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RESEARCH ARTICLE

Green Synthesis of Silver Nanoparticles using *Lantana camara* Leaves Extract and *In Vitro* Evaluation of Oral Anti-cancer Activity

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Abstract

At present, green synthesized based drug delivery systems play a major role in the bio medical field. *Lantana camara*, also known as Unni chedi in Tamil, is an evergreen shrub that is involved in a variety of cancer diseases due to its pharmacological activity. In addition, the leaf extract of *Lantana camara* was found to have excellent anti cancer activity. The green synthesized AgNPs of *Lantana camara* leaves extract were more efficient against oral cancer. In this report, green synthesized nano-formulations in presence of AgNPs were formulated. The as-prepared formulation was confirmed by UV spectroscopy, particle size and zeta potential, Scanning Electron Microscopy (SEM). In UV Spectroscopy the absorbance of the peak appears between the range of 200 to 300 nm corresponding to the formulation of AgNPs. The Zeta potential shows high colloidal stability; the average potential value is -12.6mV. The particle size results shown that the average size of AgNPs is 268.1 nm. The morphology and particle size determined using SEM analysis indicate spherical shaped particles. Further, the oral anti-cancer activity was evaluated on Squamous Cell Cancer (SCC-25) cell line. The cell line result shown is that the IC 50 value for AgNPs was 39µg/ml and the plant extract shown 145µg/ml, this indicates that the silver nanoparticles have more potential when compared to the leaf extract.

Keywords: AgNPs, Green Synthesis, *Lantana camara*, Oral Carcinoma, Plant Extract, SCC-25

1. Introduction

Plants are a vital source of medicinal compounds. Also, many health issues were treated with medicinal herbs in antiquity. The examination of plants yields a wide range of bioactive compounds. Numerous plants have been studied and reported on for a variety of therapeutic properties[1].

Lantana camara is also known as *Lantana*[2]. It is a flowering ornamental plant. It belongs to the family: Verbenaceae, a kingdom: Planate, division: Magnoliophyta, Class: Magnoliopsida, Order: Lamiales, genus: *Lantana*, species: *Lantana camara* Linn shown in Figure 1. It is commonly called as "Unni chedi" in Tamil[3]. It has also been used as a folk remedy. Lantadene A and Lantadene B were two major constituents found in the plant. Extracts from the leaves have been reported scientifically with many pharmacological activities such as antibacterial, antioxidant, antifungal, antidiabetic, wound healing and anti-cancer properties[4,5].

Figure 1. *Lantana camara* plant.

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A cancer that starts in the tissues of the mouth or throat is called oral cancer[6]. Oral cancer is affected by various bio-environmental factors such as fatal illness, and in the mouth, it might result in plaques, foul breath, or ulcers. Moreover, coughing and swollen lymph nodes in the neck are typical signs[7]. The *Lantana camara* Linn plant is being studied for its potential as an oral anticancer because of its inherent ability to treat mouth ulcers. Squamous cell cancer makes up more than 90% of oral and oropharyngeal cancer cases[8].

In general, silver (Ag) has excellent wound healing properties[9]. Silver nanoparticles (AgNPs) are used in antimicrobial applications[10]. These can be pragmatic in food science and anticancer medicines. AgNPs are synthesized by physical, chemical and biological route[11]. When compared to the biological synthesis of nanoparticles, the physiochemical method has difficulty applying to large-scale production of high temperatures and harmful chemicals[12]. According to recent research, nanoparticles can be synthesized by using plant extracts such as leaf extract, root extract, seed extract, flower extract, fruit extract[13].

Hence, green synthesis of AgNPs using leaf extracts of *Lantana camara* might be an effective drug for oral cancer. The pharmacological activity of *L. camara* is shown in Figure 2.

Figure 2. Pharmacological activities of *L. camara*.

2. Materials

Fresh leaves of *Lantana camara* have been picked in Echanari, Coimbatore. Milli-Q water was used as a solvent for the plant extract. Silver nitrate ($AgNO_3$) was procured from Jayam Chemicals, Coimbatore.

3. Methods

3.1 Preparation of Leaf Extract

Lantana camara Linn plant leaves were freshly collected and dried. Using a motor, the leaves were crushed into a fine powder. 100 ml of Milli-Q water should be added to 10g of leaf powder[7]. The plant aqueous mixture was then heated at 60°C for 15 minutes. Plant extracts were obtained and then passed through Whatman No. 1 filter paper. The extracted final product was kept at room temperature. The preparation of leaf extract is shown in Figure 3.

3.2 Synthesis of Silver Nanoparticles (AgNPs) using $AgNO_3$

0.001 M $AgNO_3$ (50 ml) solution was prepared in a 100 ml volumetric flask for the synthesis of AgNPs. Further, 10 ml of aqueous extract of *Lantana camara* Linn were added to the $AgNo_3$ solution. The prepared solution was stirred for 5–7 minutes and allowed to cool to room temperature. To avoid photo-inactivation of silver nitrate, the reaction of the mixture solution is incubated in a dark room at 37°C. The yellowish brown appearance confirms the formation of AgNPs[14].

3.3 UV Spectroscopy

UV–vis spectroscopy is generally employed for confirming the wavelength number of metallic Nanoparticles (NPs) formulations due to their physico-chemical properties. At first, NPs were visualized through the color change from colorless to yellow-brown immediately after the addition of leaf extract due to the collective oscillation of the free conduction electrons caused by the electromagnetic field in the interaction between Surface Plasmon Resonance (SPR) and UV-Vis. Aqueous spectra of the AgNPs were prepared and confirmed at maximum value[12].

3.4 Particle Size

The mean diameters and distribution sizes of nanocomposites were determined by using an analyser sizer.

3.5 Zeta Potential

A zeta sizer was used to measure the electrophoretic mobility of the nano-formulation. The electrophoretic

mobility (zeta potential) values were resolved using a zeta dip cell after the nanocomposites were placed in a polystyrene cuvette[7].

3.6 SEM

The surface morphology of prepared nanoparticles was characterized using SEM. SEM analysis was carried out using a Zeis EVO analyzer[15].

3.7 Cell Line Studies

3.7.1 Cell Culture and Cytotoxicity Assay

The SCC-25 cell was procured from NCCS, and the cells were cultured medium in DMEM. MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazodium bromide) cell proliferation assay was performed to findout the cytotoxicity on SCC-25 cell lines. The prepared formulation was trypsinized and the cells were counted using a hemocytometer. In 96-well plates, ~ 10,000 cells per well were seeded, and they were incubated for one day. After that, the cells were transferred to fresh culture medium. Different concentrations of sample (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml) were added in triplicates to the cells. After incubation at 37±1 °C for 18 h, the MTT (1mg/1ml) was added to the wells and incubated for 4 hours. After incubation, DMSO was added to the wells and read at 570 nm using a photometer.

Figure 3. Preparation of AgNPs.

4. Results and Discussion

4.1 Characterization of Nano-formulation (UV)

Initially, the formation of synthesized nanoparticles was identified by monitoring a change in the solution's colour from pale yellowish green to brown. When *Lantana camara* leaf extract was treated with silver nitrate, the colour changed from pale yellowish green to brown, indicating that the silver ion was reduced, indicating the creation of silver nanoparticles. The excitation of surface plasmon vibrations in AgNPs causes this colour. They are surface-active compounds that are extremely important in the reduction and stabilisation of silver nanoparticles.

4.2 UV Absorption

The preliminary evaluation of AgNPs was based on the UV-VIS absorbance spectra. The λ max (maximum 200 to absorbance) appears at the range of 300 nm.

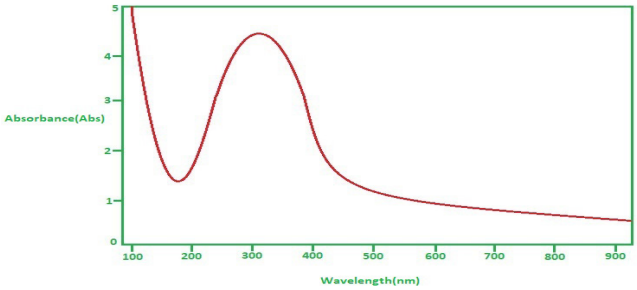

Figure 4. UV spectra of AgNPs.

This range from 200 to 300 nm indicates very small nanoparticles, as shown in Figure 4. The absorbance intensity indicates the formation of silver nanoparticles.

4.3 Zeta Sizer

The anti-solvent effect of the formulation on the particle size was studied using particle size measurement at regular intervals, viz. 7, 14, 30 and 45 days. Prepared AGNPs were kept at room temperature, where they were dissolved in the aqueous phase, and their size was measured by using zeta seizer[15].

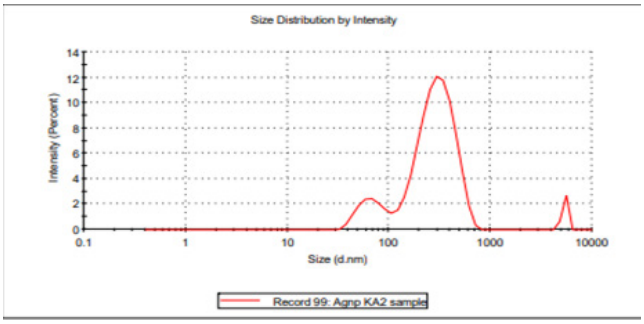

Figure 5. Zeta sizer for AgNPs.

From this result, the average size of the prepared AgNPs is around 268.1nm. The size of the particles was determined and ranges from 200 to 400 nm.

4.4 Zeta Potential

The zeta potential of the prepared AgNPs is also illustrated below, as per the formulated nano-formulation.

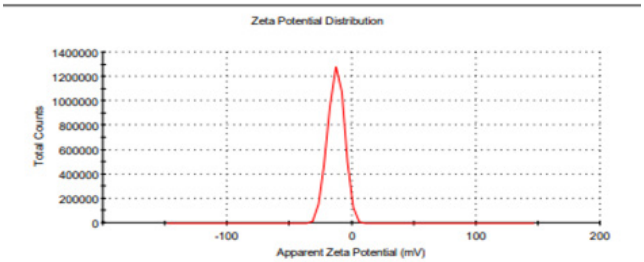

Figure 6. Zeta potential of AgNPs.

According to the graph, the average values of zeta potential were -12.6 mV, which indicates that the AgNPs (Figures 5 and 6) were very stable. The capping layer of phytoconstituents found in the leaf extract may be responsible for the higher negative value. Negatively charged particles repel one another, preventing agglomeration. As a result, nanoparticle stability increases[14].

4.5 Scanning Electron Microscopy (SEM)

Scanning electron microscopy analysis indicates the morphology of prepared nanoparticles. In this report, without adding any reducing agent, the reducing potential of *Lantana camara* leaf extract was examined for synthesizing silver nanoparticles. The morphological image shown in Figures 7, 8 and 9 with various magnifications. This indicates the prepared formulation was spherical in shape.

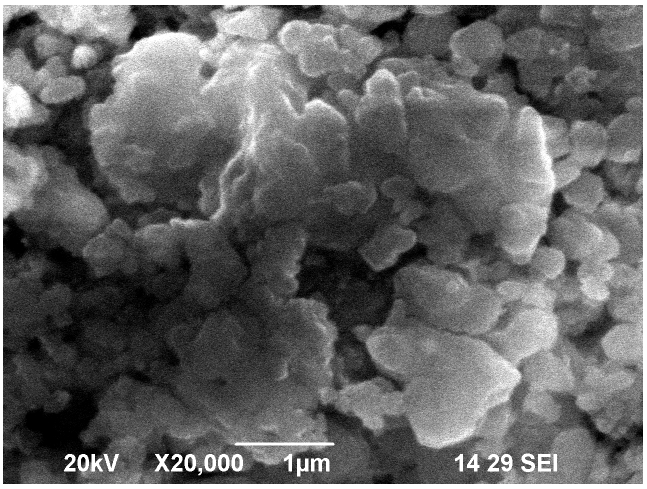

Figure 7. SEM analysis 20,000 X magnification.

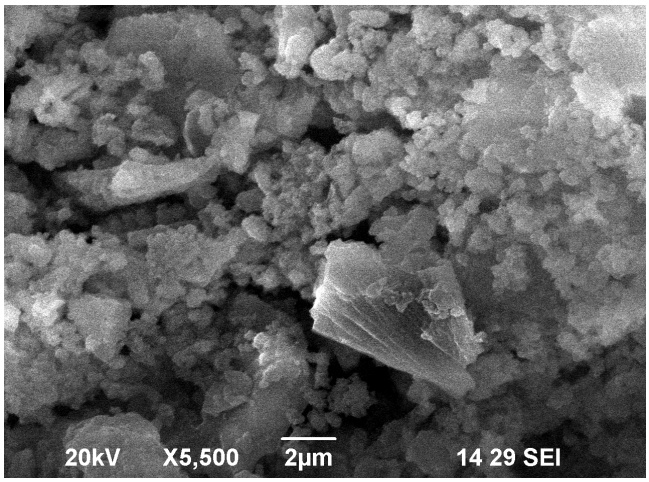

Figure 8. SEM analysis 5500 X magnification.

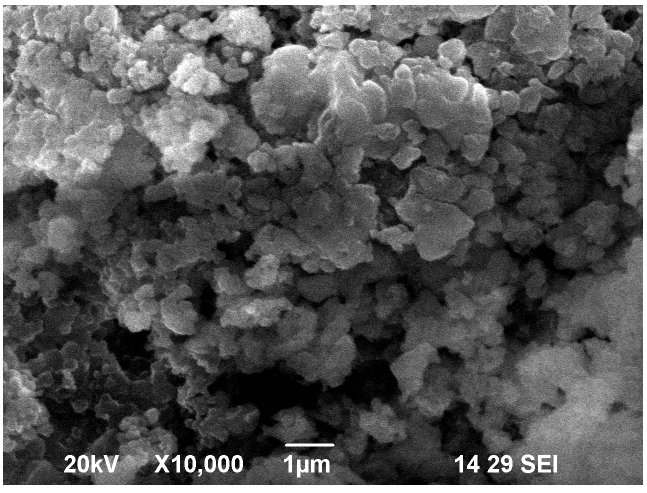

Figure 9. SEM analysis 10000 X magnification.

4.6 Cell Line Studies

The results of *in vitro* tests to assess the cytotoxicity profile of synthesized silver nano-formulation and plant extract on SCC-25 cells were 145 g/ml and 39 g/ml, respectively, as shown in Figure 10. When compared to plant extract, silver nanoformulation clearly shows threefold increases in cell inhibition against the SCC-25 cell line of oral cancer.

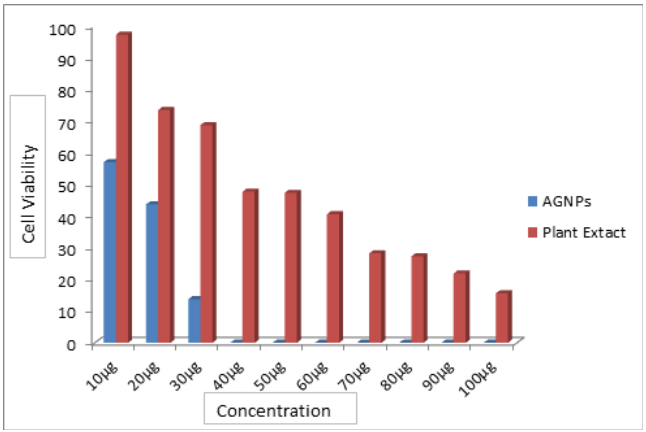

Figure 10. Cell line *in vitro* study (Inhibition).

5. Summary and Conclusion

The goal of this work was to develop silver nanoparticles from *Lantana camara* leaf extract that could be used to overcome oral cancer. Toxicity, surface plasmon resonance, and electrical resistance of silver nanoparticles are also known to have particular functions. The toxicity of AgNPs extracted from *Lantana camara* leaves will be effective in causing cancer cell death. Since the plant extract was synthesised with silver nitrate to make silver nanoparticles, *Lantana camara* is effective against several carcinomas. These particles were green synthesized and evaluated by UV spectroscopy, Zeta sizer, zeta potential, Scanning Electron Microscopy and *in vitro* cell lines studies. The results indicated that the formed nanoparticles were 268.1 nm in size and the potential was found to be -12.6 mV, which infers the stability of the formulation. The morphology of the particles was found using SEM results. These AgNPs were very effective against the oral cancer cell line SCC-25 with a minimum concentration. These merits attribute to the need for further *in vitro* studies and diagnosis of the *in vivo* system in various models.

6. Acknowledgements

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7. References

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