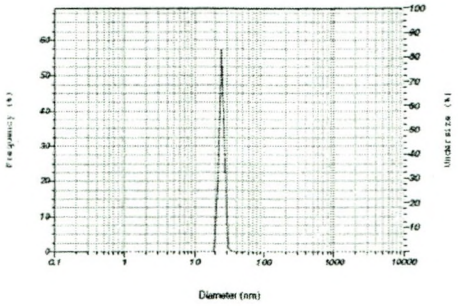

Plate III – Particle size

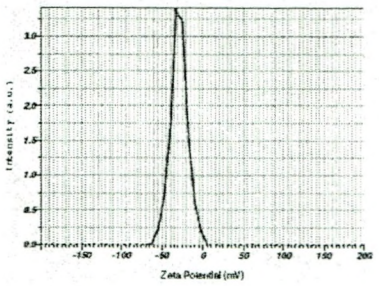

Plate IV –Zeta Potential of *Leucas aspera*

2.3.4.1. Particle Size and Zeta Potential

The particle size distribution is observed by Diffuse Light Scattering (DLS) method. The particle size analysis reflects the retention of Ag particle and also the activity towards the agglomeration settlement. The particle size has been tested for freshly prepared *Leucas aspera* extract Silver Nanoparticles solution was observed with the stability solution 50% and size of 23 nm which are shown in Plate III. The Zeta Potential value reveals that surface charge and stability of synthesized silver nanoparticles was about -28.90 depicted in plate IV. This indicates the sustainment of silver nanoparticles and its activity under steady state condition. The Zeta deviation of the freshly prepared *Leucas aspera* is highly active resulting in chaotic motion. This indicates that the colloid prepared preserves the conductivity over a period of time reflecting the fact that Ag nanoparticles are likely to be encapsulated by the plant extract that protects that activity of the particles

2.3.4.2. Antimicrobial Assay

The Antimicrobial assays were done for the *Leucas aspera* nanoparticle solution against *E.coli* (MTCC 40), *S.enterica*(MTCC 3219) and *S.dysenteriae(*PSGIMS &R) and were presented in Tables I, II, III, IV and Plates V, VI, VII, VIII and IX.

Table I – Enumeration of Bacterial Count –*Leucas aspera*

S.No	Parameters	E.coli (MTCC 40)	Salmonella enterica (MTCC 3219)	Shigella dysenteriae (PSGIMS &R)
1	Agno3	10	16	246
2	NP	7	8	94
3	AB Disc	25	5	66
4	AB Disc+NP	8	2	14

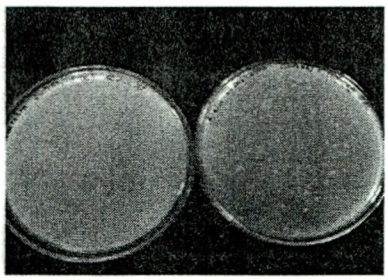

Plate V – Enumeration of E.coli (MTCC 40)

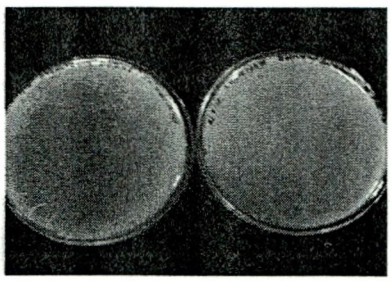

Plate VI– Enumeration of Salmonella enterica (MTCC3219)

Table II - Agar Well Diffusion Method (*E.coli-MTCC 40*)

S.No	Zone of Inhibition				
	Method	Plate I (24 Hrs)	Plate II (48 Hrs)	Plate III (24 Hrs)	Plate IV (48 Hrs)
1	AgNO3	3mm	3mm	4mm	4mm
2	NP	6mm	6mm	7mm	7mm
3	AB Disc	5mm	5mm	8mm	8mm
4	NP+ AB Disc	6mm	6mm	7mm	7mm

Table III - Agar Well Diffusion Method *(Salmonella enterica –MTCC 3219)*

S.No	Zone of Inhibition				
	Method	Plate I (24 Hrs)	Plate II (48 Hrs)	Plate III (24 Hrs)	Plate IV (48 Hrs)
1	AgNO3	3mm	3mm	4mm	4mm
2	NP	4mm	4 mm	6mm	6mm
3	AB Disc	3mm	3 mm	4 mm	4 mm
4	NP+ AB Disc	4mm	4mm	5mm	5mm

Table V- Agar Well Diffusion Method *(Shigella dysenteriae–PSGIMS &R)*

S.No	Zone of Inhibition				
	Method	**Plate I (24 Hrs)**	**Plate II (48 Hrs)**	**Plate III (24 Hrs)**	**Plate IV (48 Hrs)**
1	AgNO3	3mm	3mm	4mm	4mm
2	NP	8 mm	8 mm	10 mm	10 mm
3	AB Disc	6 mm	6 mm	8 mm	8 mm
4	NP+ AB Disc	12 mm	12 mm	15 mm	15 mm

Plates– Agar Well Diffusion method

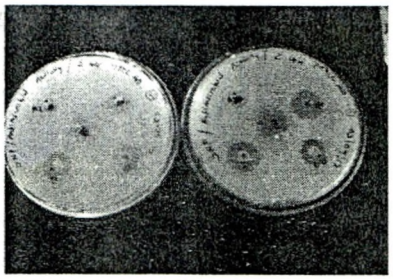

Plate VII - E.coli (MTCC 40)

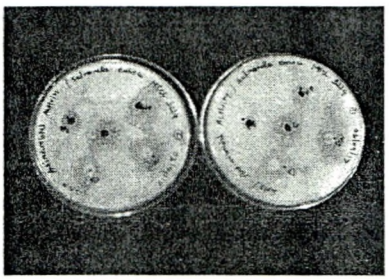

Plate VIII- Salmonella enterica(MTCC 3219)

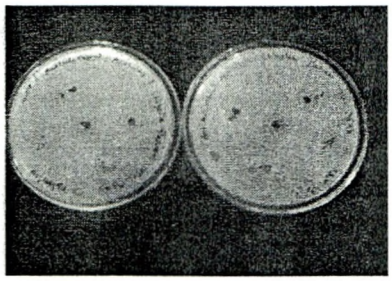

Plate IX– Shigella flexneri (PSG IMS&R)

The Medicinal Plant *Leucas aspera (23nm)* have sustained smaller particles in the colloidal state. Table II –V compares the inhibitory profile of the nanoparticles from the *Leucas aspera* plant. The extracts show inhibition zone of 6mm (after 24 hrs) and 7mm (after 48 hrs) against *E.coli* (MTCC 40) compared with Antibiotic Disc Tetracycline of 5mm (after 24 hrs) and 8mm (after 48 hrs) PlateII. The inhibitory zone of 4mm (after 24 hrs) and 6mm (after 48 hrs) compared with Tetracycline Antibiotic Disc which was 3mm (after 24 hrs) and 4mm (after 48 hrs) against *Salmonella enterica* (MTCC 3219) which are depicted in table II & Plate III. Further, inhibition zone was 8 mm (after 24 hrs) and 10 mm (after 48 hrs) respectively.

It is demonstrated that such inhibitory activity of *Leucas aspera*, the regions of Zeta potential of -28.90 which could bring maximum inhibition for different species of organisms namely *E.coli* (MTCC 40), *Salmonella enterica* (MTCC 3219) and *Shigella flexneri* (MTCC 9543) which are depicted in the plate VII, VIII and IX respectively. Further investigations also provides the inhibitory

mechanisms which may be due to Zeta potential, conductivity and particle size also offers inhibition capability of the plant extracted silver nanoparticle. It is also evident that the phytochemical ingredients are encapsulated around the silver nanoparticles which will be responsible for either enhancing or depriving the inhibition quality of silver nanoparticles. The phytochemical compound encapsulating the silver nanoparticles might offer steric action towards the organisms.

Conclusion

It is concluded that the bio-reduction of aqueous Ag+ ions by the plant leaf extract *Leucas aspera* has been demonstrated. The reduction of the metal ions through leaf extracts leading to the formation of silver nanoparticles of well – defined dimensions. Hence, in the present study it was found that plant leaf extract contains the phytochemical ingredients which encapsulate around the silver nanoparticles which will be responsible for enhancing inhibitory quality of silver nanoparticles. Therefore, this green chemistry approach towards the silver nanoparticle synthesis possess many advantages such as easy for scaling up, eco-friendly and economic viability. Antimicrobial studies of silver nanoparticles on different human pathogens open a new range of antimicrobial agents in food packaging.

Nanotechnology could *boost antimicrobial* packaging – *coating* to the inner surface of packages to provide the following aspects of food safety during disaster:

- deliver longer shelf life
- improve barrier functions
- Provide light weight design
- reduce gas and moisture exchange and UV light exposure (Sorrentino *et al.*, 2007).
- make production more efficent and more sustainable by using less water and chemicals
- Produce less waste, consume less energy and drive towards production efficiency

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