GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM SYZYGIUM AROMATICUM AND THEIR ANTIBACTERIAL EFFICACY

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ABSTRACT

In this present work ,we wish to report the antimicrobial efficiency by silver nanoparticles from the plant extract of Syzygium aromaticum under atmospheric conditions through green synthesis. A systematic characterization of silver nanoparticles was performed using UV, SEM,TEM and antimicrobial studies. After exposing the silver ions to ginger extract, rapid reduction of silver ions is observed leading to the formation of silver nanoparticles in solution. UV–VIS spectrum of the aqueous medium containing silver nanoparticles showed absorption peak at around 417 nm. SEM and TEM studies show the high density silver nanoparticles with size range within 20 nm.The antibacterial studies of so obtained silver nnaoparticles against bacterial and fungal pathogens prove as an alternative for the development of new antimicrobial agents to combat resistance problem.

KEYWORDS Silver nanoparticles ; green Synthesis; UV, SEM, TEM,antimicrobial

1. INTRODUCTION

Metal nanoparticles have received considerable attention in recent years because of their unique properties and potential applications in catalysis [1], plasmonics [2], optoelectronics [3], biological sensor [4,5] and pharmaceutical applications [6]. With the development of new chemical or physical methods, the concern for environmental contaminations are also heightened as the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts. Thus, there is a need for 'green chemistry' that includes a clean, nontoxic and environment-friendly method of nanoparticle synthesis [7]. As a result, researchers in the field of nanoparticle synthesis and assembly have turned to biological systems for inspiration.Various plant extracts eg neem,onion,lemon [8] have been used as potential reductants for silvernano synthesis.

However, it is well known that inorganic nanomaterials are good antimicrobial agents.Current research in bactericidal nanomaterials has opened a new era in pharmaceutical industries.Silver nanoparticles are the metal of choice as they hold the promise to kill microbe's effectively2 .The silver nanoparticle act on a broad range of target sites both extracellularly as wellintracellularly. In fact, microbes generally have a harder time developing resistance to silver thanthey do to antibiotics[9,10,11]. Silver nanoparticles take advantages of the oligodynamic effect thatsilver has on microbes, whereby silver ions bind to reactive groups in bacterial cells, resulting in their precipitation and inactivation.

Here in the current work we have reported the synthesis of green silver nanoparticles using the extract of the plant bud – Syzygium aromaticum(common name- cloves , Lavang). Aqueous silver nitrate solution, after reacting with datura leaf extract, led to rapid formation of highly stable, crystalline silver nanoparticles. The rate of nanoparticle synthesis was very high, which justifies use of plants over microorganisms in the biosynthesis of metal nanoparticles through greener and safer methods. In the subsequent sections we have described the synthesis of silver nanoparticles based upon the change in color, change in pH, change in absorbance and the particle size formed after reduction with antimicrobial studies followed with various microbial strains.

2. Plant Description

Syzygium aromaticum
Family: Lauraceae
Common name: cloves , Lavang

It contains about 14-21% of volatile oil (l0-13% of tannin. Various triterpene acids and esters and glycosidethe following: aliphatic and monoterpenoid alcohols, eugen (Isoeugenol, farnesol, nerolidol, sitosterols, stigmasterol and campestrol. Cloves are used as a stimulant aromatic,The sesquiterpenes of clove have been cited as potential anti-carcinogeni compounds. Medicinally cloves show Anti-oxidant, anti-fungal, anti-virul, anti-microbial, anti-diabetic, anti-inflammatory, anti-thrombotic, anesthetic, pain reliving and insect repellent, anti-platelet, anti-stress, anti-pyretic. Clove oil contains 81-95% of phenols (eugenol with about 3% of acetyleugenol), sesquiterpenes (α- and β-calyophyllenes) and small quantities of esters, ketones and alcohols.

Fig 1 .Lavang Plant (Syzygium aromaticum)

3. Material and Experimental methods
3.1 Reagents and Chemicals

Silver Nitrate was obtained from Sigma Aldrich. Freshly prepared triple distilled water was used throughout the experiment.

3.2 Collection of extracts

Lavang were collected from the local region. They were washed and cleaned with triple distilled water and dried with water absorbent paper. Then it was cut into small pieces with an ethanol sterilized knife and crushed with mortar and pestle dispensed in 10 ml of sterile distilled water and heated for 2-3 minutes at 70-80°C. The extract was then filtered using Whatman No.1 filter paper. The filtrate was collected in a clean and dried conical flask by standard sterilized filtration method and was stored at 4°C.

3.3 Synthesis of Zero Valent Silver Nanoparticles

During the synthesis of silver nanoparticles both the precursor and the reducing agent were mixed in a clean sterilized flask in 1:1 proportion. For the reduction of Ag+ ions, 5ml of filtered plant extract was mixed to 5 ml of freshly prepared 0.001 M aqueous AgNO3 solution with constant stirring at 50-60^{0}C. The Silver Nanoparticles so prepared were stabilized by adding 1% of chitosan and 1% of PVA.

3.4 UV-Vis Spectra analysis

The reduction of pure Ag ions to Ag^0 was monitored by measuring the UV-Vis spectrum by sampling of aliquots (0.3 ml) of Ag Nanoparticle solution diluting the sample in 3 ml distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer Systronics 118 at the range of 200-600 nm and observed the absorption peaks at 400-440 nm regions due to the excitation of surface plasmon vibrations in the AgNPs solution, which are identical to the characteristics UV-visible spectrum of metallic silver and it was recorded.

3.5 pH analysis

1 mM aqueous silver nitrate ($AgNO_3$) solution shows 3.8 pH There is concerned change in pH determined silver nanoparticle synthesis using extracts of plant and spices, which was analysed using Digital pH meter Systronics.

3.6 SEM analysis

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

3.7 TEM Analysis

For transmission electron microscope (TEM) measurements, a drop of solution containing as synthesized silver nanoparticles was placed on thecarbon coated copper grids and kept under vacuum desiccation for overnight before loading them onto a specimen holder. TEMmicrographs were taken by analyzing the prepared grids onHitachi H-7650 TEM instrument using low voltage (100 kV).

3.8 Antimicrobial Activity

3.8.1 Antibacterial assay

By disc diffusion method, the antibacterial activities of the datura plant extract reduced AgNPs were studied. LB (Luria bertoni) media was used ,sterilized and solidified.Then three bacterial strains(E.coli , S.aureus , S.typhi)were swabbed on the plates.Sterile discs were dipped in silver nanoparticles solution (20 µg/ml) and placed in the nutrient media plate and kept for incubation at 37ºC for 24 hrs. Zones of inhibition for control, SNPs and silver nitrate were measured and the mean values of zone diameter were presented.

3.8.2 Antifungal assay

Potato dextrose agar plates were prepared, sterilized and solidified.After solidification ,three fungal cultures (F.oxysporum , R.arrhizus, A. niger) were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (20 µg/ml) and placed in the agar plate and kept for incubation for 7 days. After 7 days zone of inhibition was measured.

4. RESULTS AND DISCUSSION

4.1 Synthesis of silvernanoparticles

Lavang extract is used to produce silver nanoparticles in this experiment. Ag ions were reduced into Ag^0 nanoparticles when plant extract is mixed with $AgNO_3$ solution in 1:1 ratio. Reduction is followed by an immediate change in color from Dark reddish brown to Dark greyish and change in pH of the solution. It is well known that silver nitrate exhibit colorless appeareance in distilled water. On mixing the plant extract with the aqueous $AgNO_3$solution it changed the color of the solution immediately and reducing the pH, which may be an indication of formation silver nanoparticles. In this experiment it was observed that the pH changed from high acidic to low acidic.

Sr. No	Solution	Color change		Color intensity	Time
		Before Reduction	After Reduction		
1.	Lavang Extract	Dark reddish brown	Dark greyish	++	1 hr
2.	0.001 M $AgN0_3$ Solution	colourless			

Color intensity: - += Light color, ++= Dark color, +++= Very dark color

Table 1. Change in color of the solution during Iron Nanoparticle synthesis

4.2 pH analysis

Plant Extract		Plant Part Taken	Ph change		UV range	Result
Binomial Name	Local name		Before	After		
Syzygium aromaticm	Lavang	Bud	4.26	4.01	400-440 nm	+

Result: - += Positive, -= Negative.

Table 2 . Change in pH during silver nanoparticle synthesis

4.3 UV visible spectroscopy and color change for the Green synthesized silver nano particles

UV spectroscopy studies report the maximum absorbance of silver nanoparticles reduced by lavang extract at 417 nm (figure 1).

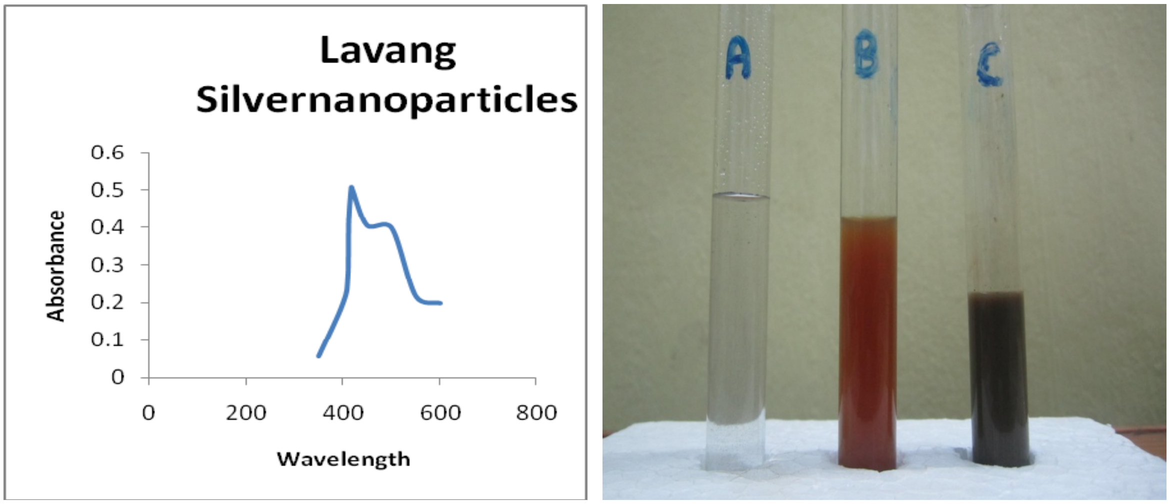

Fig.2 a)Lavang silver nanoparticle SPR at 417 nm, b)Tube A- silver nitrate, Tube B- Extract, Tube C- Lavang silvernanoparticle solution.

4.4. SEM Analysis

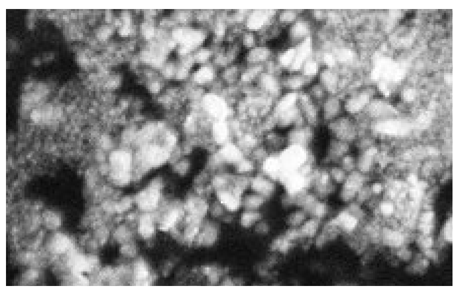

Figure 3: SEM image of Lavang reduced silver nanoparticle

The SEM image showing the high density silver nanoparticles synthesized by the lavang extract further confirmed the development of silver nanostructures by the plant extract(as shown in figure 3)

4.5. TEM Analysis

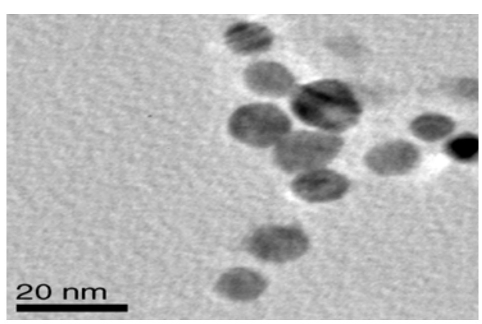

Figure 4:TEM image of Lavang reduced silver nanoparticle

The silver nanoparticles are spherical in structure. All the nanoparticles are well separated and no agglomeration was noticed. From the TEM images, we obtained the average size of silver nanoparticles of 14nm (as shown in figure 4)

4.5 Antimicrobial tests

The antimicrobial effect of silver nanoparticles reduced by lavang extract has been studied against three bacterial and three fungal strain.Results show positive resistance in bacterial growth with maximum resistance against S.aureus suggesting that the resultant structural change in the cell membrane could cause an increase in cell permeability, leading to an uncontrolled transport through the cytoplasmic membrane and ultimately cell death. Nano-silver produced by curry leaf plant extract reduction showed maximum resistance against A. niger (as seen in Table 3)

Sl no.	**Microbial Strain**	**Inhibition zone (mm)**		
		Control	**SNPs**	**$AgNO_3$**
1	E.coli	**6**	**15**	**16**
2	S.aureus	**8**	**12**	**18**
3	S.typhi	**6**	**18**	**20**
4	F.oxysporum	**6**	**10**	**12**
5	R.arrhizus	**5**	**11**	**14**
6	A. niger	**6**	**15**	**17**

Table 3: Antimicrobial Tests

5. CONCLUSION

It has been demonstrated that lavang extract is capable of producing producing silver nanoparticles at high density and stability of the nanoparticles is also observed. Silver nitrate with reducing agent i.e plant extract has shown a remarkable color change with concerned change in pH of solution. The reduction of the metal ions through plant extract leading to the formation of silver nanoparticles of fairly well-defined dimensions. Antimicrobial activities of silver nano particles reduced by lavang extract certifies the vital potential in biomedical applications and as an alternative to chemical synthesis protocols and low cost candidate as reductant for synthesizing silver nanoparticles.

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