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Research Article

Synthesis and Evaluation of the Silver Nanoparticles for the Anticancer Activity using *Cardiospermum Halicacabum* Extract.

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Abstract

Green synthesis is the safe and easiest method of producing silver nanoparticles. The silver nanoparticles found to have the antibacterial activity because of the production of the silver ions. The *cardiospermum halicacabum* is the plant specimen found to have the anticancer property by its nature. The extract was prepared by using the solvent evaporation technique. The plant extract in combination with the silver nitrate forms the silver nanoparticles. The silver nanoparticles synthesized were confirmed under the UV visible spectroscopy. The sample underwent SEM analysis to find the particle size of the silver nanoparticles. It is a vital to develop newer research studies in order to identify the novel formulations out of the natural specimens for the cancer disease. The synthesized silver nanoparticles show a marked effect on the cell lines of lung A-549 carcinoma cells in low concentration. The *in-vitro* anticancer activity of the silver nanoparticles was confirmed by using MTT Assay.

Key words: Silver Nanoparticles, Green Synthesis, Spectroscopy, Scanning Electron Microscope (SEM).

Introduction

The history of medicine dates back perhaps to the origin of human race. Throughout the ages, human beings relied upon the nature for their basic needs, be it for food, shelter or medicine. Plants have formed the basis of sophisticated traditional systems that have been in existence for thousands of years in countries such as India, China and Africa. Before modern scientific approaches to drug therapy, trial and error, cures or least preventative therapeutic experiences were drawn from herbal medicines.

Modern science has unarguably improved upon drug therapy, leading to miraculous chemical cures. Parallel to the onset of industrial revolution, the practice of allopathic medicine has gained popularity. Herbal medicine was also an effective healing method, but was viewed less enthusiastically. Herbal products were discarded from conventional medicinal use in the mid 20th century, not necessarily because they were ineffective but because they were not as economically profitable as the scientific methods, which become more advanced and preferred. The practice of herbal drug therapy was dismissed as quackery. Number of patients seeking the alternative and herbal therapy is growing exponentially. There has been a resurgence of interest on plants and plant derived products as a source of medicine in the last few decades.

In spite of advances in the modern system of medicine, there are still certain chronic and difficult diseases to treat such as Acquired Immuno deficiency Disease (AIDS), Cardio vascular diseases, liver diseases, cancer, diabetes etc which require newer and effective drug. There is an urgent need to identify novel, active chemo types as leads for effective drug development in many therapeutic areas. There is a need to re-emphasize and enhance research in natural products, especially because only a small fraction of plant species have been investigated so far.

Cancer has been recognized as a global health burden in rates of morbidity and mortality and indeed, it has been predicted that by 2030, cancer will have caused 12 million deaths worldwide. Much effort has been devoted to discover promising cancer therapeutic agents from natural sources, and the development of reliable green process for the synthesis of silver nanoparticle is an important aspect of current nanotechnology research. Nanomaterial out of Ag, Au, Pt and Pd has been synthesized by different methods. Biosynthesis using bacteria, fungi, yeast and plants were well documented. However, exploration of the plant systems as the potential Nano factories has heightened interest in the biological synthesis of nanoparticle. The present study was aimed for the rapid synthesis of silver nanoparticles using the Ethanolic leaf extract of *cardiospermum halicacabum* and to evaluate its anticancer activity against the lung cancer cell line, A-549. The leaves are used for rheumatism, nervous disease, hemorrhoids and chronic bronchitis. The juices of the leaves are put into the ear for earache.

Materials and Methods

Materials

The *cardiospermum halicacabum* plant specimen was collected and the processed. All the chemicals of A.R. grade were purchased from the Sigma – Aldrich Pvt. Ltd (Mumbai).

Methodology

Preparation of the leaf extract[5]

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The fresh leaves of the *cardiospermum halicacabum* approx. 1kg were washed thrice with the distilled water until it is completely cleaned. It was then air dried for 10 to 15 days. Followed by it was finely milled and taken in a round bottom flask. To this add 300ml of the ethanol until the powder completely soaks into it. It is macerated for 1 week. Then the extract was filtered and vacuum distillation was done to remove the excess solvent. It was then passed through the Millipore filter paper and the filtrate is stored at 4^0C for the further use.

Synthesis of the Silver Nanoparticles[5, 7]

5ml of the leaf extract was taken and added to the 50ml aqueous solution of 1mM silver nitrate in an Erlenmeyer flask (200ml). It was then stirred by using the magnetic stirrer for 1hr with 50rpm. Then it is kept aside for 24hrs. The marked color change was observed when compared to the control silver nitrate solution (without leaf extract). The color change confirms the indication for the synthesis of the silver nanoparticles.

Purification of the Silver Nanoparticles[5-7]

The synthesized silver nano particles were washed by using the distilled water until the clear solution is obtained. Then the washing was carried out by filling it into the centrifuge tube and rotated at 2000rpm for 5min. The precipitate was washed with the distilled water and this procedure was repeated until clear solution is obtained. The excess silver ions were washed out by repeated centrifugation. The nano sized particle is redispersed with Ethanol and it is used for the further analysis.

Research Design and Methodology

The silver nanoparticle was synthesized by using the green synthetic method. The synthesized samples were evaluated for stability over a period of time by storing it in room temperature for a week and simultaneous UV-Visible analysis was performed. SEM is widely useful in surface morphology of the prepared nanoparticles. Precise measurement of very small features and objects down to 50nm in size is also accomplished. The particle size was found using the SEM analysis.

Evaluation using UV- Visible Spectroscopy[6, 7]

The particles were confirmed to be in the nano size range by the UV-Vis spectroscopy with the peaks emerging around 400-500 nm which is the characteristic of silver nanoparticles as a result of Surface Plasmon Resonance.[5] It has already been reported that the absorption spectrum of aqueous $AgNO_3$ only solution exhibited λmax at about 220nm whereas silver nano particles exhibited λmax at about 420nm. The peak formed was within 400nm to 420nm respectively and it is shown in the Figure -1.

Evaluation using the Scanning Electron Microscopy (SEM) [5]

The passage of the electron beam was done in order to obtain the particle size of the silver nanoparticles. And scanning electron microscopy was performed to confirm that the silver nano particle is formed within the range i.e.350 – 390nm and it was shown in the Figure- 2. Scanning Electron Microscopy Analysis of Green Synthesized Silver Nanoparticles

Evaluation by *In vitro* cytotoxicity studies[5]

The nanoparticles evaluated for size selectivity by using UV-Vis Spectroscopy and Scanning Electron Microscopy was taken for the in vitro cytotoxicity studies. The cytotoxicity study was performed by using the lung cancer cell line A – 549 cells by MTT Assay. A-549 Lung adeno carcinoma cells were plated in 96 well plates at a concentration of 5×10^4 cells per plate and incubated for a period of 24hrs. The cells were, washed twice with serum free media, and were starved by incubation for an hour at 37°C. After an hour of starvation, cells were treated with different concentrations of silver nano particle of *cardiopermum halicacabum* and the isolated fraction in the range 7.5µg, 15µg, 30µg, 60µg, 120µg respectively for 24hours. At the end of the treatment, media from control and treated cells were discarded and 200µl of MTT containing MEM, (0.5mg/ml) was added to each well. Cells were then incubated for 4hours at 37°c in CO_2 incubator. MTT containing medium was then discarded and the cells were washed with Phosphate buffer saline (1ml). Crystals were then dissolved by adding 200µl of DMSO and the plates were placed on a shaker for 5minutes and the spectrophotometric absorbance of the purple blue formazan dye was measured using ELIZA reader at 570 nm. The percentage growth inhibition was later calculated and it was shown in the Table-1. The cell viability based on the Concentration vs. Absorbance was also reported and is shown in the Graph -1.

Discussions

Evaluation of UV- Vis Spectroscopy

The reduction of Ag+ to Ag was monitored by measuring the UV-Vis spectrum of each reaction mixture (silver nitrate solution + extract) at different time intervals within the range of 400-800nm in the UV-Vis spectrophotometer. The reduction of the silver nitrate to silver ions is due to the NADH- dependent nitrate reductase in the plant extract.[4, 9, 10]

Evaluation using the Scanning Electron Microscopy (SEM)

The particle size range for the silver nano formulation using the *cardiospermum halicacabum* extract is in the size range of 350-390nm. The particle size range seems to be effective.

Evaluation By *In -Vitro* Cytotoxicity Studies

The exposure of the cells to lesser concentration is optimum to shows characteristic features of cell shrinking, rounding and partial detachment. The minimum inhibitory concentration was noted at which no

visible growth of the cells was found to be 0.625 µg/ml. These particles penetrates through the ion channels without causing damage to cell membranes and denatures the ribosome and suppresses the expression of enzymes and protein. This could be the main reason for the anticancer activity.

Conclusion

The silver nanoparticles, possibly due to their surface Plasmon resonance activity, release a quantum confinement of energy which degrades the protein. The high metabolic activity of cancer cells need to be maintained appropriately in an order by its cellular contents. Due to the interference of the silver nanoparticles with the regular cellular activity by supplying the excess energy the mechanisms like electron transport chain which is the backbone of the signal command can be altered. By doing so the metabolite supply can be deprived or possible apoptotic cycle activation can be possible explanation behind the cell death.

Acknowledgement

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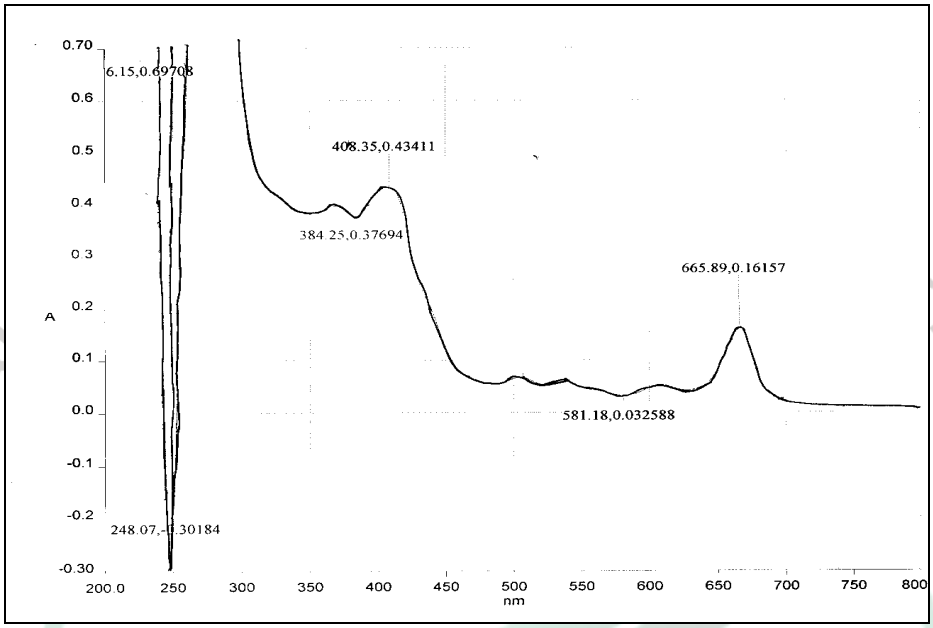

Figure -1 UV –Visible spectroscopy of formulated Silver Nanoparticles of *Cardiospermum halicacabum* extract.

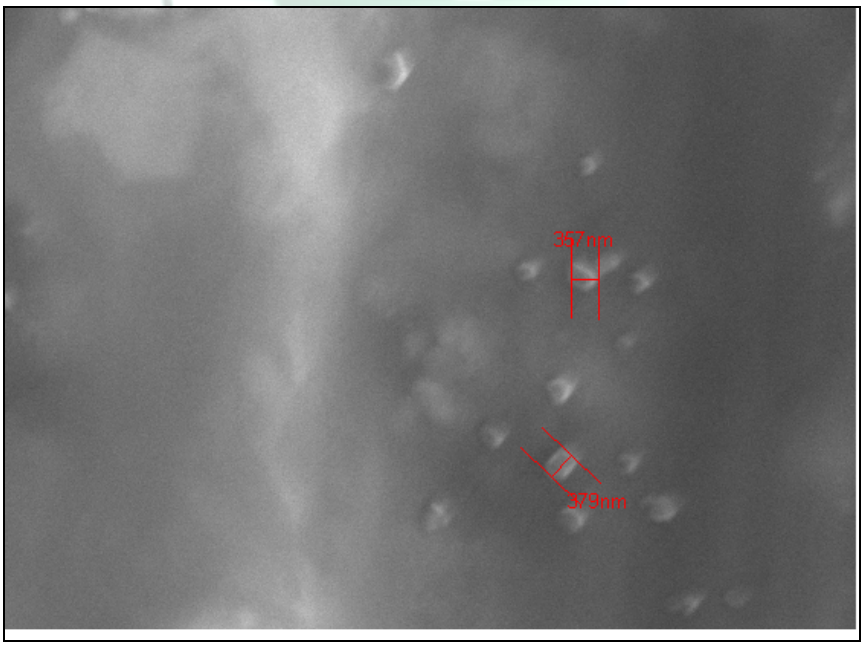

Figure – 2 Scanning Electron Microscopy of formulated Silver Nanoparticles of *Cardiospermum halicacabum* extract.

Table – 1 *In-vitro* cytotoxicity studies for the formulated Silver Nanoparticles of *Cardiospermum halicacabum* extract.

Conc (in µg/ml)	Cell Viability	% growth inhibition
Control	2.80	-
0.078	1.79	38.91
0.156	1.33	47.75
0.312	0.29	83.10
0.625	0.08	98.40
1.25	0.07	97.93
2.5	0.08	97.36
5	0.09	96.68
10	0.12	95.83
20	0.16	94.19
40	0.14	96.86

Graph no-1 *In-vitro* cytotoxicity studies for the formulated Silver Nanoparticles of *Cardiospermum halicacabum* extract.