Anti-cancer and Anti-Oxidant Properties of Silver Nano particles extracted from Acalypha indica, Azadirachta indica, Phyllanthus niruri, Coleus amboinicus and Curcuma longa

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Abstract:

The exploration of nanoparticles as potential agents for combating cancer and oxidative stress has garnered considerable attention in recent years. This study investigates the anti-cancer and anti-oxidant properties of silver nanoparticles (AgNPs) extracted from five medicinal herbs, namely Acalypha indica, Azadirachta indica, Phyllanthus niruri, Coleus amboinicus, and Curcuma longa. The synthesis of AgNPs was achieved through a green and sustainable approach, utilizing plant extracts rich in phytochemicals. The anti-cancer efficacy of the AgNPs was evaluated against a panel of cancer cell lines, revealing significant cytotoxic effects. Concurrently, the AgNPs demonstrated potent anti-oxidant activities, as evidenced by their ability to quench free radicals and attenuate oxidative stress. The findings underscore the potential of AgNPs derived from medicinal herbs as dual-action therapeutic agents against cancer and oxidative damage. This study not only contributes to the expanding field of nanomedicine but also highlights the importance of harnessing natural sources for the synthesis of therapeutic nanoparticles with multifaceted health benefits.

Keywords:*Green synthesis, Silver nanoparticles, Anti-cancer, Anti-oxidant, Botanical sources*

1. Introduction:

In the intricate landscape of oncology, cancer remains a formidable challenge, characterized by uncontrolled cell proliferation and evasion of the body's regulatory mechanisms. The diverse spectrum of cancers is underscored by the complexity of their molecular and genetic underpinnings, necessitating innovative approaches to treatment. Amidst this backdrop, silver nanoparticles (AgNPs) have emerged as potential contenders in the pursuit of effective anti-cancer strategies. Understanding the mechanisms through which cancer exerts its deleterious effects is crucial for devising targeted interventions.

Cancer initiation and progression often involve genetic mutations and dysregulation of signalling pathways that govern cell growth and survival. The evasion of apoptosis, a programmed cell death mechanism, allows cancer cells to persist and proliferate uncontrollably. Furthermore, the ability of cancer cells to induce angiogenesis and invade surrounding tissues contributes to the metastatic spread of the disease. The intricate interplay of these hallmarks necessitates a multi-faceted approach to cancer therapy. [1]

Nanoparticles, particularly silver nanoparticles, have garnered attention for their unique properties that can be harnessed in the fight against cancer. The high surface area and reactivity of AgNPs enable interactions with cellular components, making them promising candidates for targeted drug delivery and therapeutic interventions. These nanoparticles can be designed to selectively accumulate in cancer cells, capitalizing on the enhanced permeability and retention effect often exhibited by tumour tissues. Once localized, AgNPs can exert their anti-cancer effects through various mechanisms, including the induction of oxidative stress, disruption of cellular signalling pathways, and interference with DNA replication and repair processes [2]. Understanding the intricate ways in which nanoparticles interact with cancer cells provides a foundation for developing targeted and efficient therapeutic strategies.

This study focuses on the synthesis and exploration of AgNPs derived from five medicinal herbs—*Acalypha indica, Azadirachta indica, Phyllanthus niruri, Coleus amboinicus, and Curcuma longa*. The selection of these herbs is underpinned by their historical traditional uses and the presence of diverse bioactive constituents known for their health-promoting properties. In the intricate dance of chemical reactions, the phytochemicals within these herbs serve a dual role as both reducing and stabilizing agents during the AgNP synthesis process, thereby imprinting unique characteristics onto the resulting nanoparticles. This research not only explores the therapeutic potential of AgNPs derived from medicinal herbs but also underscores the importance of integrating traditional knowledge with cutting-edge nanotechnology for the development of novel and effective therapeutic agents. As we delve into the exploration of AgNPs derived from medicinal herbs, our study aims to unravel the potential of these nanoparticles in dismantling the intricate machinery that sustains and propels cancer, offering a glimpse into a future where nanotechnology converges with oncology for more effective and tailored cancer treatments.

1.1*Acalypha indica* (Indian Nettle):

Acalypha indica, commonly known as ‘kuppaimaeni’ in Tamil Nadu, is a commonly found weed plant, that contain many medicinal values to humans. It is widely used to cure eye infections, respiratory problems, rheumatism, skin problems like pimples, eczema and to decrease blood sugar. This whole plant is of great use.Ethanol extracts of *A.indica* has shown favourable antimicrobial activity against *E.coli, S.epidermidis*, and *S.typhi*. *A.indica* also has the ability to kill cancer cells as reported by many researches. One research done by [3] shows that the plant extract can help fight against breast cancer, oral cancer, and human prostate cancer.

1.2*Azadirachta indica* (Neem):

Azadirachtaindica commonly known as Neem, is a fast-growing tree found in all parts of India. It has been used a medicine since historical times, known well for their healing and anti-bacterial properties. Neem is called '*Aristha'* in Sanskrit, meaning 'perfect, complete and imperishable', and the tree, itself, called 'reliever of sicknesses. Findings from laboratory research suggest that the components of neem possess potent anticancer effects [4]. Neem limonoids also induce apoptosis through activation of the extrinsic apoptotic pathways in breast, colon, prostate, stomach, and leukemic cancer cells [5]. Neem-derived constituents is found to have abilities to block cancer growth on a cellular level, by suppressing the proliferation and growth of cancer cells, interfere with growth factor of the cancer cells, and decrease tumour cell invasion and migration [6]. Researches have concluded that this cancer cell killing property of neem could be due to the presence of various compounds present in the said species such as azadirachtin, gedunin, nimbin, nimbidol, nimbidin, salannin and quercetin. These properties are to be utilized in the production of the nanoparticles to enhance the efficiency in eliminating the cancer cells.

1.3 *Phyllanthus niruri* (Stone Breaker):

Phyllanthrus niruri plant originated in India, is also native to tropical coastal areas. In traditional herbal medicine, this plant has played a vital role in helping cure urinary tract stones, diarrhoea, ulcer, swelling, and diseases associated with genitals particularly urinary tract. The plant is of medicinal importance for numerous ailments like dysentery, influenza, vaginitis, tumours, diabetes, diuretics, jaundice, kidney stone, dyspepsia, anti-hepatotoxic, anti-hepatitis-B, antihyperglycemic and also as antiviral and antibacterial. Surprisingly, this plant, has phytochemicals that can help in creating a anti-HIV and anti-hepatitis drug [7].*P.niruri* gained attention in the late 1980s due to its activity against hepatitis B. Hydro-alcoholic extract of the whole plant when fed to albina mice with skin cancer, was said to have a anti-tumour activity on the mice and reduced the tumour cells in the mice's body [8].

1.4 *Coleus amboinicus* (Indian Borage):

This herb, commonly known as karpooravalli in Tamil Nadu, is an aromatic spice used in our day-to-day life, for consumption and to cure various infections and diseases. In traditional medicine of India, this herb was used to treat variety of diseases i.e., malaria, bronchitis, common cold and cough, inflammation, liver diseases [9]. Work done by [10], provides proof that the antioxidant property of this species is powered by components like rosmarinic acid, caffeic acid, thymol and chlorogenic acid. Most commonly used in cancer line research, ethanolic leaf extract of *C.amboinicus* has the potential of killing the cancer cells efficiently from the within by supressing NF-κB signalling pathway [11]. With so many health benefits and many compounds to help cure common diseases to cancers, this herb is a multi-potential hero.

1.5 *Curcuma longa* (Turmeric):

Curcuma longa, or turmeric, is a common ingredient found in our food and in our home. Curcumin is being recognized and used worldwide in many different forms for multiple potential health benefits. Studies have shown that curcumin contributes in the death of cancer cells and reduce angiogenesis and metastasis. Curcumin targets multiple signalling pathways involved in the initiation, development, and growth of tumours.

Growth factors, transcription factors, protein kinases, cytokines, and genes taking part in apoptosis are the molecular targets of curcumin, which appears to significantly affect the development of various malignancies [12]. With these properties, curcumin has emerged as a potential component in production of an anti-cancer drug.

2. Materials and Methodology:

2.1 Plant Sample Extraction:

The plant extraction was conducted using the heat method, following the procedures outlined in the article by [13]. The collected plant samples of *Acalypha indica, Azadirachta indica, Phyllanthus niruri, Coleus amboinicus* and *Curcuma longa* were dried and powdered, preparing them for the extraction process. For each sample, 5 grams were mixed with 50 ml of water, and the mixture was heated to 95 degrees Celsius for 15 minutes. After cooling, the extract was strained using a filter to eliminate larger powder particles.

2.2 Phytochemical Analysis:

Phytochemical analysis of plant extracts is performed to identify and quantify the various bioactive compounds present in plants. Many phytochemicals have medicinal properties and can be potential sources of new drugs or therapeutic agents. Analysing plant extracts helps in identifying and understanding the bioactive compounds responsible for medicinal properties. The plant extracts were analysed for the alkaloids, flavonoids, phenolic components, reducing sugars, tannins, saponins, Phyto steroids, steroids, cardiac glucoside, and proteins.

2.3 Silver Nanoparticle Synthesize:

Synthesizing the silver nano particles was based on the article [14]. 15 ml of each plant extract was combined with 5 ml of silver nitrate solution and 20 ml of water to make it up to 100ml. This solution was heated at 50℃ for 15 mins. The formation of the silver nanoparticles is indicated by a change in colour of the solution. The dark brown solution turns light brown. Further, the solution is Centrifuged at 10,000 rpm for 10 mins and the nanoparticles are obtained as pellets. The pellets are resuspended into 1 ml of the supernatant and are transferred to a petri plate. We were able to obtain the silver nanoparticles in powder after the solution dried.

3. Results and Discussion:

3.1 Phytochemical Analysis:

Phytochemical analysis of plant extracts is performed to identify and quantify the various bioactive compounds present in plants. Many phytochemicals have medicinal properties and can be potential sources of new drugs or therapeutic agents. Analysing plant extracts helps in identifying and understanding the bioactive compounds responsible for medicinal properties. In this phytochemical analysis the plant extracts were analysed for alkaloids, flavonoids, phenol, reducing sugars, tannins, saponins, phyto-steroids, steroids, cardiac glycosides, and proteins. The presence of the component are indicated by the positive sign (+) and their absence is indicated by the negative sign (-) (Table 1).

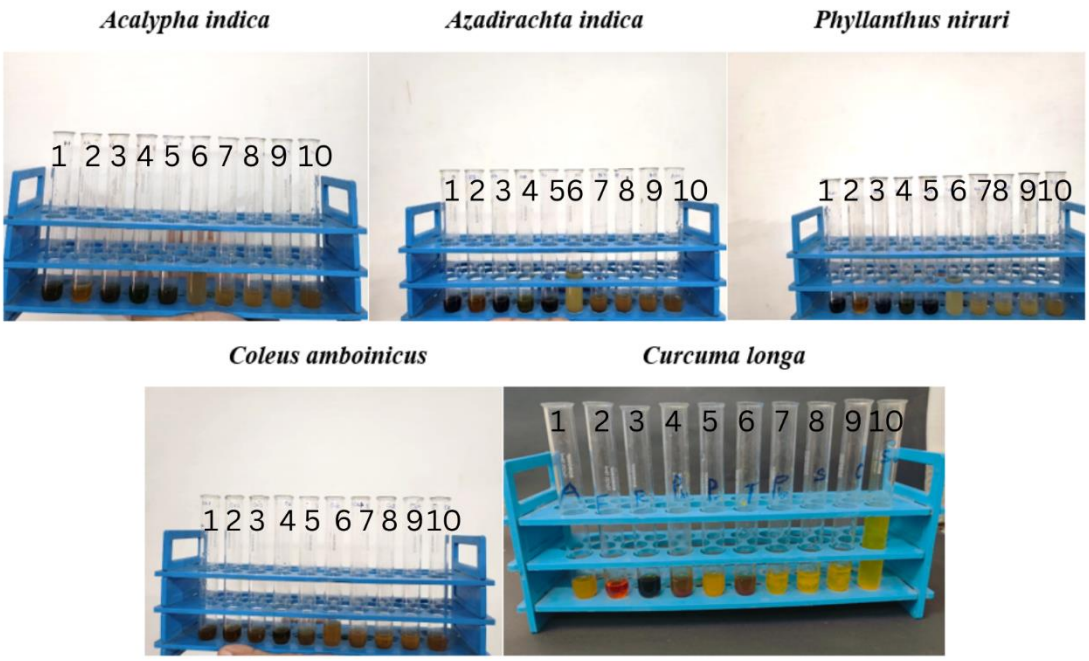

Figure1.Phytochemical Analysis

S.no	Component	*Acalypha indica*	*Azadirachta indica*	*Phyllanthus niruri*	*Coleus amboinicus*	*Curcuma longa*
1.	Alkaloids	+	+	+	-	-
2.	Flavonoids	+	+	+	+	+
3.	Phenols	+	+	+	+	+
4.	Reducing Sugars	+	+	+	+	+
5.	Tannins	+	+	+	+	+
6.	Saponins	+	+	+	+	+
7.	Phyto-steroids	+	+	-	+	-
8.	Steroids	+	+	-	+	-
9.	Cardiac Glycoside	+	+	-	+	-
10.	Protein	+	+	+	+	-

Table 1. Result of Phytochemical Analysis

3.2 Anti-Oxidant Activity:

3.2.1 DPPH and FRAP Assay:

DPPH Assay was done based on the article [15]. A solution was prepared by dissolving 1g of powdered silver nanoparticles extracted from the herbs in 900 µL of water. This solution was serially diluted to prepare the sample solutions, with 50 µL of this solution being the maximum concentration. DPPH and ethanol were added to make up the solution to 1 ml. The spectroscopic reading was taken at 517 nm after incubating it in dark for 30 mins.

FRAP Assay was done by serially diluting the solution similarly as in DPPH and was made up to 1000 µL with FRAP solution. The Spectroscopic reading was taken at 593nm after 30 mins. The decrease in the absorbance as the increase in concentration of the plant extract containing AgNPs indicate the presence of Anti-oxidative property (Table 2).

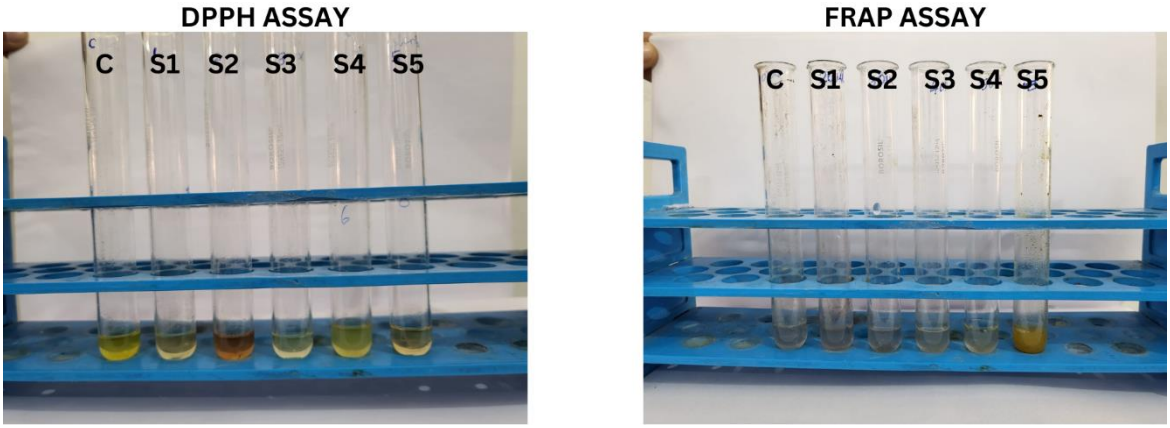

Figure2.DPPH and FRAP Assay

		DPPH Assay		**FRAP Assay**	
Sample	**Concentration (µL/mL)**	**Absorbance**	**Antioxidative Property %**	**Absorbance**	**Antioxidative Property %**
Control		0.993		0.763	
S1	10	0.55	44.6%	0.605	20.7%
S2	20	0.478	52%	0.570	24.1%
S3	30	0.456	54%	0.502	34.20%
S4	40	0.399	65.80%	0.435	42.90%
S5	50	0.333	66.46%	0.231	69.70%

Table 2.Results of DPPH and FRAP Assays

3.3 UV Spectral Analysis:

Confirmation of the synthesis of silver nanoparticles mediated by leaf extracts was achieved through spectral analysis. UV-VIS spectra of the synthesized silver nanoparticles were obtained using a spectrometer, with continuous scanning ranging from 220 nm to 800 nm. Distilled water served as the reference for baseline correction during the spectral analysis process.From the spectral analysis, it is observed that the AgNPs peak was obtained at 300 nm with the highest peak (Fig 1).

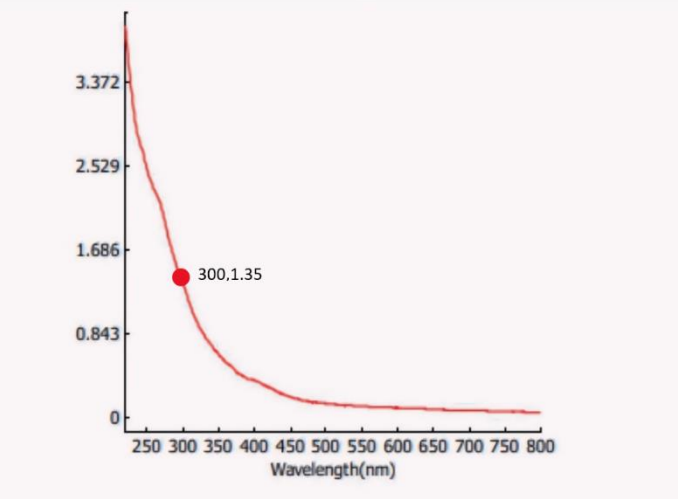

Figure3.UV Spectral Analysis graph

Wavelength (nm)	OD
220	3.937
300	1.356
350	0.637
400	0.369
450	0.203
500	0.134
700	0.065
800	0.040

Table 3.Result of UV spectral analysis

3.4 Fourier Transform Infrared Spectroscopy (FTIR):

- FTIR spectroscopy serves as a valuable technique in identifying distinct functional groups based on spectral bands, facilitating the assessment of the interaction between nanomaterials and adsorbed biomolecules. This enables comprehensive surface characterization of nanoparticles, allowing for the determination of surface chemical composition under specific conditions. Moreover, FTIR analysis can pinpoint reactive surface sites responsible for surface reactivity. The spectrum generated by FTIR comprises absorption peaks corresponding to the frequencies of vibration between the bonds of atoms within the nanoparticle, providing valuable insights into its structural properties.The results of FTIR analysis confirm the presence of a total 4 functional groups which show the major peak values that are **399.26** This frequency range typically corresponds to bending vibrations in metal-oxygen bonds or metal-halogen bonds. It could be indicative of metal complexes or metal-containing compounds, **698.23** This region often corresponds to the bending vibrations of substituted aromatics or out-of-plane bending vibrations in aromatic rings. It could indicate the presence of aromatic compounds or molecules with benzene rings, **1643.35** corresponds to stretching vibrations of carbonyl groups (C=O) in ketones and aldehydes, and **3336.85** corresponds to the stretching vibrations of O-H bonds, usually indicating the presence of alcohols or phenols (Fig 2).

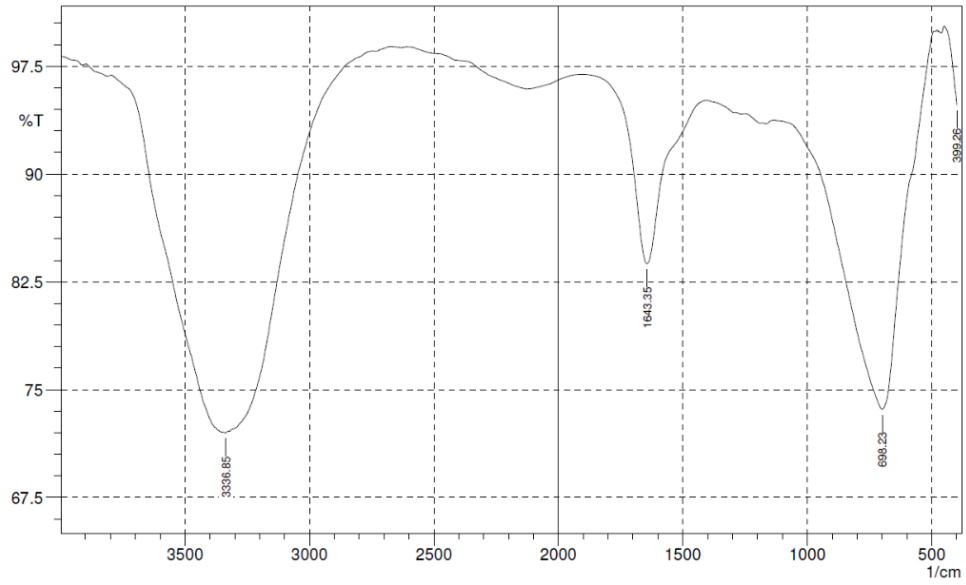

Figure4.FTIR Result

3.5 Anti-cancer Assay:

3.5.1 Cell Line:

The human Liver Cancer cell line-HepG-2 was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 370C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

3.5.2 MTT Assay:

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water-soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

The percentage cell viability was then calculated with respect to control as follows

% Cell viability = [A] Test / [A]control x 100

The % cell inhibition was determined using the following formula.

% Cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using GraphPad Prism software.

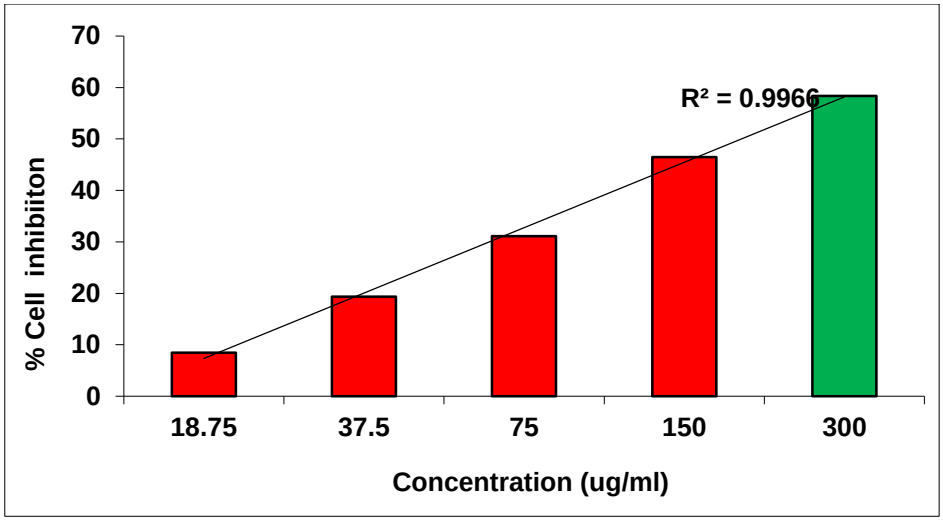

Figure 5. Nonlinear regression graph between % Cell inhibition and Log concentration

Conc	18.75 µg	37.5 µg	75 µg	150 µg	300 µg	Cont
ABS	0.065	0.151	0.244	0.367	0.456	0.781
	0.066	0.151	0.245	0.362	0.457	0.785
	0.067	0.152	0.242	0.363	0.457	0.782
Avg	0.066	0.151333	0.243667	0.364	0.456666667	0.782667

Conc (µg/ml)	% cell inhibition			IC 50 174.29 µg/ml
18.75	8.432709			
37.5	19.3356			R^2 0.996
75	31.13288			
150	46.50767			
300	58.34753			

Table 4. Calculation table of MTT Assay

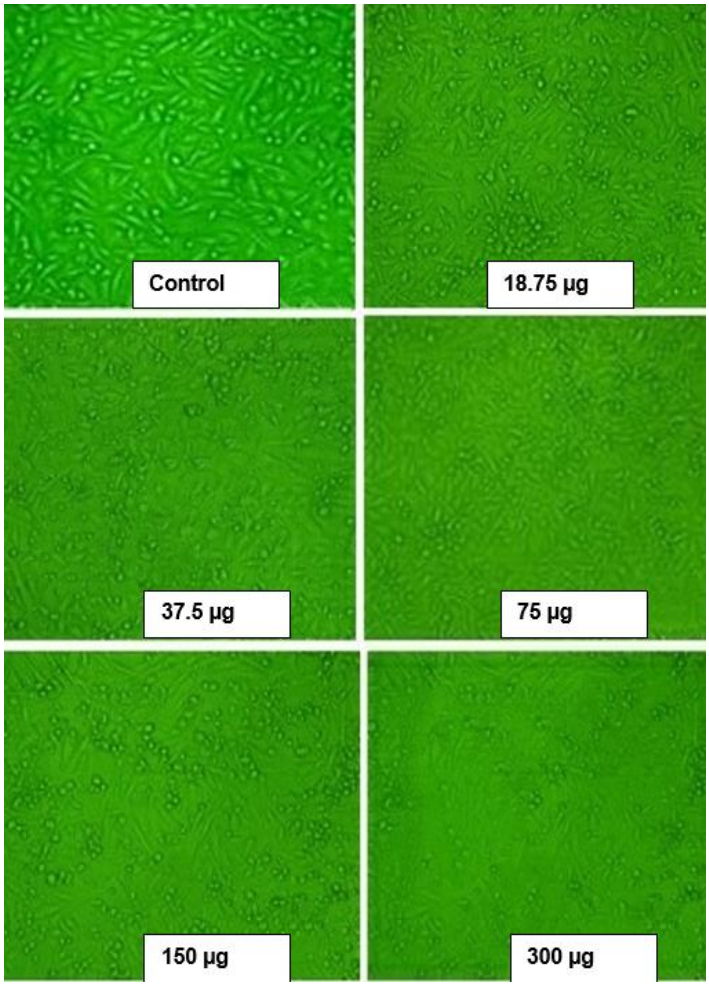

Figure 6. Cell viability checked under microscope after MTT Assay

4. Acknowledgement:

This is the B.Tech final year project of first, second, third, and fourth author. We acknowledge Department of Biotechnology, Sri Shakthi Institute of Engineering and Technology for providing an ambient environment for the completion of project.

5. Conclusion:

In conclusion, this research project has successfully demonstrated the antioxidant activity of the extract containing silver nanoparticles through DPPH and FRAP assays. Confirmation of silver nanoparticle synthesis mediated by leaf extracts was achieved via spectral analysis, with UV-VIS spectra revealing a prominent peak at 300 nm. FTIR spectroscopy proved instrumental in identifying distinct functional groups and facilitating surface characterization, highlighting the presence of key functional groups at specific peak values. Furthermore, the cytotoxicity assays, specifically MTT assays, underscored the potential anti-cancer properties of the plant extract containing silver nanoparticles. The observed decrease in cell viability, particularly in prostate cancer cell lines, suggests a promising avenue for further exploration of these nanoparticles as potential anti-cancer agents.

Additionally, morphological changes and cell shrinkage observed in the cancer cell lines further corroborate the cytotoxic effects induced by the plant extract. Overall, this study underscores the multifaceted therapeutic potential of silver nanoparticles derived from plant extracts, offering insights into their antioxidant and anti-cancer properties, and paving the way for future research in the field of nanomedicine.

6. Declarations:

Conflict of Interest: The authors report no conflicts of interest.

Funding: The source of funding is NIL.

Ethical Clearance: NIL.

Permission to reproduce: NIL.

7. References:

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