GREEN SYNTHESIS OF SILVER NANOPARTICLES USING SEED EXTRACT OF *MUCUNA PRURIENS* AND ITS ANTICANCER ACTIVITY AGAINST PC-3 AND HeLa CELLS

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Abstract: Seeds of *Mucunapruriens*, a commonly used plant in Ayurvedic medicine, was chosen for this study. A novel green source was opted to synthesize AgNPs using Silver nitrate. *M. pruriens*was found to exhibit strong potential for rapid reduction of silver ions as ($AgNO_3$ + extract) changed its color from light to dark brown. The formation of AgNPs was confirmed using analytical techniques. UV-vis spectroscopy result showed maximum adsorption between the range of 400-500nm. The particle size investigated using SEM analysis was found to be in the range of 70-80nm. X-Ray crystal analysis showed that the silver nanoparticles exhibit face centered cubic lattice structure. Cytotoxicity assay showed that the drug was effective in killing HeLa cells more efficiently at lower concentration (168.51µg/mL) as compared to PC-3. Apoptotic activity of silver nanoparticles on HeLa cells demonstrated significant cell death which suggests that the plant could be a potential anticancer drug.

Index terms- Apoptotic activity, cytotoxicity, HeLa, *Mucunapruriens*,PC-3,silver nanoparticles.

I. INTRODUCTION

Nanotechnology is a field of science which deals with production, manipulation and use of materials ranging in Nano meters. With the advancement of technologies and improved scientific knowledge a way for research and development in the field of herbal and medicinal plant biology towards intersection of nanotechnology has been observed. One such interference is applying plants source in the green synthesis of nanoparticles [1]. Most of the chemical methods used for the synthesis of

nanoparticles involve the use of toxic, hazardous chemicals that create biological risks and sometime these chemical processes are not eco-friendly [2]. This enhances the growing need to develop environmentally friendly processes through green synthesis by using microorganisms, enzymes or plant extracts. The plant *M. pruriens*, widely known as "velvet bean," is a vigorous annual climbing legume originally from southern China and eastern India, where it was at one time widely cultivated as a green vegetable crop. It is one of the most popular green crops currently known in the tropics; velvet beans have great potential as both food and feed as suggested by experiences worldwide.The seeds of *Mucunapruriens* have been used for treating many dysfunctions in Tibb-e-Unani (Unani Medicine) [1, 2]. During the germination process, plant seeds release a variety of metabolites including carbohydrates, vitamins, amino acids, and other organic compounds as a result of enzymatic activity [3]. Some of these act as reductants, complexants, and stabilizers (either individually or collectively), which in turn dictate the size and shape of the nanoparticles formed. Cancer is the uncontrolled growth of abnormal cells anywhere in a body.Prostate canceris the most common forms of malignancy in men, particularly in developed countries where the majority of cases are diagnosed in men aged above 50 years.Cervical canceris a major health problem worldwide and is the most frequent cause of cancer in women in India. Cervical cancer is caused by the Human Papilloma Virus (HPV) which forms warts in the throat and genital area. Natural products are being tested for the treatment of cancer as conventional cancer treatments as chemotherapy destroy cancerous as well as healthy cells [4]. Preparation of metallic nanoparticles using plant seed extracts was reported in the early 20th century [5], however the products were not morphologically characterized due to the absence of suitable analytical techniques at the time. It was hypothesized that other (edible) seed exudates can provide the vital biocomponents and chemical stability needed for the formation of biocompatible metallic nanoparticles [6]. This procedure is advantageous from a green engineering perspective [7]; the extraction process is simple, kinetically favorable, and energy efficient as it is carried out at ordinary temperature and requires no boiling or physical rupturing of the plant tissues to release the biomolecules into the solvent, as in the case with leaf broth [8]. Hence, in this work, we present data on the viability and efficacy of colloidal Ag nanoparticle synthesis using *M. pruriens* seed.

II. MATERIALS AND METHODS

Plant material

Based on the literature survey, *Mucunapruriens* was selected for the evaluation of cytotoxic and apoptotic activity on PC-3 and HeLa cell lines. The seeds of *M. pruriens* were collected from local market of Bengaluru.

Preparation of Seed Extract

The seeds were cleaned thoroughly in fresh water followed by distilled water and then dried for 5 days in Hot air oven. Dried seeds were ground to powder and used for

further analysis. Soxhlet extraction was carried out using 60g of seeds and approximately 350mL of ethanol. The extraction was performed for 72 hours until the solvent decolorized. The sample was concentrated to obtain crude extract.

Synthesis of Silver Nanoparticles (SNPs) using seed extract of M. pruriens

The SNPs were synthesized using 5 mM and 10 mM silver nitrate solutions and hydro-alcoholic seed extract of M. pruriens. Equal volumes of silver nitrate was added drop wise to seed extract at room temperature and dark conditions under constant stirring. The mixed solutions were incubated in dark for 1-2 hours until colour change was observed from light brown to dark brown. Sample was kept in oven for drying. The complete drying of this solid mass resulted in a black coloured material which was powdered in mortar and sampled for characterization purpose.

Anti-cancer activity of SNPs

Haemolysis Assay

5 mL blood was collected from a healthy individual and RBCs were separated by centrifuging fresh blood at 1000 rpm for 10 minutes. Supernatant was removed and collected RBCs were washed using PBS. Diluted RBCs were added to different concentrations of SNPs. The cells were incubated at 37 °C for 1 hour after which the reaction was centrifuged at 300 rpm for 5 minutes. Supernatant of each reaction was transferred to a micro titre plate and absorbance was measured at 590nm. Percentage haemolysis was calculated by the formula:

[% Haemolysis = (Control - sample)*100/Control] (1)

MTT Assay

PC-3 and HeLa cells (20,000 cells per well) were plated in 96 well plates with different concentrations of SNPs made in complete DMEM media (25, 50, 100, 200 and 400µg/mL) and incubated for 24 hours at 37˚ in a 5% CO2 incubator. Next day the media was removed and 100 µL of MTT reagent was added to each well and incubated again for 4 hours. MTT reagent was removed and 100 µL DMSO was added to each well for solubilizing the formazan product. The plate was shaken well and absorbance reading was taken at 570nm using microplate reader. IC_{50} values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell.

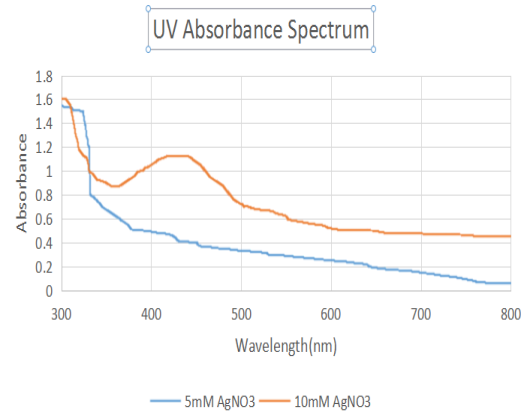
Apoptotic Study

After seeding about 1×10^6 cells in 6 well plate, cells were treated with 168.51 µg/mL concentration of SNP and incubated at 37°C in CO_2 incubator overnight. Cells were

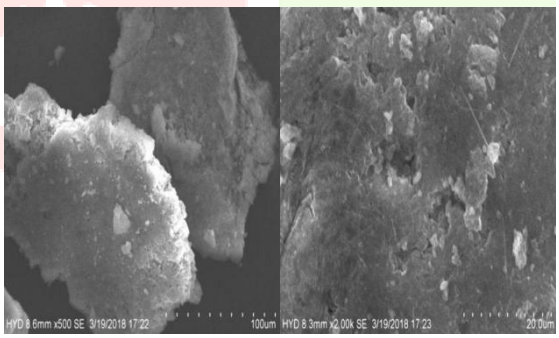
harvested by trypsinization and washed twice by cold 1X PBS. Cell pellet was resuspended in 1X PBS at a concentration of ~1 x 10^6 cells/mL. To the 100 µL of cells transferred to a 5-mL FACS tube, 5 µLAnnexin V and 5 µL PI was added, gently mixed and incubated for 15 minutes at room temperature in the dark. 400 µL of 1X Binding Buffer was added to each tube and analyzed by using FACS Caliber (BD FACS Calibur).

III. RESULTS AND DISCUSSION

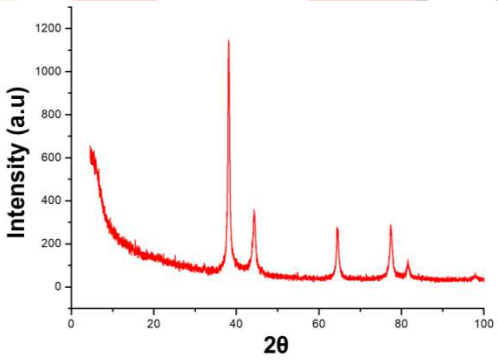
Characterization results



(A)



(B)



(C)

Fig. 1: Characterization of SNPs using (A): UV-vis spectrophotometer; (B): SEM; (C): XRD

The UV-vis absorption spectra of SNPs shows that the Surface Plasma Resonance (SPR) zone of the extract using 10mM $AgNO_3$ concentration falls within the desired range (400-500nm) compared to 5mM $AgNO_3$ concentration. It was observed from SEM images that surface morphology of AgNPs synthesized were in irregular shapes. There were observed few traces of AgNPs clusters due to aggregation of nanoparticles which might be induced by solvent evaporation during sample preparation. Furthermore, the particle range of SNPs was found to be 70-80 nm. The XRD pattern of the silver nanoparticles obtained after reduction of seed extract showed five intense

peaks at the 2θ angles of 38°, 44°, 64°, 77° and 82° respectively for 10mM $AgNO_3$ sample. A number of fcc structures of silver Bragg reflections corresponding to (111), (200), (220), (311) and (222) planes were observed (Figure 5.3.3). The XRD pattern thus clearly indicates that the SNPs are crystalline in nature. The patterns show good match with JCPDS-file-No-04-0783 [Fig. 1(A-C)].

Haemolysis Assay

Table I: Absorbance values of haemolysis caused by SNPs on RBCs

Sample	**Treat**	**Absorbance**	**% Haemolysis**
Negative Control	PBS	0.790	0.000
Positive Control	1% SDS	0.160	80.260
Sample	**Conc.(µg/mL)**	**Absorbance**	**% Haemolysis**
Control	0	0.790	0.000
SNPs using *M. pruriens*	25	0.930	0.000
	50	0.810	0.002
	100	0.680	0.005
	200	0.640	0.006
	400	0.560	0.010

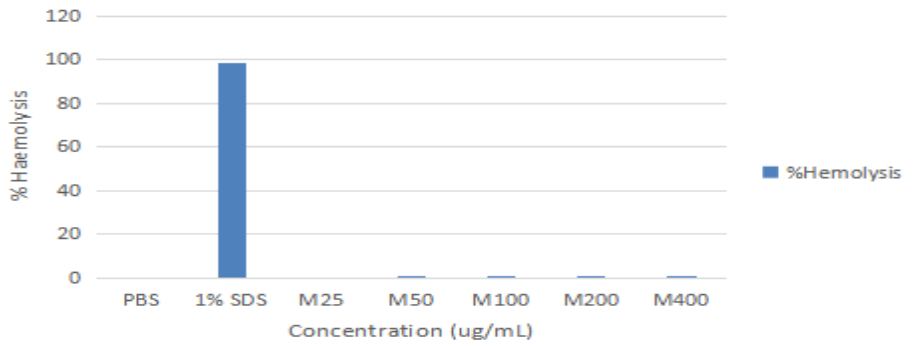
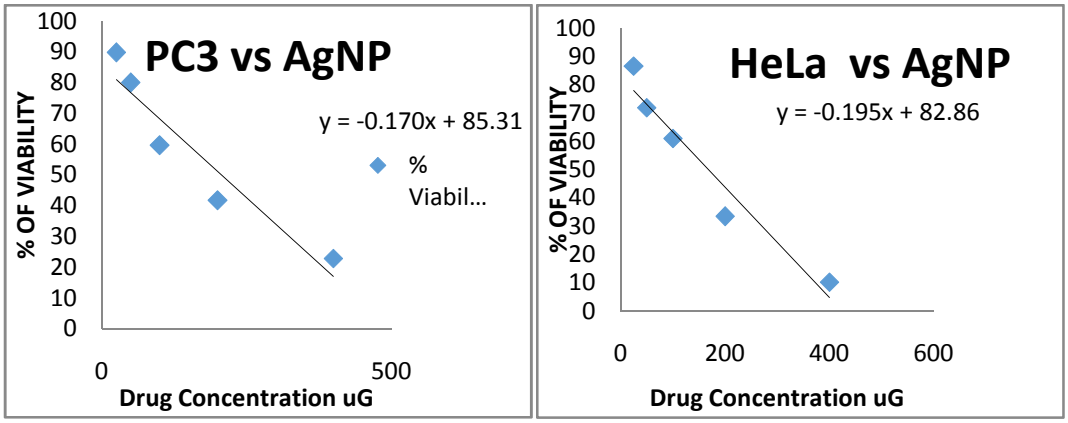


Fig. 2: % Haemolysis of SNPs (M25-M400- concentration of *M. pruriens*)

The selected plant extracts tested for their haemolytic abilities showed no haemolysis of red blood cells (Figure 2). This suggests that the SNPs samples can be used for therapeutics.

Cell cytotoxicity



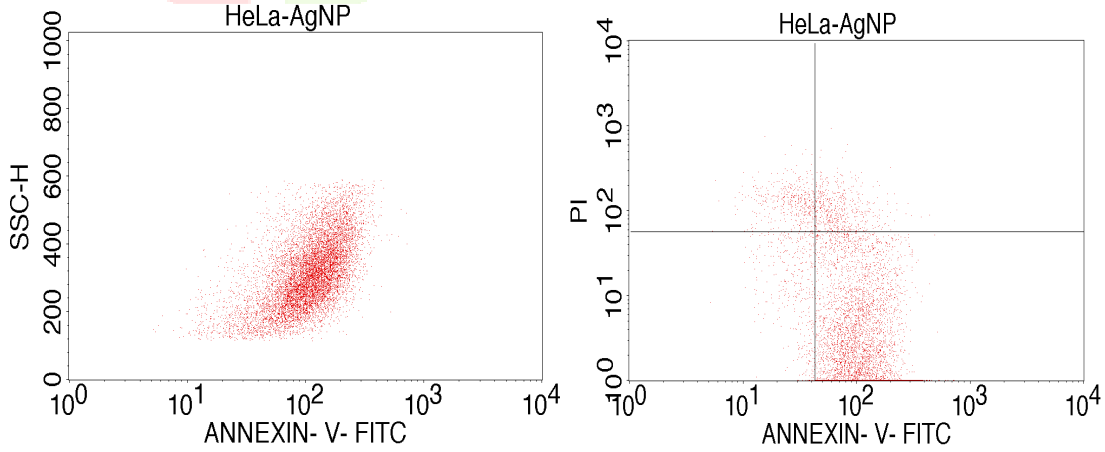
(A) (B)

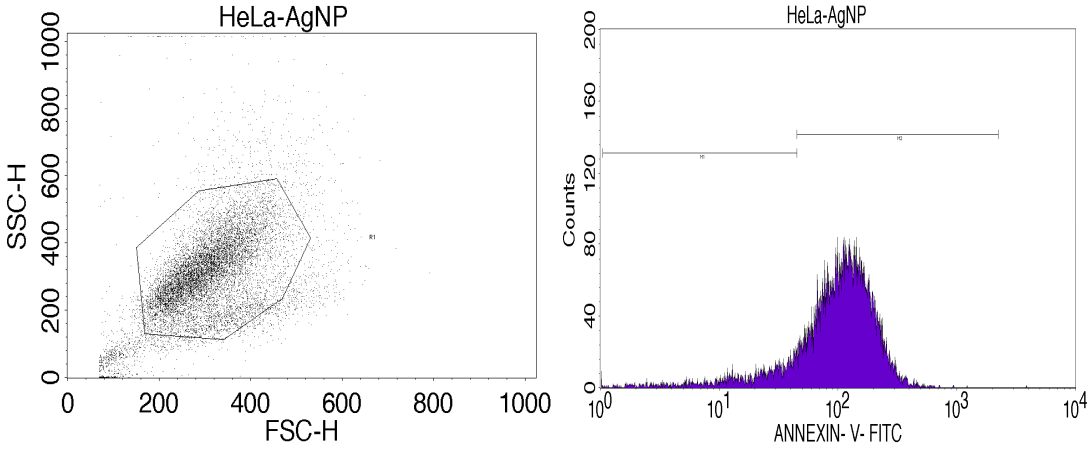
Fig. 3: (A)- % Viability of PC-3 cells by *M. pruriens*extract. The IC_{50}value of the sample was found to be 207.71µg/mL; (B)-% Viability of HeLa cells by *M. pruriens*extract.The IC_{50} value of the sample was found to be 168.51µg/mL.

Comparing theIC_{50} value of HeLa with that of PC-3 cell line, it was found that HeLa cells showed 50% inhibition at a lesser concentration of the test sample. Hence, the HeLa cell line was used for apoptotic studies.

Apoptosis Study

The effect of selected SNPs extract on cell cycle in HeLa cells as analyzed by the flowcytometry are depicted in the figures below:





Histogram Statistics

File: HeLa-AgNP
Sample ID: HeLa-AgNP
Tube: Untitled
Acquisition Date: 20-Apr-18
Gated Events: 11793
X Parameter: ANNEXIN- V- FITC (Log)

Log Data Units: Linear Values
Patient ID:
Panel: Untitled Acquisition Tube List
Gate: No Gate
Total Events: 11793

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	11793	100.00	100.00	116.25	89.13	70.20	105.54	98
M1	1, 45	1712	14.52	14.52	23.05	17.76	56.55	23.29	43
M2	45, 2227	10093	85.58	85.58	131.64	117.43	51.99	117.57	98

Fig. 4: Flow cytometry plots of HeLa treated with 168.51 µg/mL SNPs of *M. pruriens*

To investigate whether SNPs induce apoptosis in HeLa cells, flow cytometry was performed. It is observed that at the concentration of 168.51 µg/ mL, SNPs arrest cell cycle significantly (85.58% cells) in M2 phase.

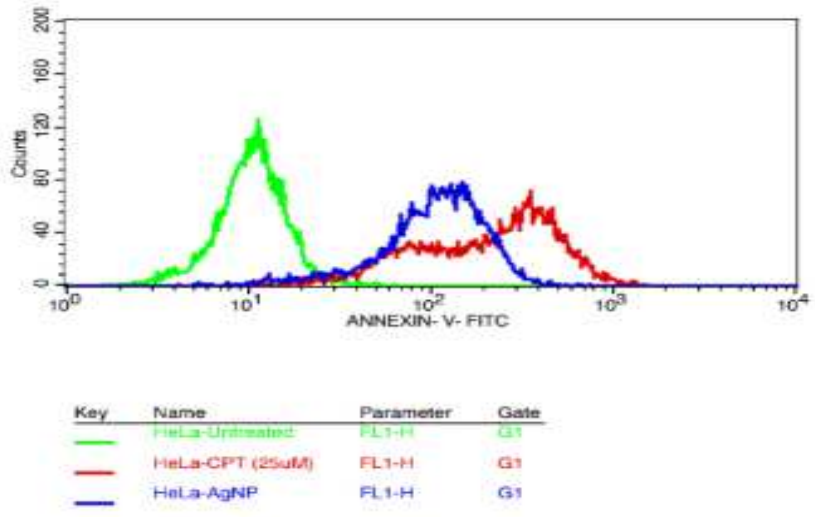


Fig. 5: Overlay of untreated cells, Campothecin treated and SNPs treated cells with HeLa

The HeLa cells treated with 168.51µg/mL of *Mucunapruriens* SNPs has induced apoptosis when compared to untreated HeLa control cells of only 0.10% cells and Campothecin treated HeLa cells.

IV. CONCLUSION

M. pruriens seed extract was effective in reducing Ag salts to form Ag nanoparticles. Benefit of this green approach is that it is an easy, extremely low energy based, eco-friendly and economic process. The characterization results obtained from various techniques showed that the SNPs synthesized were composed of crystalline fcc lattice structures and were of the size ranging from 70-80nm. From the study, SNPs were observed to have strong and almost equal apoptotic activity when compared with the standard drug- Campothecin (CPT).

V. ACKNOWLEDGEMENT

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