ANTIMICROBIAL ACTIVITY OF BIOSYNTHESIZED SILVER NANOPARTICLES PREPARED FROM THE LEAF EXTRACT OF *LANTANA CAMARA*

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Article Received on: 16/03/13 Revised on: 07/04/13 Approved for publication: 11/05/13

DOI: 10.7897/2230-8407.04541

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ABSTRACT

Lantana camara commonly known as weed or red sage is found throughout India. It was reported to be used in traditional medicine system for infectious diseases. The present study designed to evaluate antimicrobial activity of aqueous extract of *Lantana camara* and bio synthesized silver Nanoparticles from aqueous extract of *Lantana camara*. When the extract of the plant was treated with silver nitrate (1mM) solution, a yellowish brown color developed indicating the formation of silver Nanoparticles. The anti microbial activity of biosynthesized silver Nanoparticles of the extract and aqueous extract of *Lantana camara* was investigated against two gram positive and two gram negative human pathogenic bacteria and fungi by using disc diffusion method. The biosynthesized silver Nanoparticles showed promising anti bacterial activity at 300µg/ml concentration compared with aqueous extract of *Lantana camara*. Similarly the silver Nanoparticles at the same level of concentration also exhibited significant antifungal activity against *Candida albicans* and *Aspergillus niger*. Conclusion: The results indicate that the biosynthesized silver Nanoparticles from *Lantana camara* have better antimicrobial activity than with that of aqueous extract of *Lantana camara* and almost equal antimicrobial activity when compared with the standard.

Key words: Antibacterial activity, Antifungal activity, Silver nanoparticles (Ag-Np's), TEM, UV- Vis spectrum.

INTRODUCTION

The medicinal uses of silver have been documented since 1000 B.C. Silver is a health additive in traditional Chinese and Indian Ayurvedic system of medicine. Silver together with copper, is commonly used to inhibit bacterial and fungal growth in chicken farms and in post harvested cleaning of oysters. Silver resistance is important to monitor because modern technology has developed a wide range of products that depend on silver as a key microbial component. In late 1970s, Robert O. Becker has discovered that silver ions promote bone growth and kill surrounding bacteria¹. Silver kills some 650 different disease causing organisms. Silver based topical dressing has been widely used as a treatment for infections in burns, open wounds and chronic ulcers. Nanomaterials are the leading requirement of the rapidly developing field of Nanomedicine, bio nanotechnology. Nanoparticles are being utilized as therapeutic tools in infections, against microbes and thus understanding the properties of Nanoparticles and their effect on microbes is essential for clinical applications². Recently, there has been a great deal of interest surrounding the discovery that silver Nanoparticles (Ag-Np's) are significantly more effective antimicrobial agents in terms of the minimum effective concentration than their Ag⁺ counterparts³. Biological approach for the synthesis of Ag-Np's (Green synthesis) is an eco-friendly and cost-effective method as compared to the other chemical and physical methods. This has promoted research in the well known activity of silver ions and silver-based compounds, including Ag-Np's⁴. The synthesis of Ag-Np's using chemical and physical methods requires high pressure, energy, temperature and toxic chemicals. The results of chemical synthesis method showed some unfavorable impact in the medical applications. When compared with chemical and physical methods, green synthesis method provides a low cost, environment friendly, easily scale up for large scale synthesis, and no need to use high pressure, energy, temperature and toxic chemicals⁵.

Hence, Ag-Np's have been applied to a wide range of healthcare products, such as scaffold, water purification systems and medical devices. The most important application of silver and Ag-Np's is in pharmaceutical industry for the preparation of topical ointments to prevent infection against burn and open wounds. The toxic effects of silver on bacteria have been investigated for more than 60 years and the acting mechanism of silver has been known to some extent. Therefore; the preparation of uniform nanosized Ag-Np's with specific requirements in terms of size, shape, and physical and chemical properties is of great interest in the formulation of new pharmaceutical products⁶. *Lantana camara* commonly known as weed or red sage or "Unnichedi" in Tamil, "Pulikampa" in Telugu and "Caturang" in Hindi is a significant weed commonly found throughout India. Since, very long time *Lantana camara* reported in traditional medicine for the treatment of infectious diseases⁷. Hence, the aim of the present study was to develop a novel approach for the green synthesis of Ag-Np's using aqueous extract of *Lantana camara* and exploring its antimicrobial activity against some selected Gram positive and Gram negative organisms and comparing their antimicrobial activity with the leaf extract of *Lantana camara*.

MATERIALS AND METHODS

Plant collection

The plant was collected from the Botanical garden of Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India. The collected plant was authenticated by the Head, Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India and a voucher specimen (No.1956) was kept in the Pharmacognosy Lab, Department of Pharmacy, Annamalai University, India, for future reference. The healthy leaves were separated from the collected plants and dried under shade.

Preparation of aqueous extract of *Lantana Camara*

The dried plant material was powdered using a disintegrator to get a coarse powder. The powdered samples were kept in sealed containers for extraction purposes. About 10gm of the sample was shaken with 40 ml of double distilled sterilized water in a 100ml Erlenmeyer stopper flask for 15 min and then the mixture was boiled for 5 min. The extract was cooled and filtered through Whatman no 1 filter paper. The resultant filtrate was kept in a refrigerator⁷.

Collection of Microorganism

The microorganisms used in this experiment were *Bacillus subtilis* (10877), *Staphylococcus aureus* (29838), *Pseudomonas aeruginosa* (27854), *Escherichia coli* (1130) and fungus culture *Candida albicans* and *Aspergillus niger*. They were obtained from Boss Laboratories, Madurai, Tamil Nadu and India.

Bio synthesis of silver Nanoparticles

In a typical reaction procedure, 10 ml of refrigerated filtrate was treated with 90ml of AgNO₃ (1mM) solution. The resulting solution was incubated in dark (to minimize the photo activation of silver nitrate), at 37°C under static condition. The development of yellowish brown color solution indicated the formation of Ag-Np's (Figure 1). the colored Ag-Np's solution was centrifuged at 10,000 rpm for 10 min, the supernatant liquid was decanted. The resulting suspension was re dispersed in 10 ml sterile distilled water and centrifugation process was repeated for three times. Thereafter, the purified suspension was used for characterization of Ag-Np's⁸.

Characterization of silver Nanoparticles

The reduction of pure Ag⁺ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 h after diluting a small aliquot of the sample into distilled water. The color change in reaction mixture (metal ion solution + *Lantana Camara* extract) was recorded through visual observation. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu) at the wavelength of 200– 800 nm⁹. The particle size range of the Ag-Np's was determined by using particle size analyzer, Mastersizer 2000. The particle size was determined based on the Brownian motion of the nanoparticles. Transmission Electron Microscope (TEM) was used to view the morphology and size of bio synthesized Ag-Np's in Philips model CM 200 instrument operated at an accelerating voltage of 200 kV¹⁰.

Assay for antimicrobial activity of Ag Nanoparticles against microorganisms

The antimicrobial activity of aqueous extract of *Lantana Camara* and Ag-Np's synthesized from *Lantana Camara* was determined by using disc diffusion method. Two gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) and two gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) were used for this study. The organisms were sub-cultured on Mueller Hinton Agar medium (MHA), incubated at 37°C for 24 h and stored at 4°C in the refrigerator to maintain stock culture. Petri plates were prepared with 20 ml of sterile MHA medium. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted with

three different level of concentrations at 100,200 and 300 µg /ml respectively of aqueous extract and Ag-Np's suspension prepared from the extract. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Amikacin (50 µg/ml) was used as a standard positive control. The plates were incubated for 24 h at 37°C. A zone of inhibition was recorded in millimeters¹¹. The results of both crude extract and Ag-Np's from crude extract of *Lantana Camara* given in Table 1& 2; Figure 5 & 6.

Anti fungal screening

Fungus cultures of *Candida albicans* and *Aspergillus niger* were used for this study. The anti fungal activity was performed according to the standard reference method. The initial concentration of aqueous extract and Ag-Np's suspension of *Lantana Camara* was 100µg/ml. The initial test concentration was serially diluted twofold. Each one was inoculated with 50µg/ml of suspension containing 104spore/ml of fungi. The anti fungal agent ketokonazole was included in the assays as a standard positive control. The plates were incubated between 24 h and 72 h at 27 °C¹². The results are given in Table 3& 4; Figure 7 & 8.

RESULTS AND DISCUSSION

UV-Vis spectra analysis

It is well known that Ag-Np's exhibit yellowish brown color in aqueous solution due to excitation of surface Plasmon vibrations in Ag-Np's. The plant *Lantana camara* aqueous extract when mixed with the aqueous solution of the silver nitrate, silver ion complex produced and this complex was responsible for changing the color from watery to yellowish brown due to reduction of silver ion, which may be the indication of formation of Ag-Np's¹³. The UV- spectrum of *Lantana camara* Ag-Np's was recorded from the reaction medium. The results showed maximum absorption peak was exhibited between 322 – 350 nm (Figure 2).

Transmission electron microscopy (TEM)

The pictures below obtained by TEM shows the Ag-Np's in aqueous extract of *Lantana camara* leaves. TEM analysis reveals that the Ag-Np's are predominantly spherical. The overall morphology of the Ag-Np's produced by reduction of Ag⁺ ions with 1mM AgNO₃ is composed of almost uniform Nanoparticles⁹.

Particle size distribution

The particle size range of Ag-Np's synthesized from *L. camara* was monitored by using particle size analyzer Mastersizer 2000. The obtained graph showed that Ag-Np's average size range is 0.772 µm.

Antimicrobial activity of biosynthesized Ag-Np's using *Lantana camara* leaf extract

The antimicrobial effects of silver salts have been noticed since ancient times¹⁴. The mechanism of Ag-Np's is not well known, it may attach to the cell wall, thus disturbing cell-wall permeability and cellular respiration. The Nanoparticles may also penetrate inside the cell causing damage by interacting with phosphorus and sulfur containing compounds such as DNA and protein.

Table 1: In vitro antibacterial activity of aqueous leaf extract of *Lantana camara*

Drug	Con:µg/ml	Zone of inhibition (mm)			
		<i>B. subtilis</i>	<i>S.aureus</i>	<i>P.aeruginosa</i>	<i>E.coli</i>
Aqueous leaf extract <i>Lantana camara</i>	100	10	10	R	10
	200	11	12	R	11
	300	13	13	12	13
Standard (Amikacin)	50	20	16	18	17

Table 2: In vitro antibacterial activity of Ag-Np's using *Lantana camara* leaf extract

Drug	Con:µg/ml	Zone of inhibition (mm)			
		<i>B. subtilis</i>	<i>S.aureus</i>	<i>P.aeruginosa</i>	<i>E.coli</i>
<i>Lantana camara</i> Ag-Np's	100	11	10	12	14
	200	14	12	14	14
	300	14	12	14	14
Standard (Amikacin)	50	20	18	17	17

Table 3: In vitro antifungal activity of aqueous leaf extract of *Lantana camara*

Drug	Con:µg/ml	Zone of inhibition (mm)	
		<i>C.albicans</i>	<i>A.niger</i>
Aqueous leaf extract <i>Lantana camara</i>	100	9	10
	200	11	11
	300	14	14
Standard (ketokonazole)	50	16	16

Table 4: In vitro antifungal activity of biosynthesized *Lantana camara* Ag-Np's

Drug	Con:µg/ml	Zone of inhibition (mm)	
		<i>C.albicans</i>	<i>A.niger</i>
<i>Lantana camara</i> Ag-Np's	100	17	25
	200	18	25
	300	18	25
Standard (ketokonazole)	50	17	20

Figure 1: the photograph showing color change of plant extract after adding AgNO₃ (a) *Lantana Camara* extract (b) AgNO₃ solution (c) Ag-Np's Figure 2: UV-Vis spectra of Ag Nanoparticles biosynthesized from leaf extract of *Lantana camara* Figure 3: TEM images of Ag-Np's synthesized using leaf extract of *Lantana camara*. Particle size distribution Figure 4: Particle size distribution of Ag-Np's from leaf extract of *Lantana camara*

Figure 5: Anti bacterial activity of aqueous leaf extract of *Lantana camara*, A- *B. subtilis*, B - *S. aureus*, C- *P. aeruginosa*, D- *E. coli* '1','2' and '3' represents zone of inhibition of the extract at the concentration of 100, 200 and 300 µg/ml respectively. The centre zone 'S' represents zone of inhibition of standard antibacterial agent.

Figure 6: Anti bacterial activity of Ag-Np's biosynthesized from leaf extract of *Lantana camara*, A- *B. subtilis*, B - *S. aureus*, C- *P. aeruginosa*, D- *E. coli*; '1','2' and '3' represents zone of inhibition of Ag-Np's using *L. camara* leaf extract at the concentration of 100, 200 and 300 µg/ml respectively. The centre zone 'S' represents zone of inhibition of standard antibacterial agent

Figure 7: Inhibition of fungal growth of aqueous leaf extract of *Lantana camara*, E-*C. albicans*, F-*A. niger* '1','2' and '3' represents zone of aqueous leaf extract of *L. camara* at the concentration of 100, 200 and 300 µg/ml respectively. The centre zone 'S' represents zone of inhibition of standard antifungal agent (ketokonazole) at the concentration of 50 µg/ml and 'C' represents zone of control

Figure 8: Inhibition of fungal growth by *Lantana camara* Ag-Np's, E-*C. albicans*, F-*A. niger* '1','2' and '3' represents zone of inhibition of biosynthesized Ag-Np's using *L. camara* leaf extract at the concentration of 100, 200 and 300 µg/ml respectively. The centre zone 'S' represents zone of inhibition of standard antifungal agent (ketokonazole) at the concentration of 50 µg/ml and 'C' represents zone of control

Another possible contribution to the bactericidal properties of Ag-Np's is the release of silver ions from particles¹⁵. Here the antimicrobial activity of Ag-Np's prepared from leaf extract of *Lantana camara* have been investigated against *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli* and fungus cultures *C. albicans* and *A. niger* by Disc diffusion method on Mueller-Hinton broth. The antibacterial activity of aqueous extract of *Lantana camara* and Ag-Np's from *Lantana camara* showed great response against all investigated microorganism. The diameters of growth inhibition zone were analyzed by different concentration of aqueous extract of *Lantana camara* and Ag-Np's. The inhibition zone ranged from 100 to 300 µg/ml with highest inhibition zone values observed in 300 µg/ml in both prescribed samples, when compared with values from Table 1 & 2; Ag-Np's from *Lantana camara* had greatest level of resistance showed *B. subtilis*, *P. aeruginosa*, *E. coli* (the zone inhibition 14 mm), but aqueous extract of *Lantana camara* showed (13mm is the zone of inhibition). It is clearly revealed that aqueous leaf extract of *Lantana camara* Ag-

Np's at the concentration of 300 µg/ml showed highest activity on Gram-positive and Gram-negative bacteria which are comparable with the aqueous extract of *Lantana camara* and Amikacin. The antifungal effects of the aqueous extract of *Lantana camara* and Ag-Np's of the extract of *Lantana camara* are shown in Table 3&4. The results showed both prescribed samples had significant effect against the above two organisms (*C. albicans* and *A. niger*). The zone of inhibition of Ag-Np's extract of *Lantana camara* showed 18 and 25 mm respectively at 300 µg/ml concentration, which is a greater than that of the aqueous extract of *Lantana camara* (14 and 14 mm respectively at 300 µg/ml concentration). From this study, it is clearly revealed that Ag-Np's prepared from the extract of *Lantana camara* showed better antifungal activity against selected organisms which is comparable with the aqueous extract of *Lantana camara* and standard control, ketokonazole.

CONCLUSION

In the present study, it was disclosed that aqueous leaf extract of *Lantana camara* can be converted into Ag-Np's by green synthesis method. The synthesized Ag-Np's from *Lantana camara* aqueous leaf extract was compared with aqueous leaf extract of *Lantana camara* for antibacterial activity with various concentrations. Based on the results we conclude Ag-Np's from *Lantana camara* showed better antimicrobial activity than *Lantana camara* aqueous leaf extract on pathogenic microorganisms. The reported results suggested that green synthesized *Lantana camara* -Ag-Np's could be used in the medical field for their efficient antimicrobial activity after undertaking proper clinical trial.

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Cite this article as:

Kumarasamyraja D, Jeganathan N. S. Antimicrobial activity of biosynthesized silver nanoparticles prepared from the leaf extract of *Lantana camara*. Int. Res. J. Pharm. 2013; 4(5):203-207

Source of support: Nil, Conflict of interest: None Declared