BIOSYNTHESIS OF SILVER NANO PARITICLES FROM THE *CLITORIA TERNATEA* AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Silver nano particle research plays an important role in modern science for eradicating diseases. The Clitoria ternatea flowers are collected and the Silver nano particle is biosynthesized. The present study was aimed to assess the for its antimicrobial activities. The Clitoria ternatea Silver nano particles, showed antimicrobial activity was evaluated using disc diffusion and minimum inhibitory concentration. The Clitoria ternatea flowers synthesized-AgNPs at different concentration (5, 10, 15 and 20 µl/ml) was tested against Vibrio cholerae and Pseudomonas aeruginosa. Clitoria ternatea flowers synthesized-AgNPs inhibited (MIC) the growth of Vibrio cholerae and Pseudomonas aeruginosa at 20 µl/ml concentrations. the present study provides evidence that Clitoria ternatea flowers extract-AgNPs exhibit interesting antimicrobial properties, expressed either by their capacity to phytochemical compound activity.

Key words: *Clitoria ternatea, antimicrobial activity, nanoparticle and minimum inhibitory concnetration.*

I. INTRODUCTION

Nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology [1]. However, there is still need for economic, commercially viable as well environmentally clean synthesis route to synthesize silver nanoparticles [2]. The use of environmentally benign materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol [3]. The synthesis of noble metal nanoparticles attracts an increasing interest due to their new and different characteristics as compared with those of macroscopic phase, that allow attractive applications in various fields such as antimicrobials [4], medicine, biotechnology, optics, microelectronics, catalysis, information storage and energy conversion [5].

Green chemistry approach emphasizes that the usage of natural organisms has offered a reliable, simple, nontoxic and eco-friendly [6]. Therefore, researchers in the last years have turned to biological systems for nanoparticle synthesis [7]. The bactericidal efficacy of silver-containing polymers is based on the release of silver ions (Ag^+) through interaction with a liquid watery phase [8]. The inhibitory action of silver nanoparticles

is also based on the release of (Ag^+) [9]. Exposure of microorganisms to silver nanoparticles was shown to result in strong antimicrobial activity [10]. This bactericidal activity also appears to be dependent on the size and shape of the silver nanoparticles [11]. *Clitoria ternatea* has a long history in herbal and folk medicinal systems to possess various beneficial properties referred to as its pleiotropic properties. The alcoholic extract of *Clitoria ternatea* revealed antimicrobial activity against *Staphylococcus aureus, Bacillus subtitlis, E. coli, Candida albicans, Trichophyten memtagrophytes, and Aspergillus* [12]. *Clitoria ternatea* have extracted the antibacterial acivity was screened.

II. MATERIALS AND METHOD

Clitoria ternatea flowers were collected in early morning from Garden at St Peters university Campus, Avadi, Chennai 600 054, a recognized institution of Government of Tamil nadu, Government of India. The sample was washed thoroughly with distilled water. About 50 gm of flowers were slashing into small pieces. Finely cut Flowers were dipped into a beaker containing 200 ml distilled water. After that the mixture was boiled for 10 to 12 minutes. The extract was filtered using Whatmann filter paper and filtrate was collected.

Synthesis of silver nanoparticles: The extract of *Clitoria ternatea* Flowers was mixed with aqueous solution of 1 mM Silver nitrate (99.99%) in 1:4 ratio in conical flask under aseptic conditions. The pH was adjusted to 8.0. The conical flasks were then incubated at 37^{o}C for 24 hours. A change in the color of the solution was observed.

Antibacterial activity of *Clitoria ternatea* synthesized silver nanoparticles: The antibacterial activities of the synthesized nanoparticles were assayed using the disc diffusion method. Bacteria were grown overnight on Mueller Hinton agar plates, five colonies were suspended in 5 ml of sterile saline (0.9%) and the bacterial population in the suspension was adjusted to ~3x10^8 CFU/ml. A sterile cotton swab was dipped into the suspension and the swab rotated several times with firm pressure on the inside wall of the tube to remove the excess fluid. The swab was used to inoculate the dried surface of MH agar plate by streaking four times over the surface of the agar, rotating the plate approximately by 90^o to ensure an even distribution of the inoculums. The medium was allowed to dry for about 3 min before adding a sterile disc of 9 mm diameter. Each disc was placed firmly on to the agar to provide uniform contact with the bacteria. Bioactive compound (50 µg) was weighed and dissolved in 1 ml of 7% ethanol. The different concentration of bioactive compound was introduced on to each disc and the control disc received only 7% ethanol. The plates were incubated at 37°C for 24 h and the inhibition zone was measured and calculated. The experiments were carried out in duplicate three times. The results (mean value, *n*=3) were recorded by measuring the zones of growth inhibition surrounding the discs.

Minimum inhibitory concentrations (MICs) of *Clitoria ternatea* synthesized silver nanoparticles: The minimum inhibitory concentrations of the isolated compounds were determined by dilution method (Brantner and Grein, 1994). The strains were grown in Mueller Hinton broth to exponential phase with an *A*560 of 0.8, representing 3.2×10^8 CFU/ml. Different dilutions of the compounds were prepared to give solutions of 5, 10, 15, and 20 µg/ml. 0.5 ml of each concentrationwas added into separate test tubes containing 4ml of MHbroth inoculated with 0.5 ml bacterial suspension at a final concentration of 106 CFU/ml. Each MIC was determined from five independent experiments performed in duplicate. The tubes containing 4.5 ml of bacterial inoculates and 0.5 ml of 7% acetone used as bacterial control, 4.5 ml of uninoculated MH broth and 0.5 ml PBS served as a blank. The tubes were incubated at 37 ◦C for 18 h; inhibition of bacterial growth was determined by measuring the absorbance at 560 nm.

III. RESULTS

The absorption spectra of aqueous flowers extracts obtained from *Clitoria ternatea* were compared with the absorption spectra of silver nanoparticles prepared using these extracts in order to reveal the formation of silver phyto-nanoparticles. The absorption spectra of silver phyto-nanoparticles were recorded after 24 hours after their preparation and exhibited absorbance peaks at 450 nm. FTIR spectra of silver nanoparticles synthesized using aqueous flowers extract from *Clitoria ternatea* were recorded in order to study the formation of herbal silver nanoparticles and to identify the possible biomolecules responsible for Ag^+ bioreduction and for capping the resulted silver phyto-nanoparticles.The FTIR spectra of silver nanoparticles exhibited IR bands located in the region 1300-1350 cm-1 (corresponding to asymmetric and symmetric stretching vibrations of the nitrate group): 1330 cm-1 for red *Clitoria ternatea*-AgNPs (Fig 1).

The *Clitoria ternatea* flowers synthesized-AgNPs at different concentration (5, 10, 15 and 20 µl/ml) was tested against *Vibrio cholerae* and *Pseudomonas aeruginosa*. The alkaloid and *Clitoria ternatea* flowers synthesized-AgNPs exhibited more bactericidal action in against Vibrio cholerae than Pseudomonas aeruginosa with higher inhibition zone was found at 20µl/ml concentration (Table-1).

Minimum Inhibitory Concentration (MIC) assays were also performed to determine the antibacterial activities of synthesized-AgNPs at 18 hours incubation. *Clitoria ternatea* flowers synthesized-AgNPs inhibited the growth of *Vibrio cholerae* and *Pseudomonas aeruginosa* at 20 µl/ml concentrations. *Clitoria ternatea* flowers synthesized-AgNPs inhibited growth of *Vibrio cholerae* and *Pseudomonas aeruginosa* (Graph-1).

IV. DISCUSSION

The arrival of nanotechnology provides a control at a nanometric scale creating a new class of materials in a diversity of domains [13]. In the agricultural and food fields, nanotechnology applications are in their initial stages [14]; at this point, the use of plant parts for nanoparticle biosynthesis is an unexplored and unexploited area [15]. The synthesis of silver nanoparticles using *Clitoria ternatea* flowers were detected also by UV-VIS absorption spectra showing a strong plasmon resonance which was centered between 415-458 nm, depending on the type of petal extract. The UV-VIS absorption spectra of the plant extract alone showed no absorption in the spectral window between 400-600 nm, whereas the aqueous petal extracts exposed to silver ions (from AgNO3 10^{-3} M solution) presented distinct absorption at around 415-458 nm.

In the recent past, several herbal drugs, which have free radical scavenging potential, have gained importance in treating such chronic diseases. In the present experiment we have investigated the antioxidant activity of the AgNPs synthesized *Clitoria ternatea* flowers extract and the possible mechanisms, by assessing their role on reducing power, metal chelating activity, lipid peroxidation, superoxide anion removal and hydroxyl radicals trapping potential. Earlier studies reported that AgNPs can be synthesized by plants such as *Azadirachta indica, Capsicum annuum, Carica papaya, Gliricidia sepium, Eucalyptus* hybrid and microorganisms such as *Aspergillus fumigatus Cladosporium cladosporioides, Fusarium oxysporum, Pseudomonas aeruginosa* and *Rhodopseudomonas capsulate* [15-17]. In the current study, aqueous silver ions were reduced to AgNPs after

mixing with *Clitoria ternatea* flowers extract followed by incubation for 24 h in the dark. The color turned to reddish brown and this change in color has been previously observed by several investigators [18].

The antimicrobial activity of AgNPs was reported in a series of reports [16 & 19]. In the current study, *Clitoria ternatea* flowers extract-AgNPs were effective against *Vibrio cholerae* and *Pseudomonas aeruginosa*. Similar to these observations, Govindaraju *et al*., [20] showed a zone of inhibition when the synthesized nanoparticles were tested against *Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus flavus and Aspergillus niger*. A number of theories for antimicrobial actions of colloidal silver solution have been proposed. For example, alteration of permeability of cell membrane [10], release of lipopolysaccharides and membrane proteins [21], generation of free radicals responsible for the damage of membrane [22], and dissipation of the proton motive force resulting in the collapse of the membrane potential [23-24]. Plant parts were useful to inhibit growth of microbes. Fruit extract of *Durio zibethinus* inhibit pathogens. Moreover, Tsibakhashvil *et al*., [7] studied the effect of silver nano balls on *Escherichia coli, S. typhimurium, B. subtilis* and *P. aeruginosa* by colony forming unit (cfu) and growth curve at a concentration of 40 µg/ml and showed a significant reduction of bacterial population and their growth pattern at the studied concentration.

V. CONCLUSION

In conclusion, the present study provides evidence that *Clitoria ternatea* flowers extract-AgNPs exhibit interesting antimicrobial properties, expressed either by their capacity to phytochemical compound activity. These effects may be useful in the treatment of pathologies in which active compound production plays a key role.

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Table-1. The antibacterial activity of the *C. ternatea* flowers synthesized-AgNPs by disc diffusion method.

Pathogenic bacteria	Alkaloid extract exhibited the Zone of inhibition (mm)[a]				
	Positive control 10 µl Ampicillin	Different concentrations Crude extract (µl/ml)			
		5 µl	10 µl	15 µl	20 µl
Vibrio cholerae	14 ± 0.3	13 ± 0.2	16 ± 0.3	19 ± 0.3	22 ± 0.2
Pseudomonas aeruginosa	16 ± 0.2	10 ± 0.1	15 ± 0.3	17 ± 0.2	20 ± 0.3

[a] The inhibitory diameter was measured by means of calipers. All the assays were duplicated, and the mean values were recorded.

Table-2. Synthesized-AgNPs of *C. ternatea* flowers against growth of bacterial pathogen.

Pathogenic bacteria	Minimum inhibition against pathogen				
	Positive control 10 µl Ampicillin	Absorbance at 650 nm			
		5 µl	10 µl	15 µl	20 µl
Vibrio cholerae	0.82	0.72	0.58	0.47	0.33
Pseudomonas aeruginosa	0.74	0.69	0.59	0.48	0.35

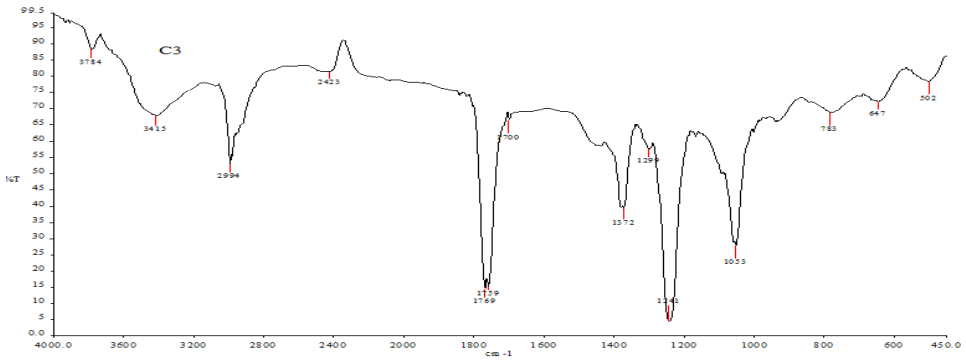

Fig-1. ATR-FTIR spectra of *Clitoria ternatea* flowers extract-AgNPs