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EXPLORATION OF ANTIMUTAGENICITY OF PLANT MEDIATED SYNTHESIS OF SILVER NANOPARTICLES USING THE PLANT EXTRACT *Anredera Cordifolia*

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ABSTRACT

Nanotechnology is a rapidly growing field with its numerous applications in science and technology. Mutation is an important factor in inducing carcinogenesis. This study reports that the antimutagenic activity of the plant extract *Anredera Cordifolia* is used to reduce the exposure of mutation and its inducing agent. The antimutagenic activity was assessed in the silver nanoparticle synthesized plant extract of *Anredera Cordifolia*. The synthesized silver nanoparticle was characterized using SEM analysis, X-Ray diffraction and UV-Visible spectroscopy. Ames test was performed. Antimutagenic activity of the silver nanoparticle synthesized plant extract *Anredera cordifolia* was tested against *Salmonella typhimurium* strains TA 98, TA 100 using direct acting mutagens and those needing activation by Ames test. Determination of antimutagenicity against mutagens was assayed using plate incorporation method. The activity indicates that silver nanoparticles of *Anredera Cordifolia* are capable of redending antimutagenic efficiency.

KEYWORDS: Silver nanoparticle, antimutagenic, *Anredera cordifolia*.

1.0 INTRODUCTION

Cancer is associated with abnormal uncontrolled cell growth or cell proliferation [1]. Cancer is caused by both external factors such as tobacco,chemicals, radiation and infectious organisms and internal factors like inherited mutations,hormones, immune conditions, and mutations

that occur from metabolism. Cancer is a significant worldwide health problem generally due to the lack of widespread and comprehensive early detection methods, the associated poor prognosis of patients diagnosed in later stages of the disease and its increasing incidence on a global scale.Indeed, the struggle to combat cancer is one of the greatest challenges of mankind.[2]

Carcinogenesis is a multistep process which accompanies molecular alterations that drive the progressive transformation of normal cells into highly malignant derivatives. One of the noticeable characteristics of malignant cancer cells is the ability to survive and grow in the absence of anchorage to an extracellular matrix.[3, 4]

Chemoprevention by plant-derived compounds or dietary phytochemicals has emerged as an accessible and promising approach to cancer control and management process.[5] Anticancer properties of many natural compounds isolated from different Indian plants have been reported. Research is being carried throughout the world to find a lead compound which can block the development of cancer in humans. Nature has always been a great contributor towards this goal. Plant-derived natural products such as flavonoids, terpenoids and steroids have received considerable attention due to their diverse pharmacological properties,which include cytotoxic and chemopreventive effects.[6]

Biomedical nanoscience has a huge potential to bring benefits in detection, diagnosis and treatment of various diseases, with fewer side effects and better quality of life for the patients [7, 8].Silver has long been recognized for having an inhibitory effect towards many bacterial strains and microorganisms commonly present in medical and industrial processes [9]. The most widely used and known application of silver and silver nanoparticles include topical ointments and creams containing silver to prevent infection wounds [10]. Many attempts have been made to use silver nanoparticles as an anti-cancer agent and they have all turned up positive [11]. The role of silver nanoparticles as an anti-cancer agent should open new doors in the field of medicine.

Mutation is an important factor in carcinogenesis. Therefore, the incidence of cancer may be reduced by decreasing the rate of mutation. The best way for humans to decrease the rate of mutation is to avoid exposure to or ingestion of mutagens and carcinogens.[12]

In the present study, we report for the first time synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the aqueous extract of *Andredera cordofolia* leaves.Morphological characterizations are performed using X-ray diffractometer (XRD). The optical absorption spectrum of silver nano particles was recorded by using UV-visible spectrophotometer and further its antimutagenic activity was tested against *Salmonella typhimurium* strains TA 98, TA 100 using direct acting mutagens and those needing activation by Ames test.

2.0 MATERIALS AND METHODS

2.1 Collection of Plant Material

The leaves of Anredera cordifolia were collected from Gudalur and has authenticated (Certificate No. BSI/SRC/5/23/2015/ Tech /406 dated: 24/2/2015) by Botanical survey of India (BSI), Coimbatore division, Tamil Nadu State, India. The leaves were washed with distilled water to remove the soil and other dust particles. After washing the leaves were shade dried and powdered. The powdered leaves were used for the assay.

2.2 Preparation of the Extract

50g of leaf powder was weighed and it is mixed with 100ml of distilled water and boiled for 5 minutes. After cooling the solution was filtered through whatsmann no.1 filter paper. The filterd samples were collected in a conical flask. The obtained extract was used for the synthesis of silver nanoparticles.

2.3 Preparation Of Silver Nitrate Solution

1Mm silver nitrate solution was prepared with and used for synthesis of silver nanoparticles. 10 ml of the plant extract was added in to 200ml of aqueous solution of 1Mm silver nitrate the colour change of the leaf extract from brown to yellow was noted periodically. Then the extract was incubated at room temperature for further incubation till 72 hours. After incubation, the silver nanoparticles were synthesized from the leaf and centrifuged at 10000 rpm for 20 minutes and the pellet was characterised using SEM analysis and X-ray diffraction.

2.4 Antimutagenic Activity

2.4.1 Salmonella mutagenicity test (Ames test)

Salmonella typhimurium strains TA 100 and TA 98 were used for antimutagenicity assays. TA 100 detected mutagens that caused base pair substitutions; TA 98 detected various

frameshift mutagens. Frozen cultures of the tester strains were stored at -20^{0}C. A fresh nutrient broth culture was grown to a density of 1-2X 10^9 cells/ml and for each 1 ml of culture 0.09 ml of dimethyl sulphoxide was added as cryoprotective agent. The bacterial culture was inoculated in fresh nutrient broth and grown for 12 hour at 37^{0}C before each experiment.

Confirming Genotypes of the Salmonella Strains

2.4.2 Histidine Requirement

The histidine character of the tester strains was confirmed by demonstrating the histidine requirement for growth on selective agar plate such as histidine/ biotin plate and biotin control plate. Biotin was also required by all of tester strains because of the UVrB deletion which extended through the biogene. Cotton swab was dipped in the 12 hour broth culture and a single sweep was made across the histidine/ biotin plate. Then, the plates were incubated overnight at 37^{0}C and the growth was examined on the next day.

2.4.3 Rfa Mutation

Strains having the deep rough (rfa) character were tested for crystal violet sensitivity (Ames *et al.*, 1973). 0.1 ml of fresh overnight culture of the tester strains (TA 98, TA 100) was added to a tube containing 2 ml of molten agar at 45^{0}C. The top agar tubes were vortexed for 3 seconds at low speed and poured on nutrient agar plate without histidine and biotin. The plates were tilted and rotated for the even distribution of the top agar on the plates. The plates were placed in a leveled surface and allowed several times for agar to become firm. 10 µl of 1 mg/ml solution of crystal violet was pipette to the centre of sterile paper disc (1/4 inch) and discs were transferred to each of the inoculated plates using sterile forceps. The discs were lightly pressed with forceps to embed it slightly in the overlay. The plates were incubated at 37^{0}C and observed for crystal violet sensitivity.

2.4.4 UVrB Mutation

The UVrB mutation was confirmed by demonstrating UV sensitivity in strains that contain this mutation (Ames *et al.*, 1973). The R- factor strains TA 98, TA 100 were streaked in parallel strips with sterile swabs across the nutrient agar plate. A piece of cardboard was placed over the uncovered plate so that half of each of bacterial streak was covered. The plates were irradiated with a 15 W germicidal lamp approximately at a distance of 35 cm and were irradiated for 8 seconds. The irradiated plated were incubated at 37^{0}C for 12-24 hours.

2.4.5 R- Factor

The R- factor strain TA 98 was tested for the presence of the ampicillin resistance factor. To test for ampicillin resistance, the cultures were streaked across of an ampicillin plate using the procedure as described for confirming the histidine requirement. The non R-factor strain TA 100 was tested on the same plate as a control for ampicillin activity.

2.4.6 Toxicity

For testing the toxicity, minimal glucose agar medium was prepared. Different concentration of the *Anredera cordifolia* extracts 5µl, was added to the minimal glucose agar and mixed well. Minimal glucose agar containing *Anredera cordifolia* extract was poured onto the petridish. Overnight culture of strains TA 98, TA 100 was streaked onto it. The plates were incubated for 24 hours.

2.4.7 Antimutagenic Activity

Antimutagenic activity of the plant was tested in *Anredera cordifolia* strains TA 98, TA 100 using direct acting mutagens and those needing activation by Ames test (Maron and Ames, 1983)[13].

2.4.8 Determination of Antimutagenicity against Acting Mutagens:

Plate incorporation method was done for antimutagenicity assay without microsomal activation. Fresh bacterial cultures of *Anredera cordifolia* strains TA 98, TA 100 (1-2 x 10^9 cells/ml) were mixed with 2 ml of molten agar containing 0.5Mm histidine/ biotin solution, in concentration of plant extract (0.5 mg/ plate) and direct acting mutagens such as sodium azide and ethidium bromide. Further it was spread over minimal glucose agar plates. Plates were incubated for 48 hours at 37^0C and the revertant colonies were counted.

% inhibition = [1-T /M] ×100

T is No. of revertants per plate in presence of mutagen

M is No. of revertants per plate in positive control

The antimutagenic effects of the extract were graded according to percentage inhibitory effect by the extract.When percentage inhibition of mutagen by plant extract was more than 40%, 25-40 and less than 25%, the antimutagenic effect considered strong, moderate and weak respectively.

3.0 RESULTS

Nanotechnology is a quick up and coming regulation not only in physics and chemistry but also in the field of biology. In view of marvellous use of nanotechnology, there is a boost between scientists to carry out research in this most vital discipline. Chemists are extremely paying attention in synthesizing nanoparticles of diverse dimensions employing many of the valuable metals.

3.1 Antimutagenic Activity

Today, bacteria are being used for the assessment of antimutagenic activities of different compounds in a short-time with excellent results. One of the methods used for assessing the mutation prevention properties of a compound in bacteria is the Ames test. Ames test is a worldwide short-term bacterial reverse mutation test specifically designed for screening a variety of new chemical substances and drugs that can produce genetic damage that leads to gene mutations . The Salmonella strains used in the test have different mutations in various genes in the histidine operon, each of these mutations is designed to be responsive to mutagens that act via different mechanisms.[14]

A strain check should also be performed whenever an experiment is performed. The strain check is usually performed with the nutrient broth overnight cultures. Histidine dependence (his), *rfa* marker, *uvrB* deletion and Presence of plasmid pKM101 (ampicillin resistance) should be followed for a complete strain check.

3.1.1 Confirming Genotypes of the *Salmonella typhimurium* Strains TA 98 and TA 1535

The figure 1 showed the growth of Salmonella typhimurium TA98 and TA100 strains on histidine-biotin plates.The growth of *Salmonella typhimurium* TA98 and TA 100 strains was observed on histidine-biotin plates after 24 hours incubation at 37°C. The presence of colony in biotin-histidine medium and absence in control biotin medium showed that these strains were dependent to histidine.

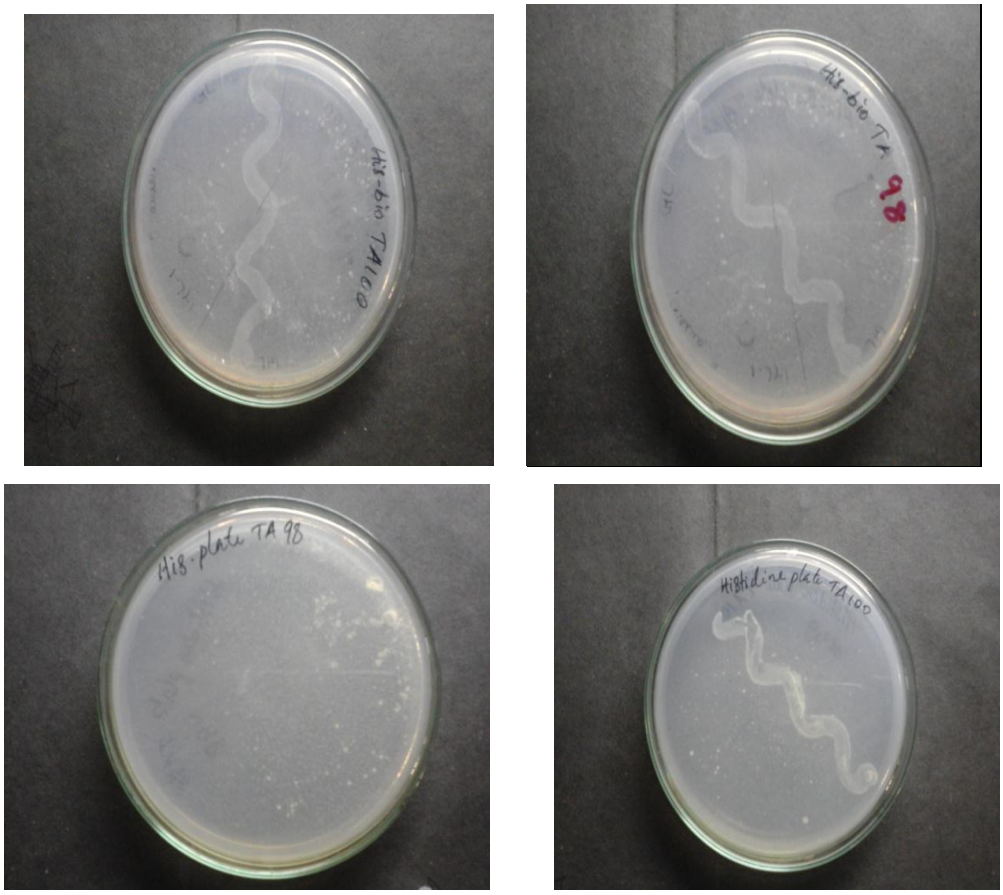


Figure 1 Histidine Requirement of *Salmonella typhimurium*

3.1.2 *rfa* Mutation of *Salmonella typhimurium*

The existence of inhibitory zone around the disk in figure 2 indicated that the tester strain TA 98 and TA 100 did not grow and the *rfa* mutation was occurred. This mutation caused relative decrease of lipopolysaccharide barriers and then, increased the cell wall permeability for bigger molecules like crystal violet.

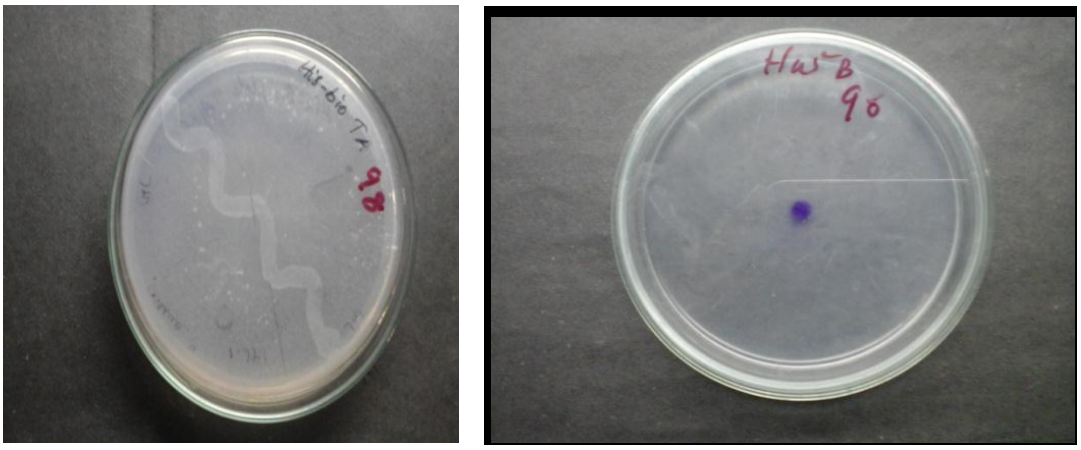
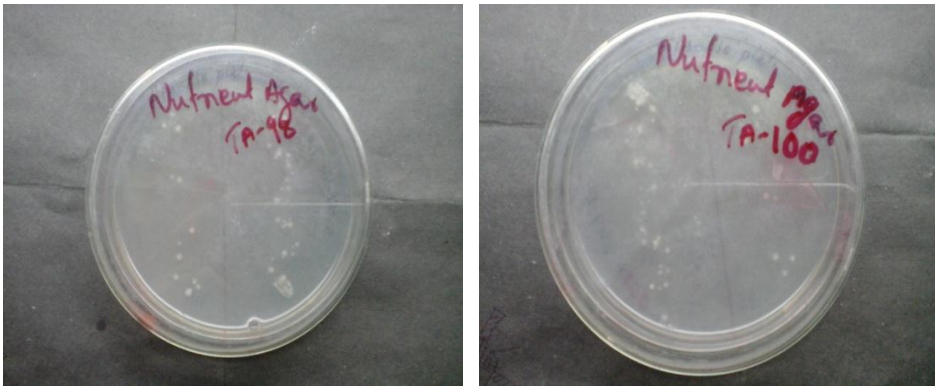


FIGURE 2 *rfa* Mutation of *Salmonella typhimurium*

3.1.3 uvrB Mutation of *Salmonella typhimurium*

The TA 98 and TA 100 strains in figure 3 showed less of growth in radiated culture region indicated that *uvrB* mutation was occurred.



3.1.4. R-Factor

Growth was observed in the ampicillin plates with TA 98 and TA 100 (Figure 4). Growth was observed for TA 100 and TA 98 is an R-factor strain. This strain possessed pKM101, Plasmid DNA essential for ampicillin resistance. The R-factor served as a convenient marker that made it possible to test for the presence of plasmid.

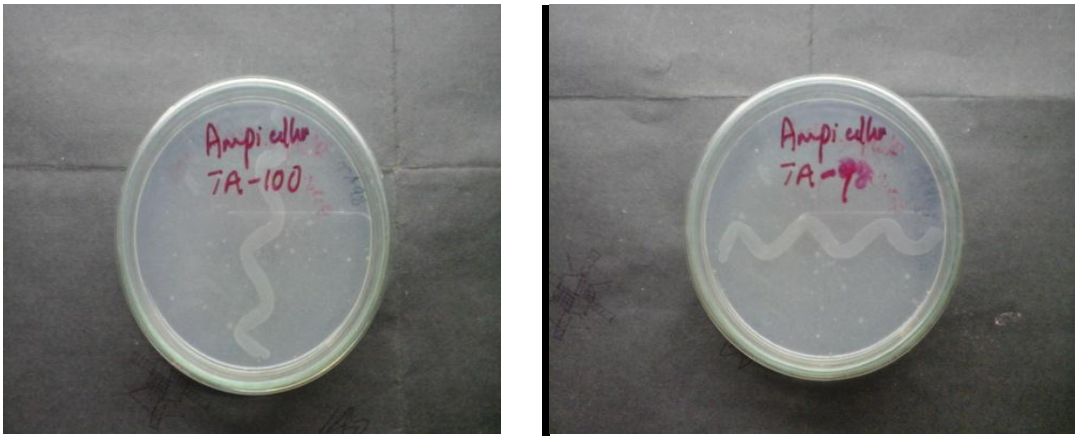


FIGURE 4. R-Factor

3.1.5 Toxicity Test of silver synthesised *Anredera cordifolia* using *Salmonella typhimurium* Strains TA 98and TA 100

The biosynthesised silver nanoparticle extract of *Anredera cordifolia* were non toxic to *Salmonella typhimurium* strains at the tested concentration 0.5mg/plate (Figure 5) .No of colonies are shown in table 1.

Table 1: Toxicity Test of Biosynthesised Silver Nanoparticle of *Anredera cordifolia*

Salmonella typhimurium	Number of colonies
TA 98	5
TA 100	21

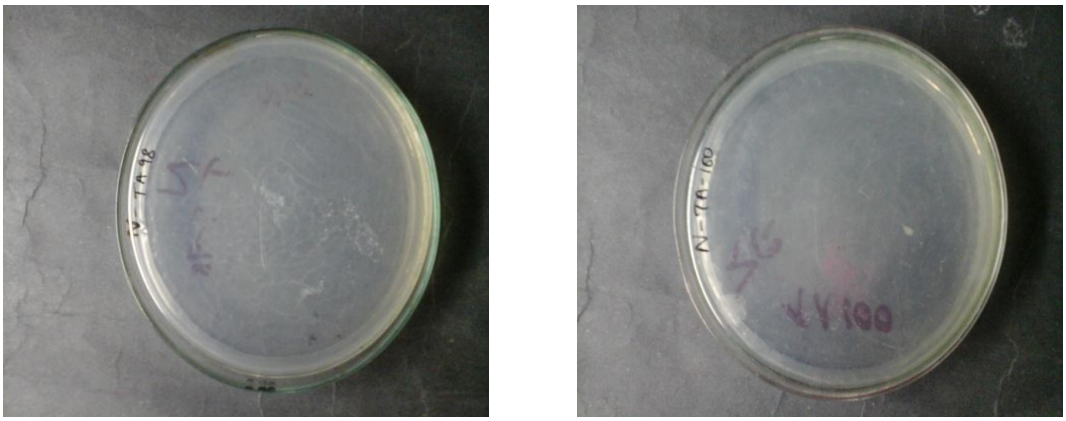


Figure 5: Toxicity Test of silver synthesized *Anredera cordifolia*

3.1.6 Antimutagenic Activity of Biosynthesised Silver Nanoparticle of *Anredera cordifolia* against Sodium Azide and ethidium bromide Induced Mutagenicity on *Salmonella typhymurium* Strain TA 98 and TA 100

The figure 6 showed the biosynthesized silver nanoparticle of *Anredera cordifolia* on direct acting mutagen sodium azide (NaN3) and ethidium bromide induced mutagenicity on *Salmonella typhimurium* strain TA 98 and TA 100. At 0.5 mg/ml plate concentration, the biosynthesised silver nanoparticle of *Anredera cordifolia* showed decrease in number of revertants colonies against sodium azide and ethidium bromide induced mutagenicity by TA 100 and TA 98.

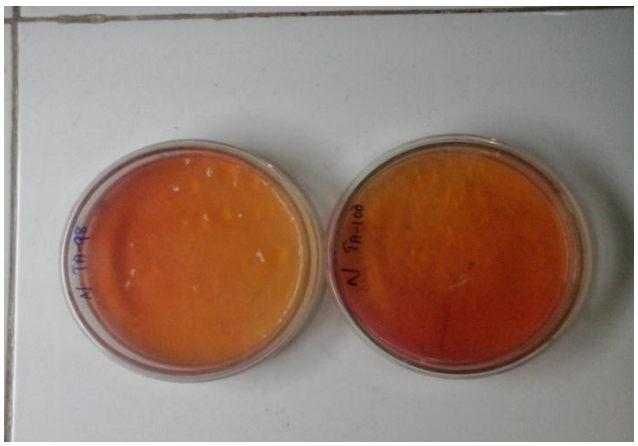


Figure 6: Antimutagenic Activity of Biosynthesised Silver Nanoparticle of *Anredera Cordifolioa* Against TA 100 AND TA 98.

4.0 DISCUSSION

Cancer is a disease or disorder in which a single normal body cell undergoes genetic transformation into a cancer cell and will begin to reproduce uncontrollable. There is a high correlation between carcinogencity and mutagenicity. Ninety percent (i.e., 156/174) of the carcinogens tested were found to be mutagenic in the Ames assay. Recently, considerable attention has been focused on identifying naturally occurring chemo-preventing substances capable of inhibiting, retarding or reversing the multistage carcinogenesis.[15]

The antimutagenic compounds can act at cellular level by enhancing the activities of enzymes involved in detoxification of mutagens, inhibiting the activities of enzymes involved in formation of mutagens metabolites, trapping of electrophils, scavenging reactive oxygen species, inhibiting metabolic activation, or protecting nucleophilic sites of DNA. Polyphenols, particularly flavonoids, have an ideal structure for scavenging free radicals and can also interact with the active groups of mutagens or protect the sites of DNA that would be affected by the mutagen.[16-18] Flavonoids and phenolics are the most likely candidate in providing antimutagenic effect and preventing oxidative damage.

In this study,consideration of antimutagenic of biosynthesized silver nanoparticle of *Andredera cordifolia* compared with positive controls (sodium azide and ethidium bromide) indicates good antimutagenic properties. The use of antimutagens and anticarcinogens in the diet has been suggested as the most effective procedure for cancer prevention.It can be concluded that the consumption of this plant may actually be giving protection to human body against mutation of cells and cancer induced processed food substances we consumed daily.

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