GREEN SYNTHESIS OF SILVER NANOPARTICLES USING SEED EXTRACT OF *MUCUNA PRURIENS* AND ITS ANTICANCER ACTIVITY

Shivashankar. M
Department of Life Science, Bangalore University, Bangalore, India.

ABSTRACT

Seeds of *Mucuna pruriens*, a commonly used plant in Ayurvedic medicine, was chosen for this study. Extraction was carried out using Ethanol, Methanol, n-Butanol and Hexane. Ethanolic extract was chosen for anticancer and antioxidant studies after comparing it's yield with that resulting from other solvents. The key phytochemicals responsible for converting silver ions into silver nanoparticles are alkaloids, tannins, flavanoids and phenolics. These were found to be present in the ethanolic extract and hence were used for synthesis of SNPs. A novel green source was opted to synthesize SNPs 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 SNPs was confirmed using analytical techniques. UV-vis spectroscopy result showed maximum adsorption between the range of 400-500nm, which represents the characteristic SPR of silver. The particle size investigated using SEM analysis was found to be in the range of 70-80 nm. X-Ray crystal analysis showed that the silver nanoparticles exhibit fcc structure with characteristic (111), (200), (220), (311) and (222) planes. FTIR results demonstrated the presence of functional groups responsible for reduction of silver ions. 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. The DPPH and Iron chelating antioxidant assays were successful as the drug showed similar results when compared with the standard Ascorbic Acid. DPPH was reduced in the range of 18.22-63.76% depending on the formulation and the extract showed 52.53-65.58% metal chelating activity. SNPs were observed to have strong and almost equal apoptotic activity when compared with the standard drug-Campothecin.

Keywords

Mucuna pruriens, silver nanoparticles (SNPs), Surface Plasmon Resonance (SPR), HeLa, PC-3, antioxidant activity.

1. 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 [1].

Nanoparticles can be easily synthesized using various methods which include chemical, electro-chemical, radiation, photochemical methods and biological techniques. But 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.

Silver is well-known since ancient time due to its medicinal value and for its preservative properties. Silver is one of the basic elements that make up our planet. It is efficient antimicrobial agent compared to others due to their extremely large surface area, which provides better contact with microorganisms. Silver nanoparticles are reported to show better wound healing capacity and better cosmetic appearance [3].

The genus Mucuna, belonging to the Fabaceae family, sub family Papilionaceae, includes approximately 150 species of annual and perennial legumes. It is a tropical legume native to Africa and tropical Asia and widely naturalized and cultivated.

The seeds of *Mucuna pruriens* have been used for treating many dysfunctions in Tibb-e-Unani (Unani Medicine). The plant and its extracts have been long used in tribal communities as a toxin antagonist for various snakebites. It has long been used in traditional Ayurveda Indian medicine[5].

Cancer is the uncontrolled growth of abnormal cells anywhere in a body. These abnormal cells are termed cancer cells, malignant cells or tumor cells. These cells can infiltrate normal body tissues. Anything that may cause a normal body cell to develop abnormally potentially can cause cancer. Many things can cause cell abnormalities and have been linked to cancer development. Some cancer causes remain unknown while other cancers have environmental or lifestyle triggers or may develop from more than one known cause. Some may be developmentally influenced by a person's genetic makeup. Many patients develop cancer due to a combination of these factors[6].

2. Materials and methods

The Experimental material *M. pruriens* was purchased from a local market of Bangalore.

2.1 Chemicals

Ethanol, N-Butanol, Methanol, Hexane, AgNO3, DMSO, DPPH, PBS, Ascorbic Acid, EDTA,Tryphan Blue, MTT, O-phenanthroline, all the chemicals used were of analytical grade.

2.2 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.

2.2.1 Extraction with Ethanol

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.

2.2.2 Extraction with Methanol

Dried milled seeds (60g) were extracted using methanol (350mL) by Soxhlet extraction method. The procedure was carried out for 72 hours to obtain decolorized sample. It was later heated at 80^{o}C in water bath in order to evaporate the methanol and obtain crude plant extract.

2.2.3 Extraction using magnetic stirrer

60 g of dried milled seeds were extracted using 350mL of n-Butanol and Hexane. The samples were stirred overnight and filtered. The filtrate was concentrated to obtain crude extract.

2.3 Phytochemical analysis

2.3.1 Screening for Alkaloids (Dragendroff's test)

100 µL of Dragendroff's reagent was added to 200 µL of extract and mixed well. Appearance of red precipitate indicates presence of alkaloids.

2.3.2 Screening for Tannins

10% and 1% FeCl3 was added to 200µL of methanol and the other three extracts respectively. Appearance of green precipitate indicates the presence of tannins.

2.3.3 Screening for Terpenoids (Salkowski's test)

200µL chloroform + 3-4 drops of conc. Sulphuric acid were added to 200µL extract. Appearance of yellow precipitate indicates presence of terpenoids.

2.3.4 Screening for glycoside (Libermann test)

200 µL of chloroform + 200 µL of glacial acetic acid + drops of conc. Sulphuric acid was added to the extract. Presence of glycoside is indicated by color change from violet to blue to green.

2.3.5 Screening for steroids

200 µL chloroform + 3-4 drops of conc. Sulphuric acid were added to 200 µL extract. Appearance of red precipitate indicates presence of steroids.

2.3.6 Screening for Saponins (foam test)

200µL of distilled water was added to 200µL of plant extract. Shook well and warmed.
Foam formation indicates presence of saponins.

2.3.7.Screening for flavonoids

Few drops of NaOH solution was added to plant extract. The change in yellow colour to colourless solution after addition of dil. HCl indicates presence of flavonoids

2.3.8 Screening for mucilages

500µL of absolute alcohol was added to 200µL of plant extract and was allowed to dry. Formation of precipitate indicates presence of mucilage

2.3.9 Screening for volatile oils

Few drops of dil. NaOH and dil. HCl were added to 200µL of plant extract and was shaken. Formation of white precipitate indicates presence of volatile oils.

2.3.10 Screening for phenolic compounds

500µL of distilled water and few drops of 5% $FeCl_3$ were added to 500µL of extract Appearance of dark green color indicates presence of phenolic compounds.

2.3.11 Screening for carbohydrates (Benedict's test)

Few drops of Benedict's reagent were added to plant extract. Appearance of blue color indicates presence of carbohydrates

2.3.12 Screening for proteins (Xanthoproteic test)

Few drops of conc. HNO3 was added to 200µL of extract Appearance of yellow color indicates presence of proteins.

2.4 Synthesis of SNPs

10mM Stock solutions of AgNO3 (incubated at room temperature in dark conditions for 24 hours for equilibration) and 1mg/mL of plant extract were prepared. Equilibrated stock solution was diluted to obtain required concentrations (5 & 10 mM). The SNPs were synthesized using 5mM and 10mM silver nitrate solutions and hydro-alcoholic seed extract of *M. pruriens*. Equal volumes of silver nitrate were 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.

2.5 Characteristics of SNP's

2.5.1 UV-vis spectral analysis

It was carried out using Shimadzu UV-1700 UV Visible Spectrophotometer. UV visible spectrums of SNPs were recorded between the ranges of 300-800nm.

2.5.2 Scanning electron microscopy

Morphology of the obtained SNPs was analyzed using electron microscope (SEM S-3700) after sonicating the SNPs in ethanol for 1 hour.

2.5.3 X-ray diffraction studies

Characterization was carried out using Bruker AXS D8 Advance X-Ray Diffractometer operated at a voltage of 40kV and a current of 30mA with Cu Kα radiation between 20 angles of (0°-100°) for analyzing the crystal structure and peak data respectively.

2.5.4 FTIR spectroscopy

FTIR spectroscopy measurements were carried out to identify the biomolecules responsible for the synthesis of SNPs using Shimadzu FTIR spectrophotometer (FTIR 8400). The samples were prepared using the KBr pellet technique and were analyzed at a resolution of $4cm^{-1}$.

2.6 **Culturing and maintenance of cell lines (PC-3 and HeLa)**

The adherent cell lines (PC3 and HeLa) were obtained from NCCS (National Centre for Cell Science), Pune and maintained in Dulbecco's Modified Eagle Media (DMEM) maintained in 5% Co_2 incubator.

2.7 Haemolysis assay

To study the negative effects caused by the synthesised SNPs were analysed by conducting haemolysis assay according to the procedure outlined in the ASTM standard E2524-08 (Standard Test Method for Analysis of Hemolytic Properties of Nanoparticles).

2.8 MTT assay/ cell cytotoxicity assay

Cell proliferation and cytotoxicity assay was performed by using MTT reagent. Briefly, 200µL of cell suspension was seeded in a 96-well plate (20,000 cells per well) without the test agent (SNP's). Cells were allowed to stand for 12 hours. Add tdifferent concentrations of test solution (25, 50, 100, 200 & 400mg/mL) and incubated for 24 hours at 37°C in a 5% CO2 incubator. After the incubation period spent media was removed and MTT reagent was added to a final concentration of 0.5 mg/mL and the absorbance was read at 570 nm.

2.8 **DPPH assay**

Antioxidant activity was performed by using DPPH reagent. Briefly, 100µL of 0.5mM DPPH was added to 200µL of plant samples of different concentrations (400, 200, 100, 50 and 25µg/mL) mix well and the reaction mixture was placed in dark at room temperature for 30 minutes. After the incubation period the absorbance was measured at 518nm. Percentage inhibition was calculated by using the formula,

Percentage of inhibition= $[(Ab_{control} - Ab_{sample}) * 100] / Ab_{contrl}$

Where, Abcontrol is the absorbance of control

Ab_{sample} is the absorbance of sample mixture.

A graph of Concentration v/s percentage was plotted and EC50 value was calculated by linear regression of the plot.

2.9 Iron Chelating Assay

Iron chelating assay was performed by using O-phenanthroline reagent. Briefly, 50µL of O-phenanthroline and 125µL of FeCl3 were added to 25µL of different concentrations of plant extract (400, 200, 100, 50 and 25µg/mL).The reaction mixtures were incubated for 10 minutes and the intensity of the colour was measured at 518nm. Standard was prepared using ascorbic acid A graph of Concentration v/s percentage of inhibition was plotted and EC50 value was calculated by linear regression of the plot.

2.10 Apoptosis study

HeLa cells in a 6-well plate at a density of 3 x 10^5 cells/2mL were incubated in a CO_2 incubator overnight at 37°C for 24 hours. The spent medium was aspirated and washed with 1X PBS. Cells were treated with 168.51µg/mL concentration of SNPs and control in 2 mL of culture medium and the cells were incubated for 24 hours. At the end of the treatment, the medium was removed from all the wells into 12 x 75mm polystyrene tubes and was washed with 500 µL PBS. PBS was removed and 180 µL of trypsin-EDTA solution was added and incubated at 37°C for 3-4 minutes. The culture medium was poured back into their respective wells and the cells were harvested directly into 12 x 75mm polystyrene tubes. The tubes were centrifuged for 5 minutes at 300 rpm at 25°C. The supernatant was decanted. The cells were resuspended in 1X Binding buffer at a concentration of 1 x 10^6 cells/mL. 100µL of the solution (1 x 10^5 cells) was transferred to a 5 mL culture tube. 5 µL of FITC Annexin V was added. The cells were gently vortexed and incubated for 15 minutes at room temperature (25°C) in the dark. 5µL of PI and 400µL of 1X Binding Buffer were added to each tube and vortexed gently. Immediately after addition of PI, it was analysed by flow cytometry.

3. RESULTS AND DISCUSSIONS

3.1 Yield of extracts

Extraction of *M. pruriens* seeds with different samples yielded different quantities of extracts It was seen that ethanol extract yielded the maximum quantity of crude sample (23.5g) compared to other of extracts from other solvents (Methanol- 20.0g, n-Butanol - 8.08g, Hexane - 7.54g) (Table 3.1).

Table 3.1: Yield of crude extract of *M. pruriens* seeds in different solvents

SL. No.	Solvent	M. pruriens seeds
1.	Ethanol	60g in 350mL. Yield = 23.5g

2.	Methanol	60g in 350mL. Yield = 20.0g
3.	n-Butanol	60g in 350mL. Yield = 8.08g
4.	Hexane	60g in 350mL. Yield = 7.54g

3.2 Phytochemical analysis of extracted sample.

Methanol extract showed the presence of maximum number of secondary metabolite (Table 3.2). But since methanol is cytotoxic towards cancer cell lines, the next best extract of ethanol (Table 3.2) was used for conducting further studies

Table 3.2: Qualitative Analysis of phyto-compounds in various extracts of Mucuna pruriens

Secondary metabolite	METHANOL	ETHANOL	HEXANE	n-BUTANOL
Alkaloids	++	++	+	+
Tannins	+++	++	-	+
Terpenoids	-	-	-	-
Steroids	+	+	+	+
Glycosides	-	-	-	-
Saponins	-	-	-	-
Flavonoids	+	+	-	-
Mucilage	+	-	-	-
Volatile Oils	+	+	-	-
Phenolic Compounds	+	+	-	-
Carbohydrate	++	++	-	+
Proteins	+	+	-	-

+++: Very high presence; ++: High presence; +: Low presence; -: No presence

3.3 Characterization of SNPs:

3.3.1 UV-visible Spectroscopy

Silver particles at nano range exhibit an unusual optical phenomenon called Surface Plasmon Resonance (SPR), due to the cumulative oscillation of the conducting metal surface electrons in resonance with the non-particle radiation. This property is largely governed by particle size, shape and local chemical ambience. The characteristic fingerprint zone which exhibits this phenomenon (by SNPs) predominantly appears in the range of ~400-500nm respectively [6]

The absorption spectra of the samples synthesized using *M. pruriens* extract are shown in Figure 3.3.1. The spectra show that the SPR zone of the extract using 10mM AgNO3 concentration falls within the desired range (400-500nm) compared to 5mM AgNO3 concentration.

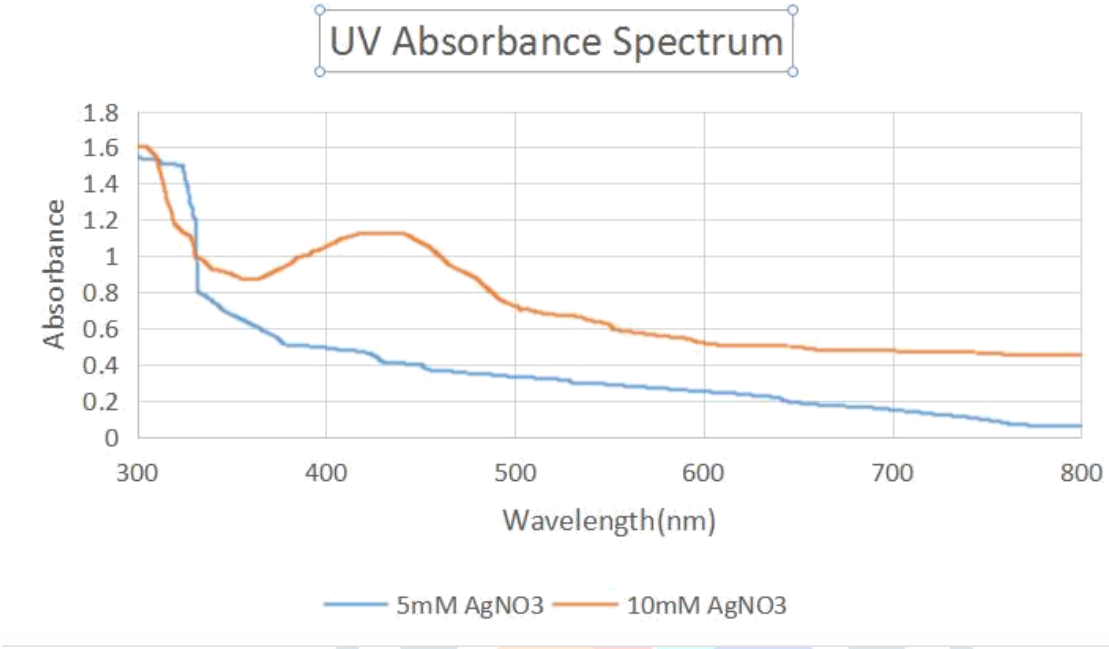
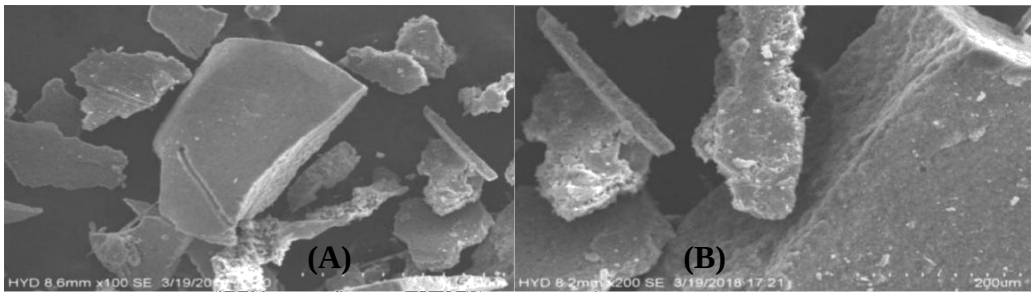
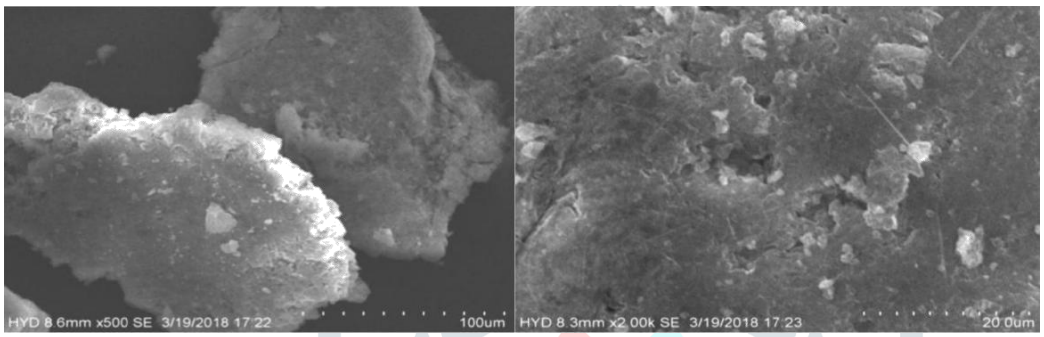


Figure 3.2.1: UV-visible spectroscopy of SNPs

3.3.2 Scanning Electron Microscopy (SEM)

Typical SEM images of SNPs synthesized using 10mM AgNO3 concentration are presented in the Figures 3.3.2 (A-D) with different magnification scale. It was observed that surface morphology of SNPs synthesized were in irregular shapes. There were observed few traces of SNPs clusters due to aggregation of nanoparticles which might be induced by solvent evaporation during sample preparation. It was observed that particles synthesized were of different sizes and particle range was found to be 70-80nm (Figure 3.3.2(e)).





C) (D)

Figure 3.3.2: SEM micro-graph: scale - A) 500µm; B) 200µm; C) 100µm; D) 20µm

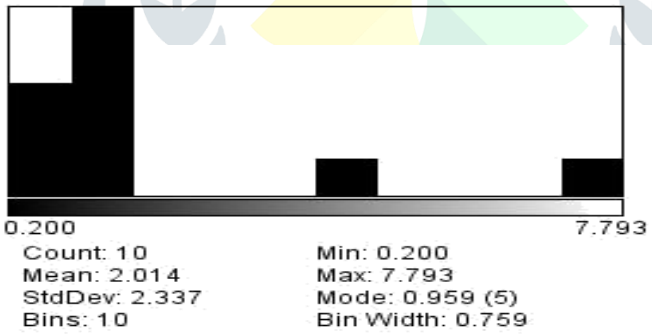


Figure 3.3.2(e): Particle size analysis of SEM image (20µm scale) using ImageJ

3.3.3 X-Ray Diffraction (XRD) Studies

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 AgNO3 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.The details of the 2θ, d-spacing and *hkl* values of obtained silver nanoparticles are shown in Table 3.3.3.

Table 3.3.3: XRD details with 2θ, D-Spacing and *hkl* values of the obtained fcc SNPs

Sample	2θ	D-Spacing	hkl values
10mM AgNO3 + Seed extract	38.23	2.35	111
	44.30	2.04	200
	64.48	1.44	220
	77.35	1.23	311
	82.07	1.01	222

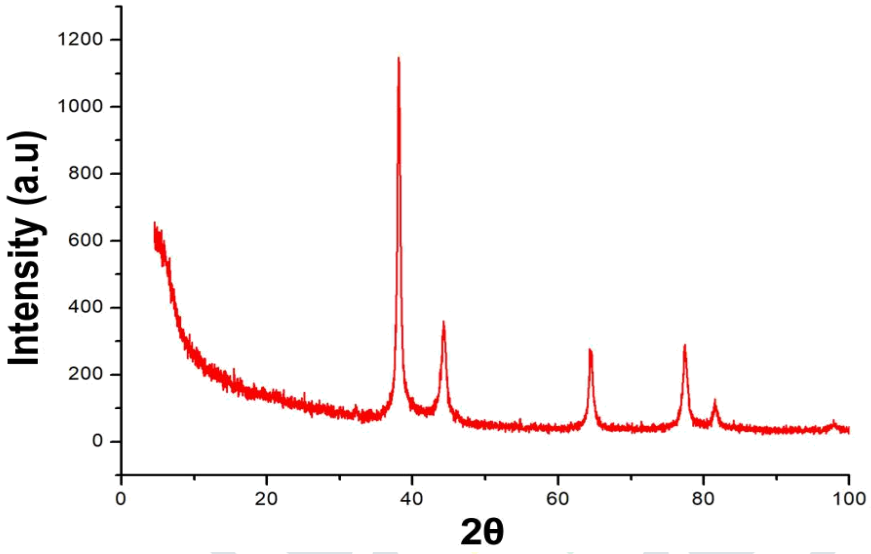


Figure 3.3.3: X-Ray Diffractogram of SNPs synthesized using *M. prurien*

3.3.4 FTIR analysis

FT-IR analysis revealed the strong bands at 3433, 2924, 1632, and 1072cm^{-1}. The band at 3433cm^{-1} corresponds to NH- amine amino groups, 2924cm $^{-1}$ alkane C-H stretching lipids, 1632cm^{-1} corresponds to amide amino groups. The band at 1384cm^{-1} corresponds to C=C stretching of aromatic amine group and weaker band at 1072cm^{-1} is arisen due to carbonyl stretch in proteins (Figure 3.3.4). It is well known that proteins can bind to silver nanoparticles either through free amine groups or cysteine residues in proteins and, therefore, stabilization of the SNPs by surface bound proteins is a possibility. This is due to the fact that one or more of these proteins may be enzymes that reduce silver nitrate ions and form SNP by reduction technique[7].

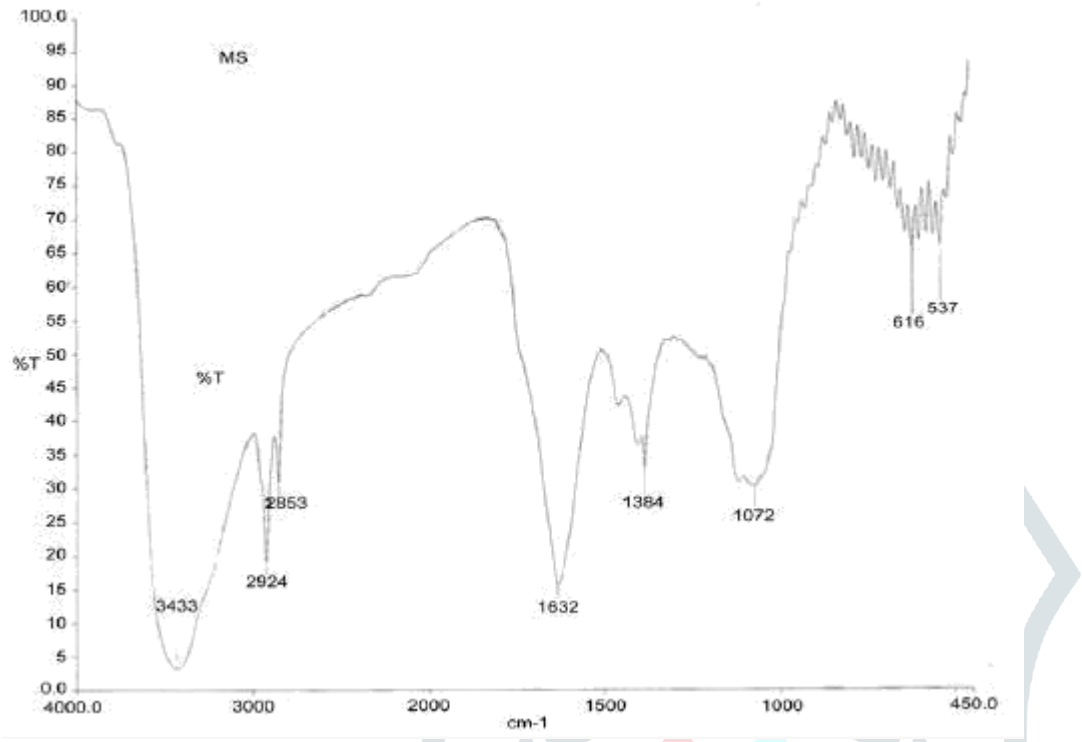


Figure 3.3.4: FT-IR spectrum of SNPs synthesized using *M. pruriens* seed extract

3.4 Haemolysis assay:

Table 3.4: Absorbance values of haemolysis caused by plant extracts 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
	25	0.930	0.000
	50	0.810	0.002
M. pruriens	100	0.680	0.005
	200	0.640	0.006
	400	0.560	0.010

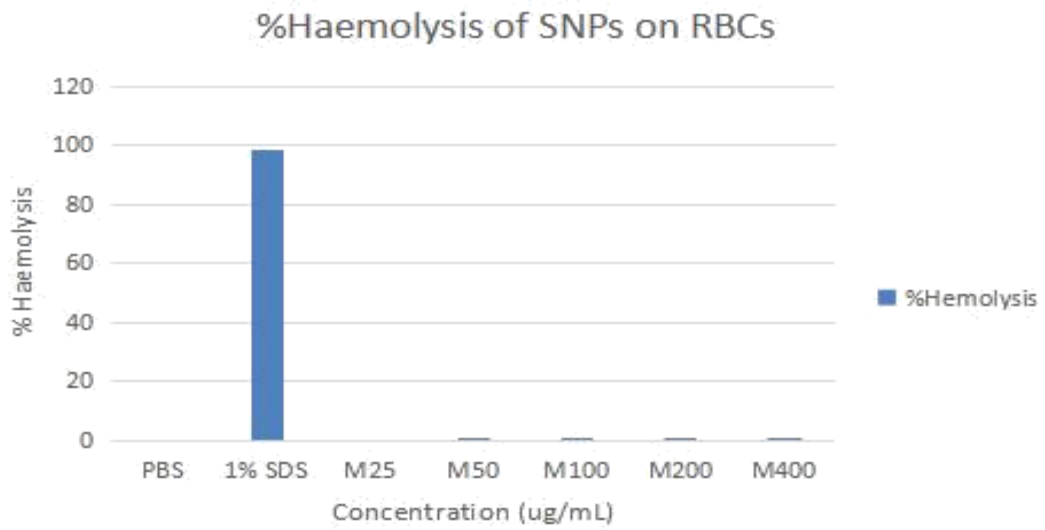


Figure 3.4: % Haemolysis of SNPs (M25-M400- concentration of *M. pruriens*)

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

3.5 CELL CYTOTOXICITY:

Table 3.5.1 Cytotoxic Effect of selected plant extract on PC-3 cell lines

	BLANK	UNTREATED	CPT 25µM	25	50	100	200	400
Reading 1	0.110	0.660	0.388	0.601	0.549	0.434	0.344	0.225
Reading 2	0.090	0.660	0.381	0.617	0.558	0.442	0.329	0.234
Mean	0.100	0.660	0.384	0.609	0.553	0.438	0.336	0.229
Mean OD-Mean B	NA	0.560	0.284	0.509	0.453	0.338	0.236	0.129
STANDARD DEVIATION		0.002	0.004	0.011	0.006	0.005	0.010	0.006
STANDARD ERROR		0.001	0.003	0.008	0.004	0.004	0.007	0.004
Viability %	NA	100	50.220	89.849	80.052	59.664	41.747	22.859

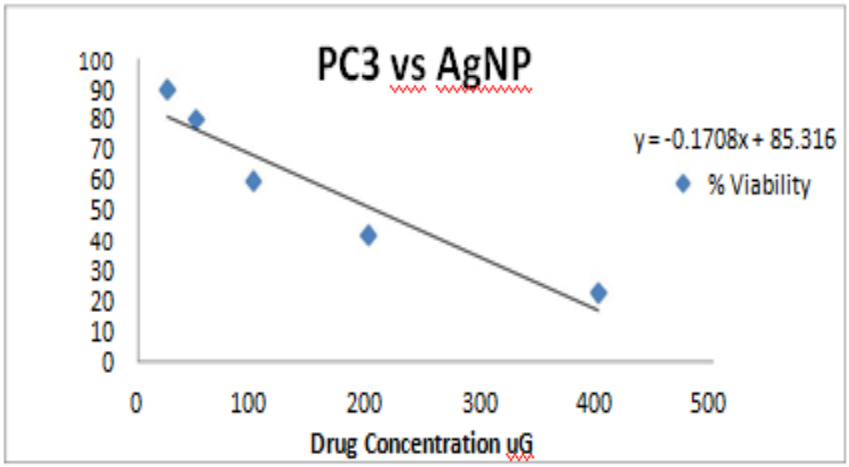
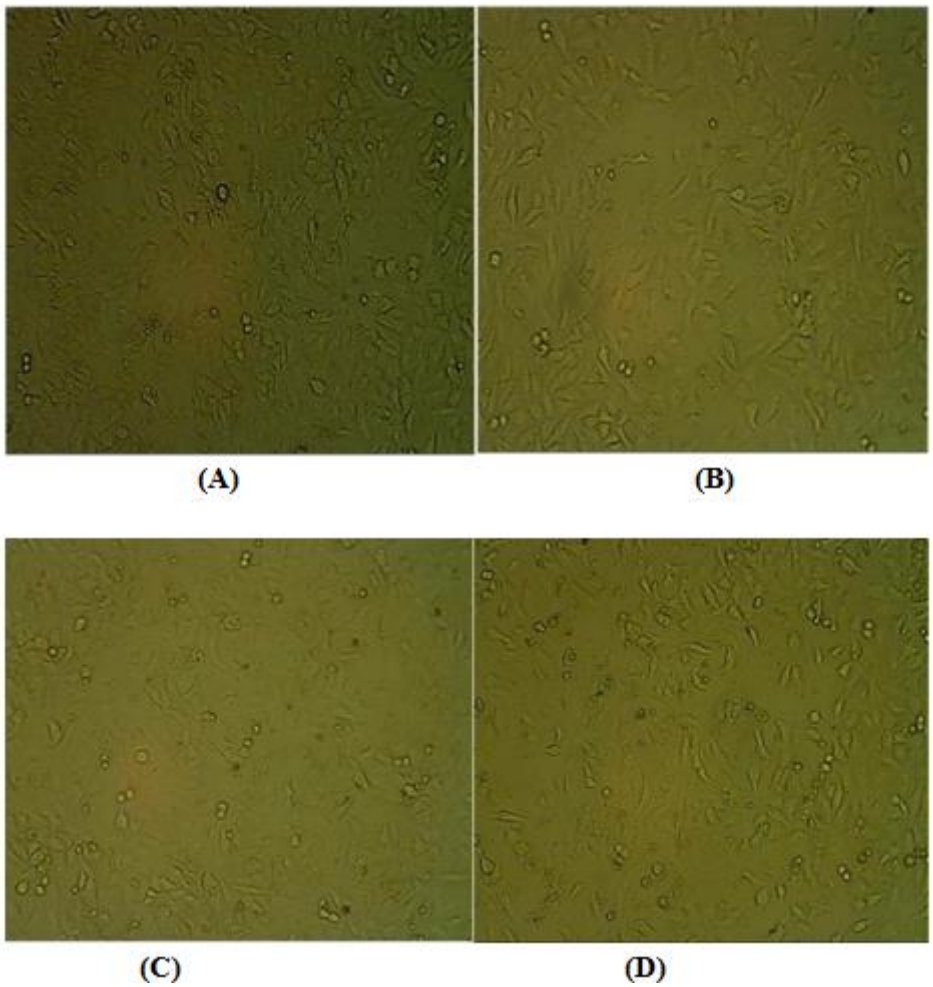
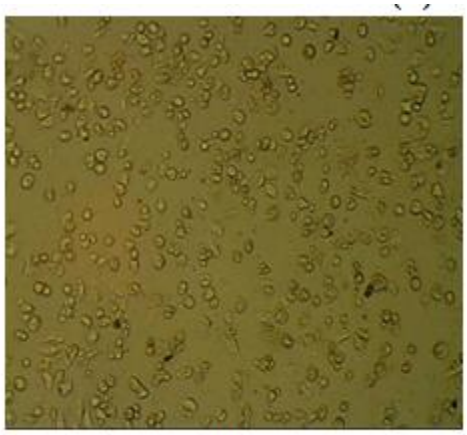


Figure 3.5.1 : % Viability of PC-3 cells by *M. pruriens* extract

The IC50value of the sample was found to be **207.71µg/mL** using y=mx+c equation.





(E)

Figures 3.5.2 (A-E): Drug treated PC-3 cells with varying concentrations (25, 50, 100, 200, 400µg/mL) of extract It was observed that at 400µg/mL, maximum cell death occurred.

	BLANK	UNTREATED	CPT 25µM	25	50	100	200	400
Reading 1	0.11	0.685	0.391	0.615	0.527	0.454	0.292	0.146
Mean	0.1	0.692	0.392	0.612	0.525	0.461	0.298	0.160
Mean OD-Mean B	NA	0.592	0.292	0.512	0.425	0.361	0.198	0.060
STANDARD DEVIATION		0.009	0.002	0.003	0.002	0.009	0.008	0.020
STANDARD ERROR		0.007	0.001	0.002	0.001	0.007	0.006	0.014
Viability %	NA	100	49.408	86.570	71.875	60.979	33.445	10.219

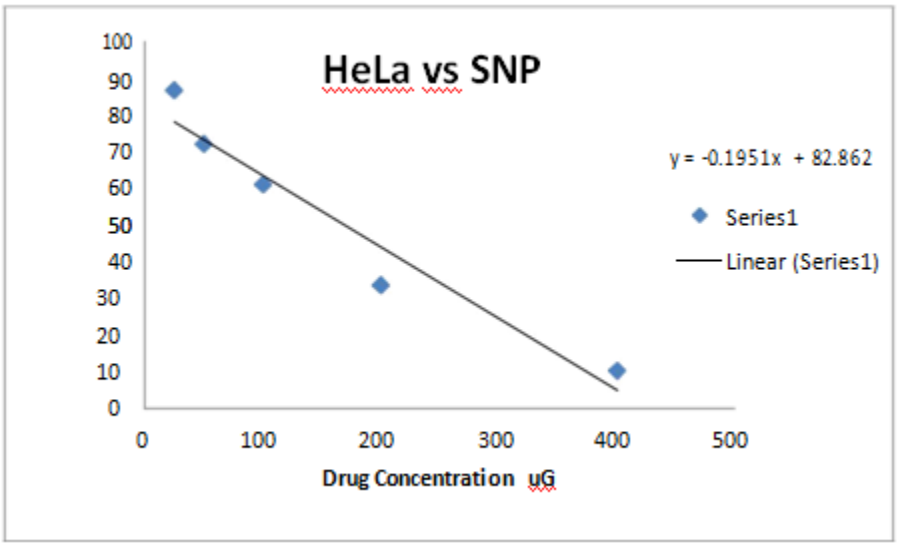
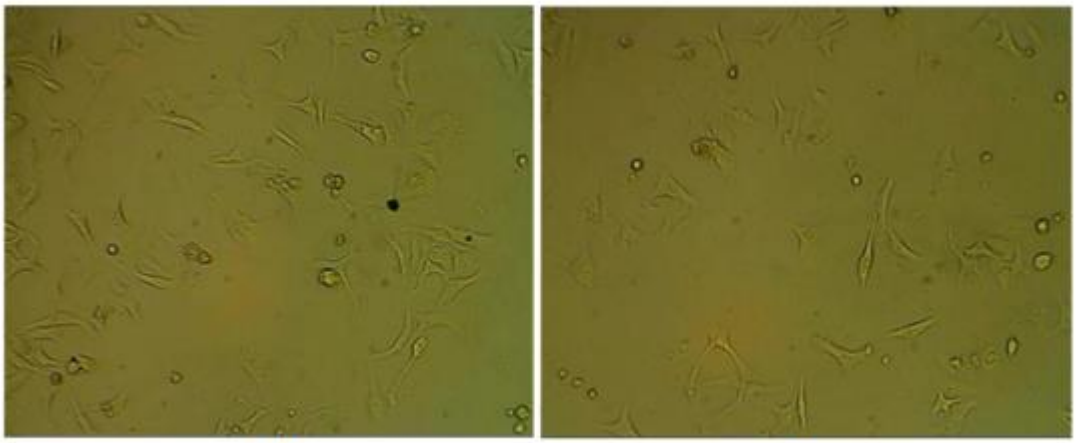
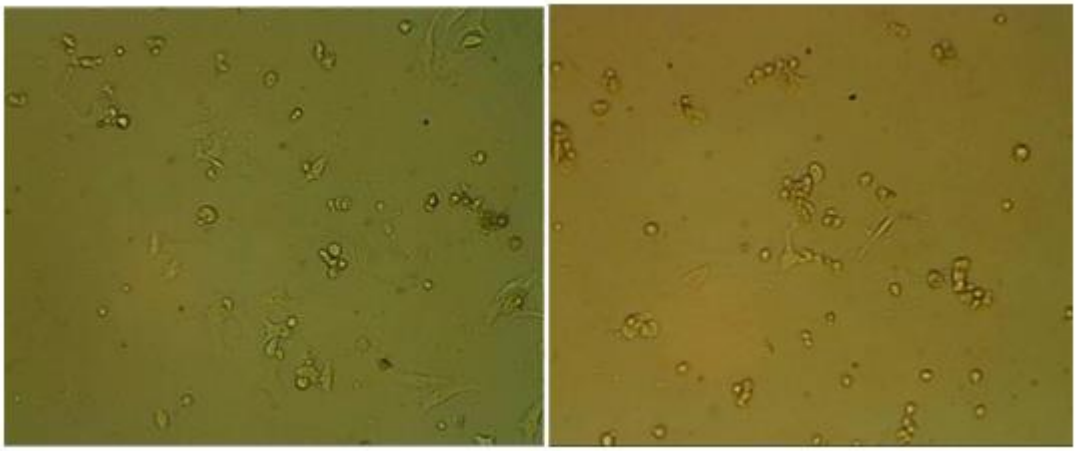


Figure 3.5.3: % Viability of HeLa cells by *M. pruriens* extract

The IC50 value of the sample was found to be **168.51µg/mL** using y=mx+c equation. Comparing theIC50 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.



(A) (B)



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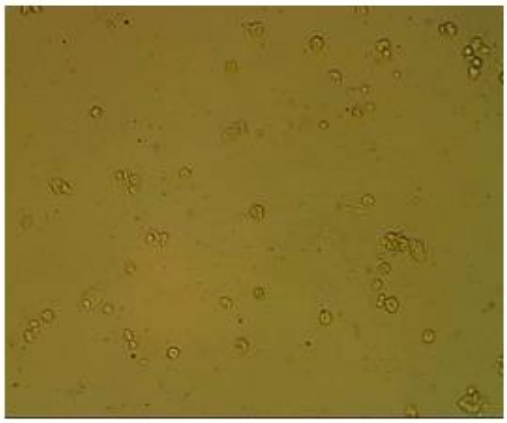


(E)

Figures 3.5.4 (A-E): Drug treated HeLa cells with varying concentrations (25, 50, 100,200, 400µg/mL) of extract

Similar to the patterns observed in PC-3 cell lines, maximum cell death occurred at 400µg/mL concentration.

3.6 Anti-oxidant activity:

3.6.1 DPPH Radical Scavenging Assay

Table 3.6.1: % Antioxidant Activity of SNPs compared with standard (Ascorbic Acid)

Concentration (µg/mL)	% A.A [Standard]	% A.A [Plant extract]
25	22	18.22
50	40.5	42
100	55.3	49.74
200	67	51.73
400	88	63.76

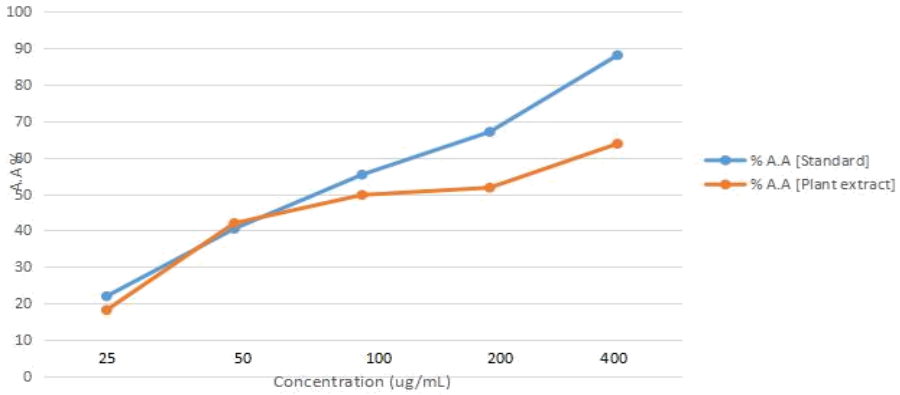


Figure 3.6.1.1: % A.A at different concentrations (Comparison of SNPs with Standard)

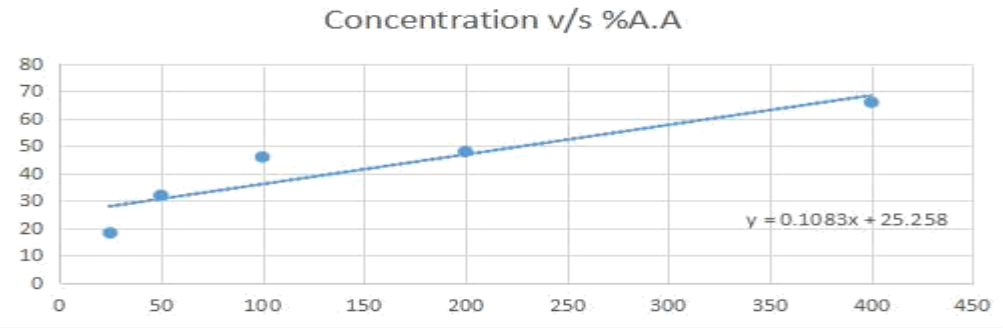


Figure 3.6.1.2: EC50 by linear regression (EC50= 71.48 µg/mL)

3.6.2 Iron Chelating Activity

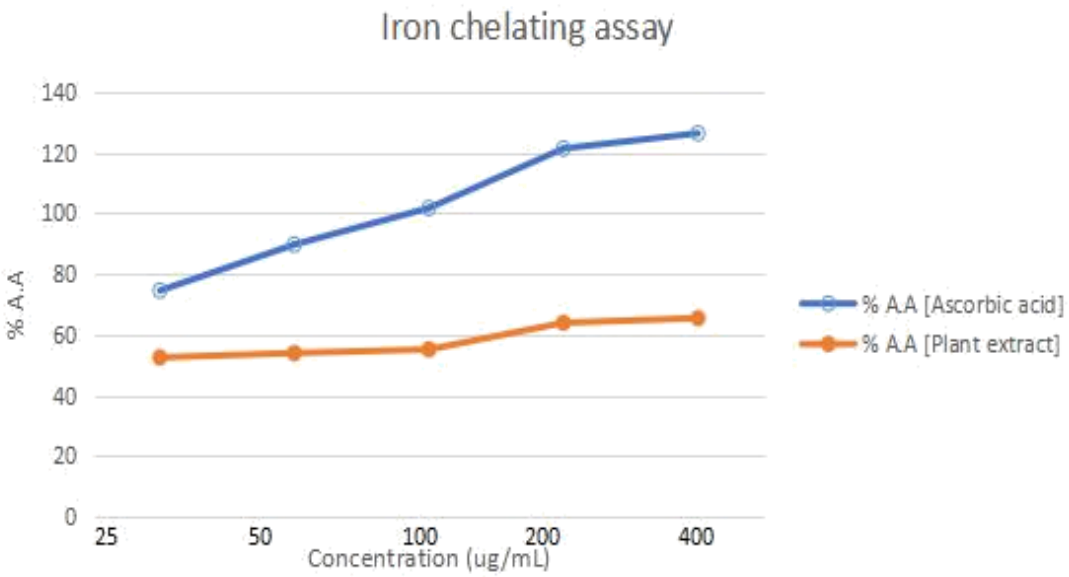


Figure 3.6.2.1: % A.A at different concentrations (Comparison of SNPs with Standard)

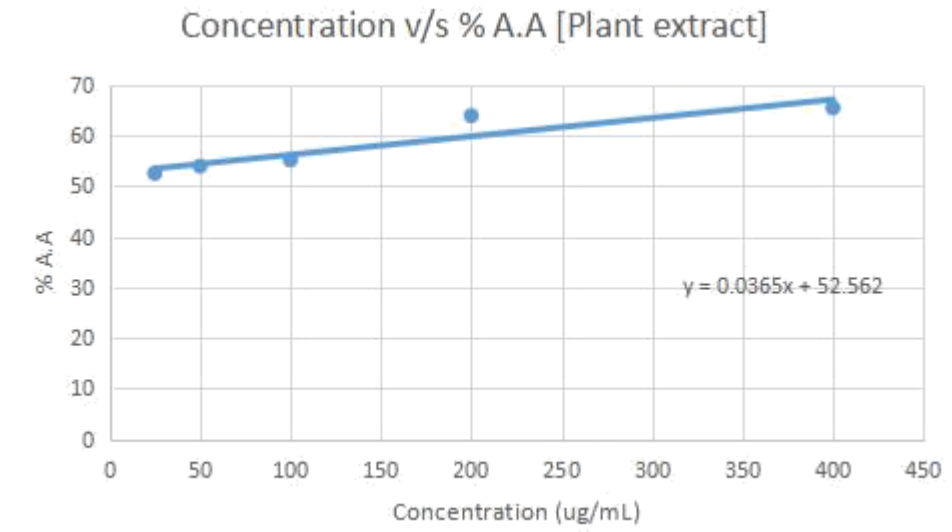
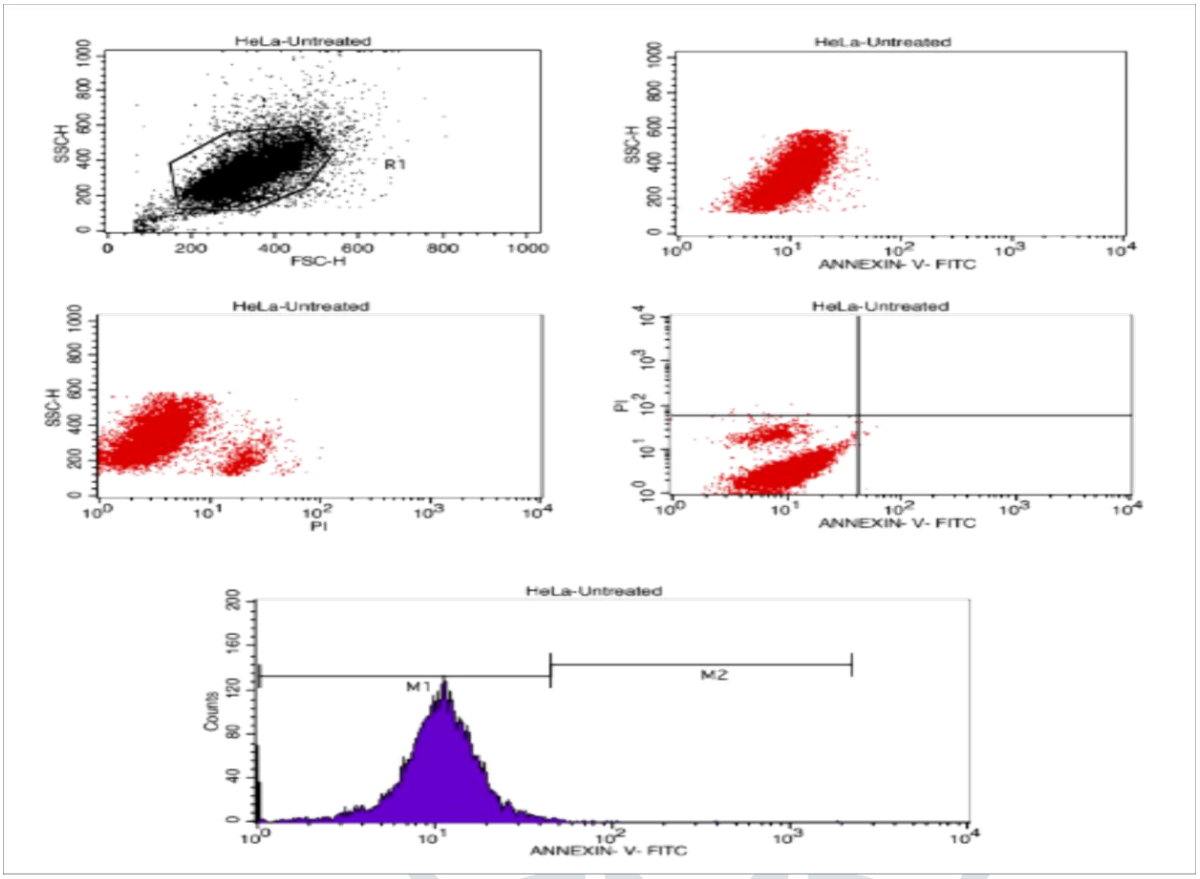


Figure 3.6.2.2 EC50 by linear regression (EC50= 70.19µg/mL)

Both the antioxidant assays (DPPH and Iron Chelating) were conducted relative to Ascorbic Acid which was used as the standard. The EC50 values obtained were 71.48µg/mL and 70.19µg/mL respectively. In both the assays, it was observed that the percentage inhibition of plant sample showed close similarity with the standard.

3.7 Apoptosis study:

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



Quadrant Statistics

File: HeLa-Untreated
Sample ID: HeLa-Untreated
Tube: Untitled
Acquisition Date: 20-Apr-18
Gated Events: 10000
X Parameter: ANNEXIN- V- FITC (Log)
Quad Location: 42, 57

Log Data Units: Linear Values
Patient ID:
Panel: Untitled Acquisition Tube List
Gate: G1
Total Events: 11175
Y Parameter: PI (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	11	0.11	0.10	6.32	5.43	70.00	68.48
UR	0	0.00	0.00	***	***	***	***
LL	9981	99.81	89.32	11.23	10.42	4.56	3.54
LR	8	0.08	0.07	50.30	50.08	21.30	15.89

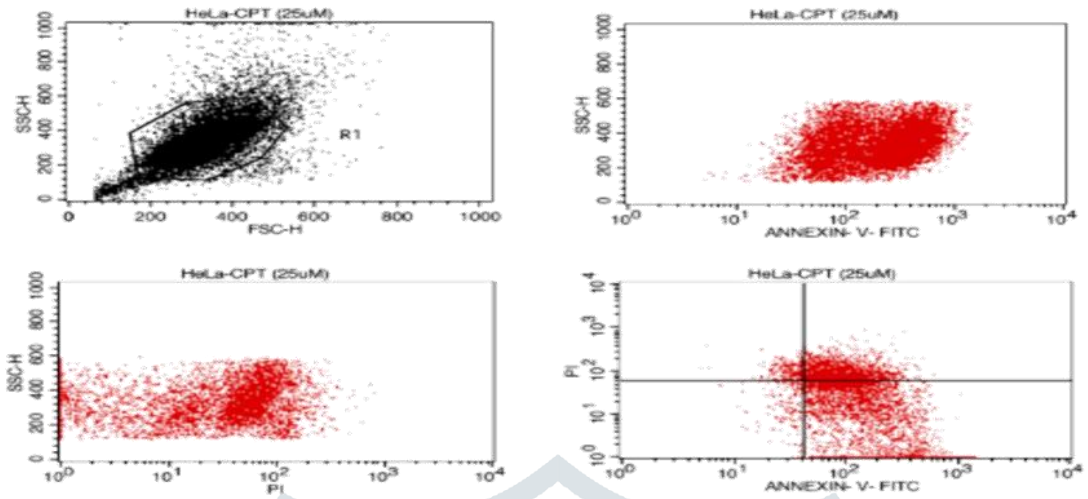
Histogram Statistics

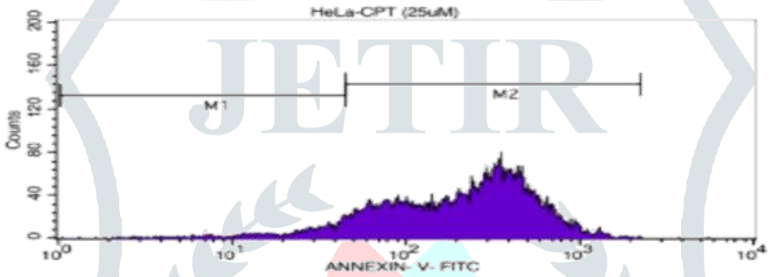
File: HeLa-Untreated
Sample ID: HeLa-Untreated
Tube: Untitled
Acquisition Date: 20-Apr-18
Gated Events: 11175
X Parameter: ANNEXIN- V- FITC (Log)

Log Data Units: Linear Values
Patient ID:
Panel: Untitled Acquisition Tube List
Gate: No Gate
Total Events: 11175

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	11175	100.00	100.00	11.91	10.43	151.69	10.75	11
M1	1, 45	11071	99.07	99.07	11.66	10.52	46.23	10.75	11
M2	45, 2227	35	0.31	0.31	115.01	64.56	256.01	51.40	45

igure 3.7.1: Flow cytometry plots of HeLa before treatment with drug





Quadrant Statistics

File: HeLa-CPT (25uM)
Sample ID: HeLa-CPT (25uM)
Tube: Untitled
Acquisition Date: 20-Apr-18
Gated Events: 10000
X Parameter: ANNEXIN- V- FITC (Log)
Quad Location: 42, 57

Log Data Units: Linear Values
Patient ID:
Panel: Untitled Acquisition Tube List
Gate: G1
Total Events: 11903
Y Parameter: PI (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	199	1.99	1.67	32.52	31.31	105.99	98.06
UR	1641	16.41	13.79	105.70	93.02	101.00	91.55
LL	171	1.71	1.44	31.55	30.20	24.02	15.61
LR	7989	79.89	67.12	314.80	255.60	8.45	2.33

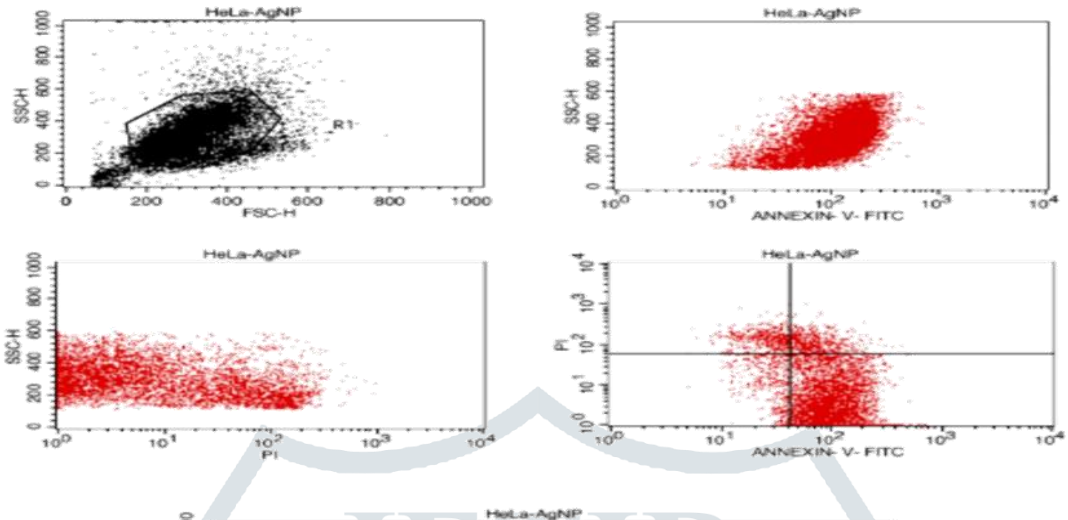
Histogram Statistics

File: HeLa-CPT (25uM)
Sample ID: HeLa-CPT (25uM)
Tube: Untitled
Acquisition Date: 20-Apr-18
Gated Events: 11903
X Parameter: ANNEXIN- V- FITC (Log)

Log Data Units: Linear Values
Patient ID:
Panel: Untitled Acquisition Tube List
Gate: No Gate
Total Events: 11903

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	11903	100.00	100.00	276.22	186.43	84.83	230.82	336
M1	1, 45	960	8.07	8.07	27.44	23.33	44.90	28.90	42
M2	45, 2227	10957	92.05	92.05	296.94	223.07	75.85	254.83	336

Figure 3.7.2: Flow cytometry plots of HeLa treated with Campothecin (25µM)



Quadrant Statistics

File: HeLa-AgNP
Sample ID: HeLa-AgNP
Tube: Untitled
Acquisition Date: 20-Apr-18
Gated Events: 10000
X Parameter: ANNEXIN- V- FITC (Log)
Quad Location: 42, 57

Log Data Units: Linear Values
Patient ID:
Panel: Untitled Acquisition Tube List
Gate: G1
Total Events: 11793
Y Parameter: PI (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	425	4.25	3.60	27.28	25.37	147.03	132.14
UR	536	5.36	4.55	78.50	71.52	121.60	108.10
LL	381	3.81	3.23	29.52	27.87	18.32	10.12
LR	8658	86.58	73.42	131.73	119.48	4.29	1.81

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

Figure 3.7.3: Flow cytometry plots of HeLa treated with SNPs (168.51 µg/mL)

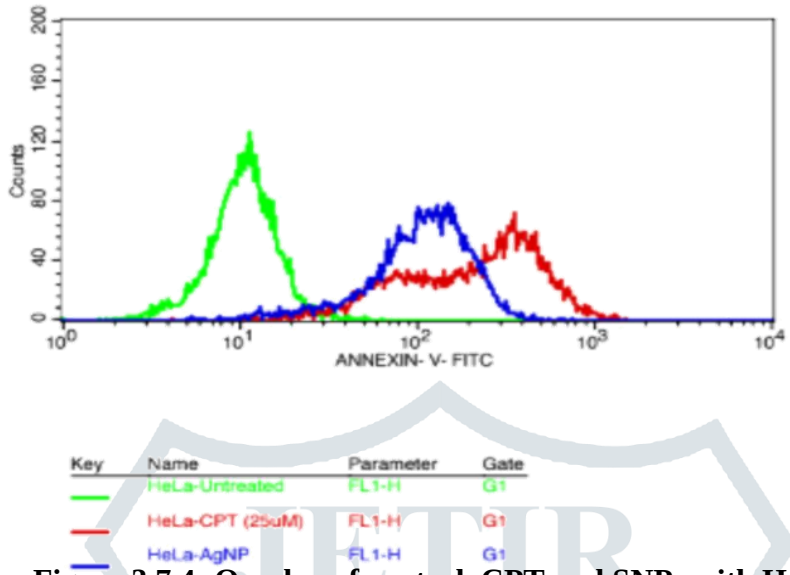


Figure 3.7.4: Overlay of control, CPT and SNPs with HeLa

The HeLa cells treated with 168.51µg/mL of *Mucuna pruriens* SNPs has induced apoptosis and 3.60% of cell death (PI positive) was observed when compared to untreated HeLa control cells of only 0.10% cells and Campothecin treated HeLa cells of only 1.67% cells. Quadrant statistics is given along with the respective figures (5.7.1-5.7.4).

CONCLUSION

Cancer is the second cause of death after cardiovascular diseases. With due attention to rapid progress in the phytochemical study of plants, they are becoming popular because of their anticancer effects. The aim of this study was to investigate the effective medicinal plants and silver nanoparticles synthesized using the plant extract *Mucuna pruriens* in the treatment of cancer. In the present investigation efforts made to synthesize silver nanoparticles using *Mucuna pruriens* seed extract based on green method was demonstrated. *Mucuna 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. The screening of SNPs using DPPH free radical method has proved to be effective in characterizing them as biological antioxidants due to the presence of radical scavengers such as flavonoids. The synthesized SNPs also showed metal chelating activity. Cytotoxic potential of SNPs as a function of its concentration was tested against two different cancer lines- HeLa and PC-3. From the study, SNPs were found to have lesser IC50 value against HeLa cells and were hence used for further apoptotic studies.From the study, SNPs were observed to have strong and almost equal apoptotic activity when compared with the standard drug-Campothecin (CPT).

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