



## ANTIMICROBIAL EFFICACY OF GREEN SYNTHESIZED SILVER NANOPARTICLES FROM THE MEDICINAL PLANT *PLECTRANTHUS AMBOINICUS*

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### ABSTRACT

The aqueous extract of *Plectranthus amboinicus* (Lour.) Spreng was used for the synthesis of silver nanoparticles. The UV-Visible absorption spectrum recorded for the solution shows the characteristic surface plasmon resonance band for silver nanoparticles in the range of 400-440nm. The SEM studies confirmed the formation of silver particles in the size range of 40-100nm, a clear indication of the formation of silver nanoparticles. The characterization of silver nano particles by XRD studies revealed the average grain size to be 41.9nm, with crystalline planes (111) and (101) corresponding to cubic shape of silver nanoparticles. On comparison with the silver nitrate and plant extracts, silver nanoparticles outperformed the antimicrobial effect on *Klebsiella pneumoniae*, *Staphylococcus aureus*, *E.coli*, *Aspergillus sp.* and *Candida albicans*. Finally it is proven that the green synthesized silver nanoparticles have potent antimicrobial effect.

**Keywords:** *Plectranthus amboinicus*, Silver nitrate, nanoparticles, antimicrobial.

### INTRODUCTION

Nanoparticles present a higher surface-to-volume ratio with decreasing size and the Specific surface area is relevant for catalytic reactivity and other related properties such as antimicrobial activity. The synthesis of silver nanoparticles using plants extracts has various human applications. Compare to other biological processes of nanoparticle synthesis using plants have been many advantages because it eliminates the elaborate process of maintaining cell cultures. Plants use can also be suitably scaled up for large-scale synthesis of nanoparticles.<sup>1-3</sup>

Because of the pathogenic organism resistance to the already available drugs, there is always need an alternative approach to search for new bioactive compounds. Plants which are natural products and present abundantly all over world can be use to search for new bioactive compounds.

The use of *Aloe vera*, *Azadirachta indica*, *Medicago sativa*, *Embllica officinalis* has already been reported.<sup>4</sup> The present investigation demonstrated that silver nanoparticles could be prepared using screened plant extract of *Plectranthus amboinicus*.

*Plectranthus amboinicus* (Lour) Spreng belongs to family Lamiaceae, and is known as country borage in English. It is found throughout India, Ceylon and Moluccas. It is popularly known as Indian oregano. The leaves have many traditional medicinal uses especially for the treatment of cough, sorethroats and nasal congestion. Upon literature review it was found that the plant contains butylaniside,  $\beta$ -caryophyllene, quercetin, ursolic acids, triterpenic acids,  $\alpha$ -pinene,  $\beta$ -pinene, thymol,

eugenol, carvacrol, 1,8-cineole,  $\beta$ -phellandrene, p-cymene, salvigenin, crismaritin and chrysoeriol. Many pharmacological properties have been reported including urolithiasis, antiepileptic, antitumor and antimutagenic, neuropharmacological, radioprotective effect, antioxidant, antiviral, cytotoxic, antimicrobial, antibacterial, antifungal properties.<sup>5-11</sup>

### MATERIALS AND METHODS

#### Screening and selection of plants

The plant leaves were collected in and around Namakkal District, TamilNadu. The plant materials were taxonomically identified and authenticated by the Botanical Survey of India. The voucher specimen was deposited there for future reference and registered with no: BSI/SRC/5/23/2011-12/Tech-933. The plant leaves were surface cleaned with tap water followed by distilled water. Initial screening was performed to synthesize the silver nanoparticles from aqueous extracts of plant leaves. *Plectranthus amboinicus* leaf showed the rapid synthesis of silver nanoparticles. Due to this reason, this plant has been chosen for the study.

#### Preparation of aqueous extract

Fresh plant materials of *Plectranthus amboinicus* were collected and the aqueous extract of sample was prepared using the freshly collected leaves (25g), by washing in running tap water and then in distilled water, followed by boiling in 100ml of distilled water, at 60°C for about 5minutes. Then the extract was filtered through gauze cloth and used for further experiments.



## Synthesis of Silver Nanoparticles

The chemical, AgNO<sub>3</sub> was purchased from HiMedia Laboratories Pvt. Limited, Mumbai, India and was used as received. In the typical synthesis of silver nanoparticles, 10ml of the aqueous extract of *Plectranthus amboinicus* was added to 90ml of 1mM (10<sup>-3</sup> M) solution of silver nitrate in 250ml Erlenmeyer flask. The reaction was performed at room temperature and was kept over orbital shaker at 120 rpm, for 5 hours. Suitable controls were maintained throughout the conduct of experiments.<sup>12</sup>

### UV-Visible spectroscopy analysis

The bioreduction of Ag<sup>+</sup> in aqueous solution was monitored by periodic sampling of aliquots (0.2ml) of the suspension, then diluting the samples with 2ml deionized water and subsequently measuring UV-Vis spectra, at the wave length of 200 to 600 nm in Elico microprocessors (μp) based UV-visible spectrophotometer. UV-Vis spectra were recorded at a time interval for about 0 minutes, 30minutes, 1hour and 24hours.

### Determination of Phenolic Compound

The phenolic compound was determined to know about the presence of phenolic compound in the plant leaf broth and in the bioreduced solution containing the silver nanoparticles. This determination was performed by pipetting out 5ml of sample in a test tube. To this add few drops of ferric chloride solution and observed for the formation of green or blue colour.<sup>13</sup>

### Recovery of Silver Nanoparticles by Ultra-Centrifugation

For characterization of silver nanoparticles formed in the aqueous extract, about 1 litre of 1mM silver nitrate solution containing 100 ml of aqueous leaf extract of *Plectranthus amboinicus* was prepared and incubated over the orbital shaker at normal room temperature. After bioreduction, the solution consisting of hydrosols of silver nanoparticles and biomolecules from the aqueous extract of *Plectranthus amboinicus*, was subjected to centrifugation at 5000rpm for 10minutes, washed twice and the pellet was discarded. Later the supernatant was subjected to centrifuge at 25900rpm (75000 x g), for 30minutes. The pellet formed was dissolved in 1.0ml of deionized water and air dried.

## Characterization of Green Synthesized Silver Nanoparticles

### Particle Size Analysis

This particular analysis was carried out for analyzing the wide range of distribution of silver nanoparticles in the bioreduced solution.

### FTIR Analysis

The dried Ag nanoparticles were subjected to FTIR analysis for analyzing the capping ligand of silver nanoparticles which act as a stabilizing agent.

## XRD Measurements

The air dried nanoparicles were analyzed for the formation of Ag nanoparticle by X-Ray Diffractometer. The diffracted intensities were recorded from 10° to 90° of 2θ angles.

## SEM Analysis

This study was undertaken to know the size and surface morphology of the biosynthesized silver nanoparticles using aqueous extract of *Plectranthus amboinicus*.

## Scanning Electron Microscope

The images of nanoparticles were obtained in a Scanning Electron Microscope. The details regarding applied voltage, magnification used and size of the contents of the images were implanted on the photographs itself.

## Antimicrobial Activity

Silver nanoparticles synthesized using aqueous leaf extract of *Plectranthus amboinicus* were tested for its potential antibacterial activity against a few bacterial pathogens such as *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas* sp. and fungal pathogens such as *Candida albicans*, *Fusarium* sp. and *Aspergillus* sp. were used as the test organisms. Agar Well diffusion assay method<sup>14</sup> was followed, which involves swabbing the cultures in pre-sterilized Muller Hinton agar plates and sabouraud dextrose agar plates and four wells were cut in the same using sterile cork borer. Each well was loaded with 100μl of the solutions in the following order: water as negative control, aqueous leaves extract of *Plectranthus amboinicus*, solution of silver nanoparticles, and silver nitrate. Then the sample loaded Muller Hinton agar plates and sabouraud dextrose agar plates were incubated at 37°C for 24 hrs and 27°C for about 48 hours respectively. Then the formation of Zone of Inhibition were observed.

## RESULTS AND DISCUSSIONS

The results exhibit that the addition of the aqueous leaf extract *Plectranthus amboinicus* to 1mm solution of silver nitrate led to the appearance of reddish brown color (Figure 1) as resultant of formation of Silver Nanoparticles.



**Figure 1:** Image showing green synthesized Silver Nanoparticles with Positive Control, Test and Negative Control silver nanoparticles in the solution.

Generally UV-Vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions. The UV-Vis absorption spectrum recorded for the solution shows the characteristic surface plasmon resonance band for silver nanoparticles in the range of 400-440 nm (Figure 2).

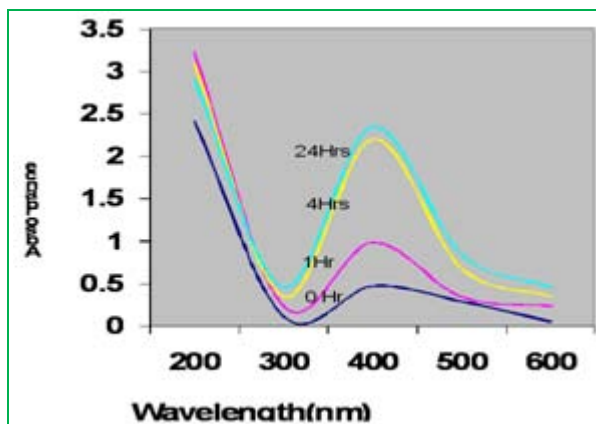


Figure 2: UV Visible Spectroscopy Analysis

The rate of formation is literally rapid, comparable to the chemical method of synthesis. Also the comparison was made by reducing the leaf broth concentration and silver nitrate concentration. The synthesis rate was found to be increased with the increase in the concentration of leaf broth. Also the synthesis rate was found to be decreased by decreasing the silver nitrate concentration to 0.5mM. The pale yellow colour appears immediately after the addition of the aqueous plant extract, and the reaction was completed in about 4hrs. This makes the investigation highly significant for rapid synthesis of silver nanoparticles.

Phenolic compound determination had revealed that the presence of phenolic compound in the plant leaf broth may enhance the bioreduction process and the presence of those compounds in the green synthesized silver nanoparticle solution may act as the capping or stabilizing molecule and the additional antimicrobial activity may be because of those compounds. As the green synthesized silver nanoparticle solution was showing the presence of phenolic compound, the silver nanoparticle may enhance the antioxidant activity.

The particle size analysis had revealed that the silver nanoparticles in the bioreduced solution were widely distributed (Figure 3). FTIR analysis was used to characterize the nature of capping ligands that stabilizes the silver nanoparticles formed by bioreduction process. The FTIR spectrum (Figure 4) showed bands at  $999\text{cm}^{-1}$  are the characteristics of alkenes or ester compounds, the spectral band at  $1365\text{cm}^{-1}$  are the characteristics of alkanes or aldehydes and phenolics. The band at  $2296\text{cm}^{-1}$  are the characteristics of alkenes and the band at  $3507\text{cm}^{-1}$ ,  $1558\text{cm}^{-1}$  revealed that these bands are the characteristics of the aromatic compound and amine groups (primary and secondary amines).

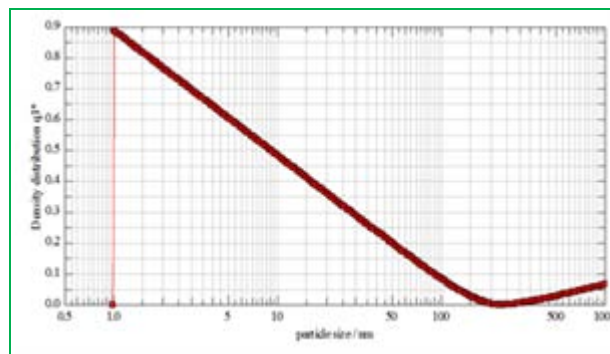


Figure 3: Particle Size Analysis

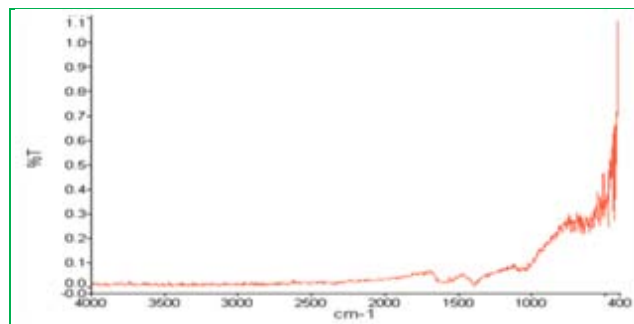


Figure 4: Fourier Transform Infra Red Spectroscopy Analysis (FTIR)

The characterization of silver nano particles by XRD studies (Figure 5) revealed the average grain size to be 41.9nm, with crystalline planes (111) and (101) corresponding to cubic shape of silver nanoparticles.

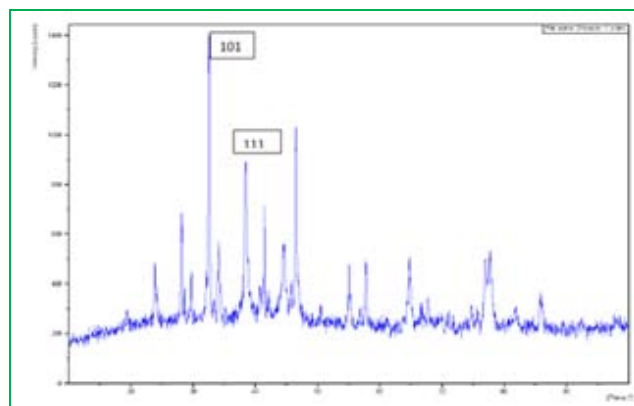


Figure 5: XRD Profile

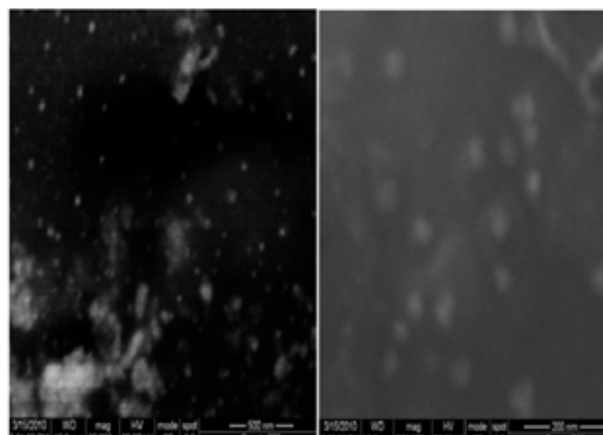


Figure 6: SEM Image of Silver Nanoparticles

The SEM studies confirmed the formation of silver particles in the size range of 40-100nm, a clear indication of the formation of silver nanoparticles (Figure 6). These data suggests that the stabilizing agents may be aromatic compounds, alkane derivatives, phenolic compound, amines or alkenes present in the aqueous leaf extract of *Plectranthus amboinicus*.

We have found that the silver nanoparticles synthesized in our study effectively inhibited the growth and

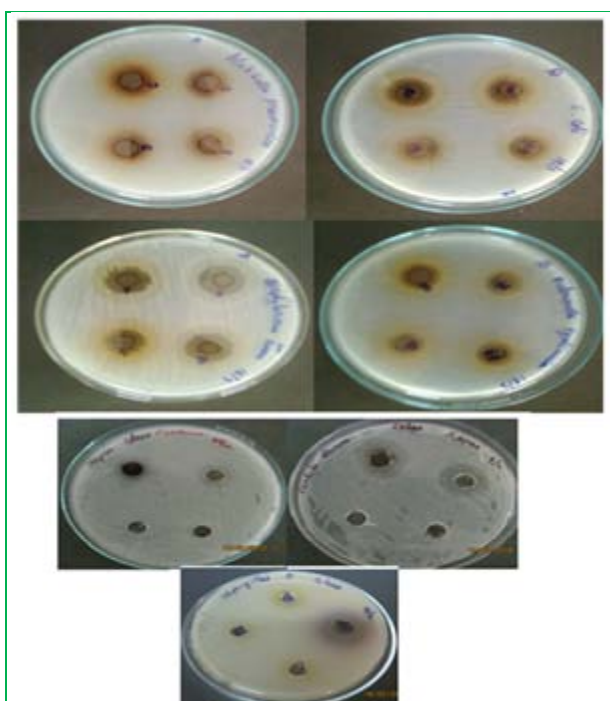
multiplication of pathogenic bacteria like *E.coli*, *Staphylococcus aureus*, *klebsiella pneumoniae*, and *Salmonella typhimurium* and fungal pathogens namely *Fusarium*, *Candida albicans* and *Aspergillus* sp. On comparison with the silver nitrate and plant extracts, silver nanoparticles outperformed in the bactericidal effect. The antimicrobial activity was checked for different concentration of silver nanoparticles. (Table 1 & Table 2).

**Table 1:** Zone of inhibition Silver Nanoparticle against bacterial test pathogens at different concentration

Bacterial test pathogens	Concentration (mg)	Volume ( $\mu$ l)	Diameter of Zone of inhibition (mm)
<i>E.coli</i>	2	40	12
	4	60	14
	6	80	16
	8	100	17
<i>Klebsiella pneumoniae</i>	2	40	17
	4	60	18
	6	80	19
	8	100	20
<i>Staphylococcus aureus</i>	2	40	13
	4	60	15
	6	80	17
	8	100	19
<i>Salmonella typhimurium</i>	2	40	11
	4	60	13
	6	80	14
	8	100	15

**Table 2:** Zone of inhibition Silver Nanoparticle against fungal test pathogens at different concentration

Fungal test pathogens	Concentration (mg)	Volume ( $\mu$ l)	Diameter of Zone of inhibition (mm)
<i>Fusarium</i> sp.	8	100	5
<i>Candida albicans</i>	8	100	14
<i>Aspergillus</i> sp.	8	100	15



**Figure 7:** Zone of Inhibition Silver Nanoparticle against Bacterial and Fungal Test Pathogens

The synthesized silver nanoparticle controlling *Klebsiella pneumoniae* effectively than other bacterial strains. Next to this, it is controlling *Staphylococcus aureus*, and *E.coli*. But it has lowest activity against *Salmonella typhimurium* compare to other bacterial strains. In fungal pathogens it showed effective activity against *Aspergillus* sp. and *Candida albicans*. But the synthesized silver nanoparticles showed least activity (figure 7) for *Fusarium* sp.

### CONCLUSION

All around the global, interest for finding bioactive compounds because of the pathogens resistance to available drugs. So to treat and prevent human diseases the way changed to search on biofriendly based products. In the present study a maiden attempt has been made to biosynthesize silver nanoparticles using aqueous leaf extract of *Plectranthus amboinicus*, a highly renowned medicinal herb in south India. The results showed that the synthesized nanoparticles showing good antimicrobial effect on *Klebsiella pneumoniae*, *Staphylococcus aureus*, *E.coli*, *Aspergillus* sp. and *Candida albicans*. So the present study accents the use of *Plectranthus amboinicus* for the synthesis of silver nanoparticles with potent antimicrobial effect.

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