Biosynthesis of Silver Nanoparticles from *Crossandra infundibuliformis* and its Anticancer activity

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Abstract: **Among the nanomaterials, silver nanoparticles in antitumor properties are expected to fight against tumors and infectious diseases. Silver nano particles are the metal of choice as they kill microbes effectively. Biosynthesis of silver nano particles was carried out using the aqueous extract of *Crossandra infundibuliformis* under various experimental condition. The aqueous extract of *Crossandrainfundibuliformis*shows the significant potential for the quick detection of silver ions. The synthesized silver nano particles were characterized with UV visible absorption spectrophotometer and ETBr-Ao staining analysis. The cytotoxic activity of synthesized nanosilver was carried out against human colon cancer cells [HT29 cells] by MTT assay and found to show significant activity. The present work of biosynthesis of silver nanoparticles using *Crossandra infundibuliformis appears* to be cost effective & ecofriendly.**

***Index terms: Crossandra infundibuliformis*, UV visible spectroscopy, MTT-Malignant Triton Tumor, ETBr-Ao staining-Ethidium Bromide**

I. INTRODUCTION

Silver nanoparticles have been used in the form of catalysts and micro-electronics. Due to their anticancer properties, silver nanoparticles are used in the field of medicine. Newly improved properties were exhibited by the nanoparticles based on the characteristics such as size, distribution and morphology. Nano crystalline silver particles have various applications in the field of catalysis, micro-electronics[8], biological systems[2] and medicine due to their anticancer effects[4]. Silver nanoparticles are synthesized by the reduction of silver ions from Ag+ to Ag0 [2] using reducing agents[10]..Medical values of *Crossandra infundibuliformis* is that it is highly effective in wounds and effective in bronchitis that is cough and the action of *Crossandra infundibuliformis* is of anti-inflammatory, antimicrobial & antiulcer. Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other part of the body. This contrast with begin tumors, which do not spread to other parts of the body. A neoplasm or tumor is a group of cells that have undergone unregulated growth. Human colon cancer or large intestine is a muscular, tube-shaped organ measuring about 4 feet long. The term Colorectal region begins at the colon and ends at the anus. Most colon Cancers are adenocarcinoma-tumors that develop from the glands lining the Colon's inner wall treatment for colon cancer is of primary treatment is to surgically remove part of your colon and the next treatment is radiation treatment does not support and it is important for people with rectal cancer.Fluorescent dyes with aromatic amino or guanidine groups, such as acridine orange (AO), emit fluorescence when they interact with nucleotides. ETBr molecules enters the DNA double helix. AO can form complexes with either double-stranded DNA or single-stranded DNA and RNA. One molecule of AO can also interact with one phosphate group of single-stranded DNA or RNA to form an aggregated, or stacked, structure that emits red fluorescence with the maximum wavelength at 650 nm. This fluorescent dye is used asfluorescent indicators of dead cells that are impermeable through the cell membranes of viable cells. A cridine orange is a vital dye and will stain both live and dead cells. Necrotic cells stain orange, but have a nuclear morphology resembling that of viable cells, with no condensed chromatin.When cytoplasmic membrane integrity is lost,Ethidium bromide (ETBr) is only taken up by cells and stains the nucleus red. ETBr also dominates over AO. Thus live cells have a normal green nucleus; early apoptotic cells have a bright green nucleus with condensed or fragmented chromatin; late apoptotic cells display condensed and fragmented orange chromatin; cells that have died from direct necrosis have a structurally normal orange nucleus. Ethidium re-emits this energy as yellow/orange light centered at 590 nm. The fluorescence of ethidium bromide in aqueous solution is significantly lower than that of the interchelated dye.

II. MATERIALS AND METHODS

Preparation of extract from *crossandra infundibuliformis*

The flower of Crossandra *infundibuliformis* were collected from the location (Mannachanallur) of latitude and longitude of 10.9145˚N, 78.6994˚E and collected flowers were weighed of 30 grams, cut into small pieces then washed using distilled water and were added to the 200 ml of distilled water. The collected flower extract were kept in 250 ml beaker , then it was autoclaved at 60 ˚C for 30mins and the flower extract were collected in 50ml tubes , the collected tubes are stored at room temperature for one day.

Synthesis of Nano materials

100 ml of aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the process of biosynthesis of silver nanoparticles. The flower extract which have been stored in tubes are observed and then the extract was taken at different ratios for the bulk production. The three ratios of 9:1**, 5:5, and 7:3** are examined. At the ratio of **7:3** the yield is high, and then the sample was prepared for 50ml of 35ml silver nitrate and 15ml flower extract and centrifuge at 3500 rpm for 10 minutes. Then discard the Supernatant

and collect the pellet, it was air dried for one day and the dried pellet was weighed. The weight of flower extract was 0.06 µg and then the extract was made upto solution for 1000 µl, the DMSO solution of 983.4 µl and 16.6 µl of stock solution were pipetted out and stored in a eppendorf tube.

MTT ASSAY FOR CELL CYTOTOXICITY

Cell culture

HT-29 (Human colon carcinoma cells) cell line were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37˚C.

MTT Assay

The *Crossandra* AgNPS sample was tested for *in vitro* cytotoxicity, using HT-29 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay. Briefly, the cultured HT-29 cells were harvested by trypsinization, pooled into a 15ml tube. Then, the cells were plated at a density of 1×105 cells/ml cells/well (200 µL) into 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hours at 37°C.

The wells were washed with sterile PBS and treated with various concentrations of the *Crossandra* AgNPS sample in a serum free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO_2 incubator for 24 h. After the incubation period, MTT (20 µL of 5 mg/ml) was added into each well and the cells incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) were aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a micro plate reader (Thermo Fisher Scientific, USA) and the percentage of cell viability and IC50 value was calculated using GraphPad Prism 6.0 software (USA).

UV –Vis SPECTROSCOPY

UV-Vis spectroscopy is an instrument tool used in the characterization of structures of silver nanoparticles. TheUV-Vis spectrum of the reaction is used to monitor the reduction Ag+ ions[10]. The physiochemical properties of nano particles are important for their behavior, bio distribution, safety and efficacy. Therefore characterization of (AgNps) was important in order to evaluate the functional accepts of synthesized particles. It performed using a variety of analytical techniques using UV-vis spectrophotometer.

ANTICANCER EFFECT OF *CROSSANDRA INFUNDIBULIFORMIS* IN SILVER NANO MATERIALS

They are effective and safer to the milieu as they show less toxic effect to normal cells and can be extensively applied in biomedical science particularly in cancer field.

ETBR-AO STAINING

Briefly, 5 x 10^5 cells/ml of HT-29 (human colon carcinoma) cells were seeded into the 24 well tissue culture plate and treated with 54.2 µg /ml of AgNPs sample in a serum free DMEM medium. The plate was incubated at 37° C at 5% Co_2 incubator for 24 hours. After incubation, 50 µl of 1mg /ml acridine orange and ethidium bromide were added to the wells and mixed gently. Finally, the plate was centrifuged at 800 rpm for 2 minutes and evaluated immediately within an hour and examined at least 100 cells by fluorescence microscope using a fluorescent filter**.**

III. RESULTS AND DISCUSSION

It is known that the Due to its excitation of surface plasmon vibrations in silver nanoparticlesit exhibits yellowish brown color in aqueous solution The flower extract was mixed in the aqueous solution of the silver ion complex, and the color changes from watery to yellowish brown due to its reduction of silver ion,this indicated the formation of silver nanoparticles. Generally it is identified uv–vis spectroscopy could be used for examining size- and shape of nanoparticles in aqueous suspensions. Absorption spectra of silver nanoparticles formed in the absorbance peak at 450 nm.In the stage of early apoptotic cells, it appears in bright green nucleus with condensed or fragmented chromatin. On the late apoptotic cells it displays in fragmented orange chromatin.

Table 1: CELL VIABILITY

S. No	Tested sample concentration (µg/ml)	OD value at 570 nm	Cell viability (%)
1	**Control**	**100**	**100**
sss2	**100µg/ml**	**0.225**	**54.347**
3	**90µg/ml**	**0.277**	**66.908**
4	**80µg/ml**	**0.304**	**73.429**
5	**70µg/ml**	**0.267**	**64.492**
6	**60µg/ml**	**0.314**	**75.845**
7	**50µg/ml**	**0.282**	**68.391**
8	**40 µg/ml**	**0.279**	**67.391**
9	**30 µg/ml**	**0.326**	**78.743**
10	**20 µg/ml**	**0.340**	**82.125**
11	**10 µg/ml**	**0.357**	**86.231**

Fig. 1: Synthesis of Nano materials

Fig.2: Control of AgNP

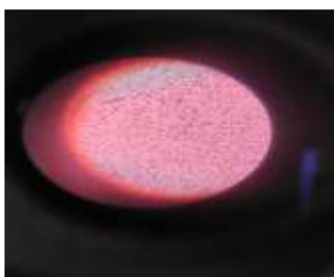

Fig. 3: 10 µl of AgNPS

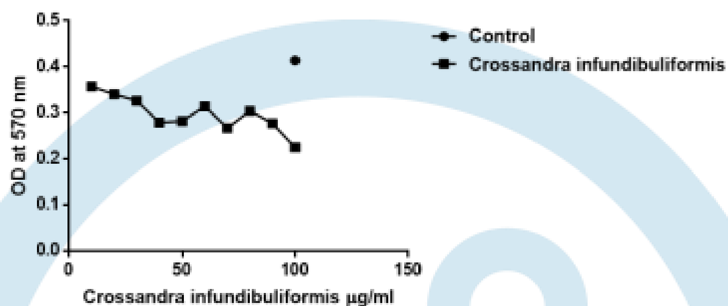

Fig. 4: Cell viability of *crossandrainfundibuliformis*

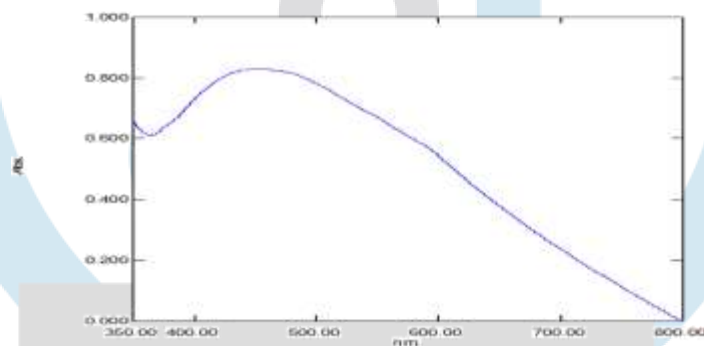

Fig. 5: UV Spectrophotometer

Fig. 6: EtBr Ao Staining in control and treated cells. (A) Before treated

Fig. 7: EtBr Ao Staining in control and treated cells (B) Treatment with 54.2 µg/ml of AgNP's sample.

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Fig. 4: Cell viability of *crossandraInfundibuliformis*

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