RESEARCH ARTICLE

Orange Peel Extract Silver Nanoparticles: Formulation and Characterization for Potential Anticancer Activity

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ABSTRACT

Objective: The current study was attempted to develop silver nanoparticles from orange peel extract designed for the management of lung cancer.

Method: Silver nanoparticles were prepared using orange peel extract and nanoparticles forming material in an organic solvent to form an effective solution. The nanoparticles were formed by the solvent evaporation method. Nanoparticles are extremely small units whose size is articulated in nano-meters (nm; 1 nm = 10−9 meters). Both naturally occurring and artificially produced nanoparticles are products of human activity. To transport medications to the target region, nanoparticles (NP) play a crucial function and can conjugate with different pharmaceuticals in a variety of ways. The prepared silver nanoparticle were characterized i.e. UV spectroscopy, FTIR, XRD, SEM, Anticancer activity.

Results: Orange peel silver nanoparticles exhibit satisfactory parameters results which shows this nanoparticle can be used for anticancer activity and both the compounds have good compatibility and effectiveness in the cancer cells and sustainability for the growth of the cancer.

Silver nanoparticles are one of the most prominent compound for cancer activity.The best average sizes of the particles were around 67.94nm from the orange peel extract.

Keywords:*Nanoparticle, Orange peel extract, anti-cancer, silver nanoparticle.*

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INTRODUCTION

A nanoparticle, sometimes referred to as a nano-scale particle, with a dimension of one to one hundred nanometers (nm). In both directions, the word is also used to describe fibres and nanotubes with diameters of 100 nm or less, as well as bigger particles up to 500 nm in size. A typical term for metal particles smaller than 1 nm is "atom clusters." The biological manufacture of nanoparticles from waste plant extracts is now being explored, with some researchers working on it and testing for anticancer properties. The dimension vary from 1 to 100 nm closely resembles the mesoscale, or range of 1 to 1,000 nm, which is generally associated with the field of colloid science. The distinction is mainly conceptual for molecules less than 100 nm. A nanoparticle is a single nano-object that has three coordinate dimensions smaller than 100 nm. Both one-dimensional (nanofibres and nanotubes) and two-dimensional (nanodiscs and nanoplates) nanoobjects are defined in the ISO standard. However, a more expansive yet scientific interpretation was approved by the European Union Commission in 2011. Innovative synthesis techniques and the investigation of new biological resources for nanoparticle production are made possible by green synthesis approaches. New plant species, microbial strains, and bioactive chemicals that can be used in green synthesis methods are constantly being discovered by researchers[1] Microorganisms used in green synthesis processes include bacteria, fungus, algae, and plant extracts[2]. The biomolecules found in these organisms can be utilised as capping, stabilising, and reducing agents when creating nanoparticles. These biomolecules include, for example, polyphenols, proteins, and polysaccharides[3]. Green synthesis is an environmentally safe and sustainable way to produce nanoparticles that could be used in a number of industries, including electronics, environmental remediation, medicine, and catalysis[4]. Green NP synthesis makes use of the compounds' reducing potential in living organisms. Both microbes and plants are global, easily accessible, and safe handling resources. Additionally, including biomass waste in the green production practice can help move NP production to circular economy principles[5].Because of their minuscule size and special characteristics, nanoparticles are useful in a wide range of scientific and technological domains[6]. Among the essential characteristics are:Biocompatibility: Green synthesized nanoparticles are frequently more biocompatible than their chemically synthesized counterparts. This qualifies them for a range of biomedical uses, including treatments, imaging, and medication delivery[7].When compared to nanoparticles made by chemical means, green-synthesized ones frequently show improved biocompatibility. However, particularly for biomedical applications, it is crucial to thoroughly evaluate their cytotoxicity and potential health implications[8]. High Surface Area to

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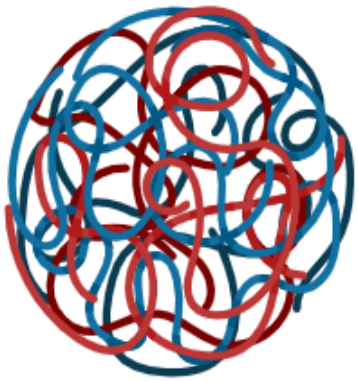


Figure 1: Nanoparticle

Figure 2: Orange peel extract.

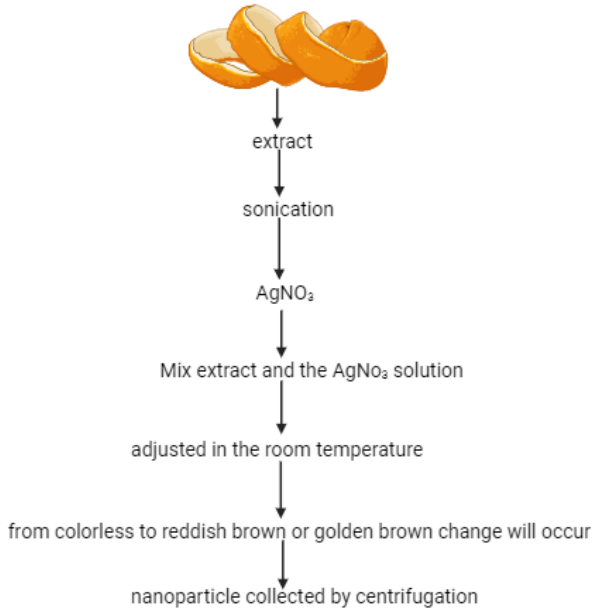


Figure 3**:** Method of solvent evaporation nanoparticles

Volume Ratio: The remarkable surface area of nanoparticles about their volume amplifies their reactivity and lends them utility in medication administration[9]. Size-Dependent Properties: The physical, chemical, and optical characteristics of nanoparticles can alter as they go closer to the nanoscale. A variety of disciplines, including materials science, electronics, and optics, make use of this size-dependent behavior[10].

Quantum Effects: Quantum effects become important at the nanoscale, resulting in distinctive optical, electrical, and magnetic properties[11]. For instance, the size-tunable fluorescence of quantum dots makes them useful in imaging and display technology. Orange peel, the outer, protective skin of an orange fruit, contains a diverse substance, including essential oils, flavonoids, vitamins, and dietary fiber[12]. The peel is part of the Rutaceae family, also known as the citrus or rue family. Citrus sinensis is the biological term for the orange plant and its peel. "Citrus" is the genus name, while "sinensis" is the species name. Citrus sinensis refers to the complete orange tree, including the fruit and peel[13]. Orange peel's many properties make it beneficial for a number of uses, such as in cooking and medicine. Its Biological name is *Citrus sinensis*, family is Rutaceae and genus- Citrus

MATERIALS & METHODS

Chemical and Solvent

Orange peel powder, $AgNo_3$(silver nitrate), and Ethanol were received by the Uttaranchal University of Pharmaceutical Sciences, Uttaranchal University Dehradun.

Glassware and Apparatus

These glassware (petri dish, measuring cylinder,test tube, funnel, volumetric flask, beaker, centrifugation tube) and apparatus (probe-sonicator, sonicator, filtration assembly, pH meter) were used for the for the formulation of silver nanoparticles.

Formulation of $AgNo_3$ nanoparticles

*Solvent Evaporation Method***:** This method involves dissolving the orange peel extract and nanoparticle-forming materials in an organic solvent to form an effective solution. The solvent is then evaporated to form orange peel extract tiny particles. To make the silver nanoparticles, 40 ml of 1 mm aqueous AgNO3 solution was carefully mixed with 3 ml of each fruit peel extract in a 250 ml flask[14-21]. The flask was then left in a dark place and left to stand at room temperature for five hours. Using solvent evaporation, one of the most common methods for creating polymeric nanoparticles—particularly drug-loaded polymeric systems—for pharmaceutical formulations is the change from colourless to reddish brown or orange[22]. Generally, the medication is mixed into a volatile natural solution that also dissolves the polymer. The final solutions are combined with the surface active agent having aqueous phase to produce an emulsion, the final solutions are incorporated with the surfactant-containing aqueous phase. Using a 250 ml flask, 40 ml of 1 mm aqueous AgNO3 solution was carefully mixed with 3 ml of each fruit concentrate to produce silver nano-particles in an ecologically sustainable way[23]. After that, the flask was kept at room temperature in a dark place for five hours. When the colour of the colloidal suspension turned from colourless to reddish brown or golden brown, it was determined that silver nanoparticles were biogenic. The organic solvent is removed when a durable emulsion has formed by stirring continuously or by raising the

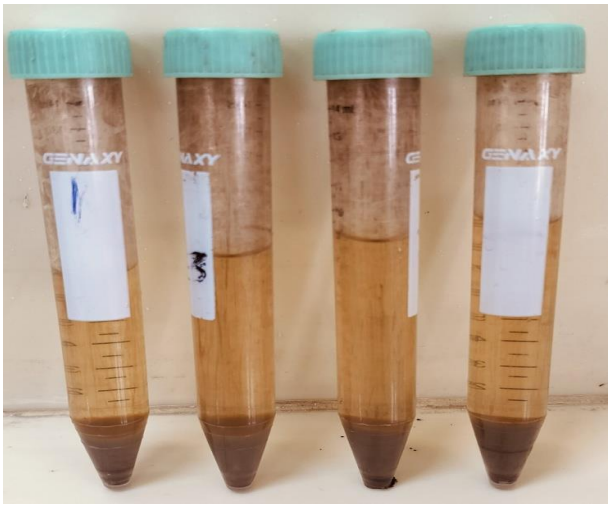


Figure 4a: Nanoparticles collected after centrifugation

Figure 4b: Solution of orange peel extract+ AGNPs

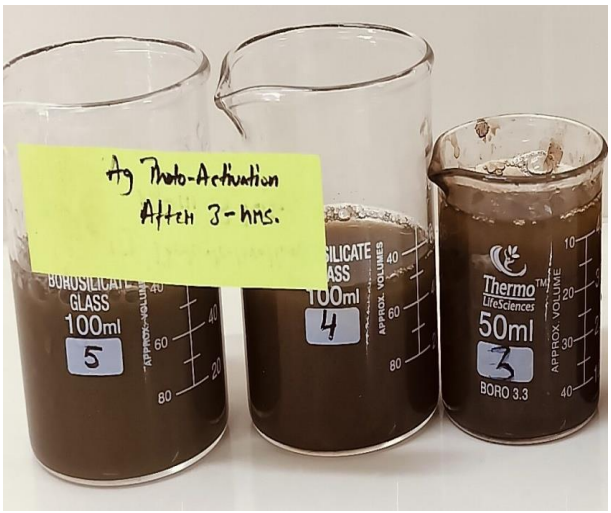


Figure 5: Solution Extract+AgNPs were kept under the dark for 3 hrs (for photo-activation)

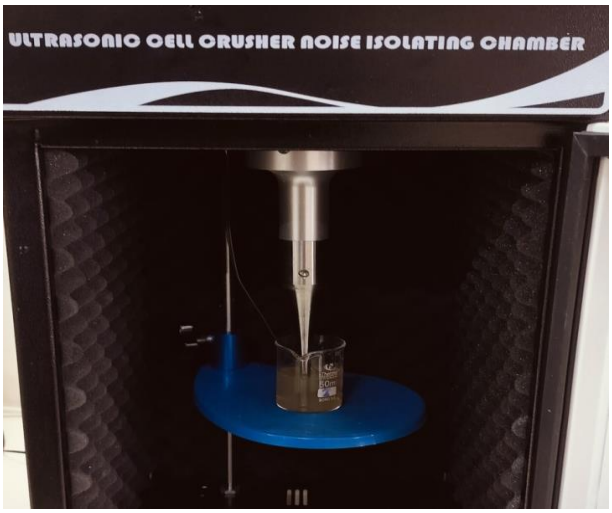


Figure 6: Probe sonication of the obtained orange peel extract+ AgNP solution.

Table 1: Formulae components

S.No	Components	F1	F2	F3
1	Extract	3ml	6ml	9ml
2	Silver Nitrate	1.69 gm	3.38 gm	6.76
3	De-ionized water	40ml	80ml	120ml

temperature while lowering the pressure[24]. As a result, the nanodroplets spread out. By forming and stabilising the core in a solvent obtained in the first step of a water-in-oil emulsion and then dispersing its final water phase in the surfactant and stabiliser golden brown of a colloidal suspension, this method was also used to create core-shell particles, confirming the biosynthesis of silver nanoparticles[25].

Phytochemical Screening

Test of Alkaloids: 1% HCL, 6 drops of Mayor's reagent, and 2 millilitres of filtrated orange peel extract were combined. The presence of alkaloids is indicated by the cramish and pale yellow colour that was seen.[(26].

Test of Tannins: 1ml of orange peel extract was used in the ferric chloride and brownish-green and blue-black colors were observed.

Table 2: Through Soxhlet apparatus orange peel extract (yield in gms)

Solvent according to the polarity	Yield (Sample 100 gm)
Acetone	1.4g
Methanol	62.6g
Hexane	1.4g
Distilled water	12.7 g

Test of Proteins: 4% sodium hydroxide was mixed to 3 ml of extract followed by some drops of 1% copper sulfate.into it which shows the violet and pink color observed.

Evaluation Parameters of Silver Nanoparticles

*Fourier Transfer-Infrared Spectrophotometer (FTIR***):** An investigative method for identifying or confirming the OH group known as the functional group present in the moiety indicative of that molecule is FTIR spectroscopy. The primary functional components found in AgNPs and the herbal extract were detected by scanning the samples between 4000 and 400 cm^{-1}. It gives the capacity to identify and examine nanoparticles, as well as macro-flaws such as secondary phases, porosity, fissures, and

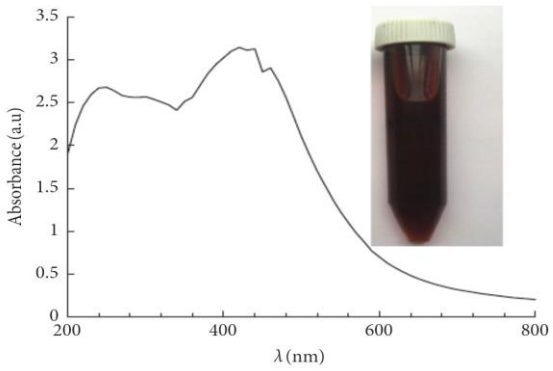


Figure 7: UV-Absorption Spectroscopy of Orange Peel Extract AgNPs at a wavelength of 430nm.

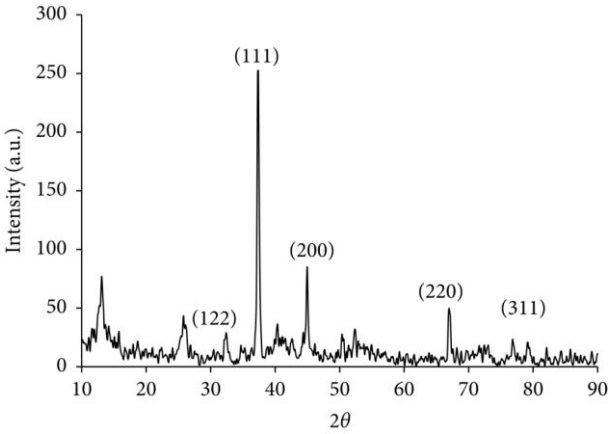
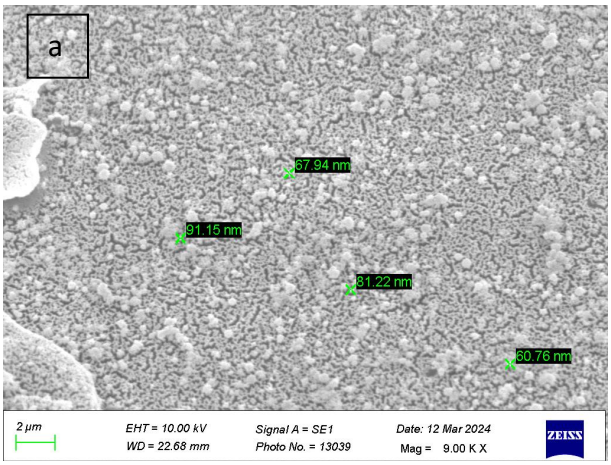


Figure 8: XRD images of obtained AgNPs.



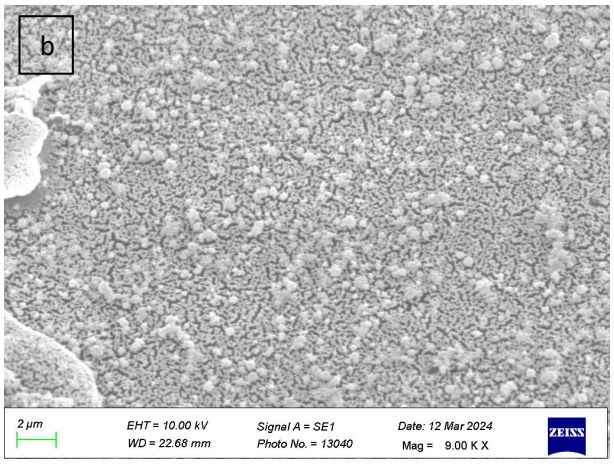


Figure 9a and 9b SEM(scanning electron microscopy) images ofobtained AgNPs show the best result.

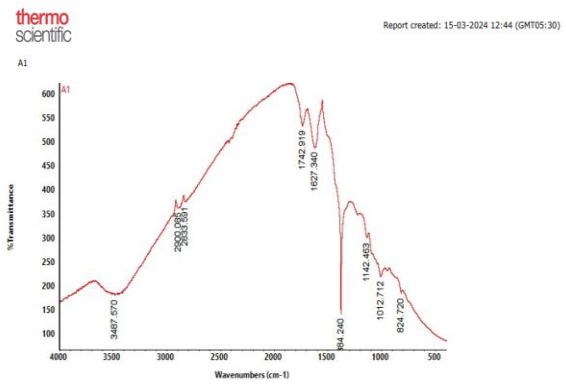


Figure 10: FTIR images of orange peel extract AgNPs (A1).

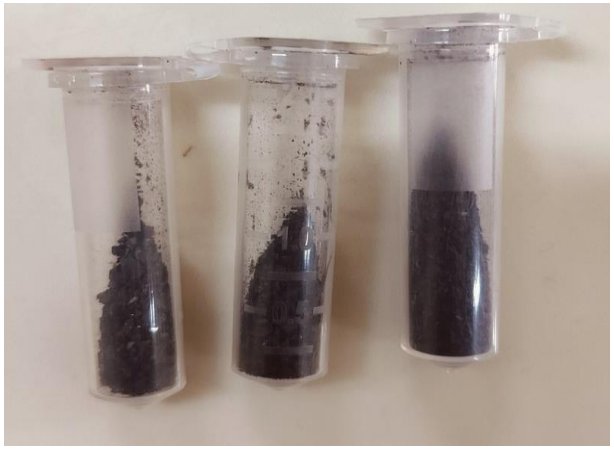


Figure 11: Obtained AgNPs after formulation.

microscopic imperfections. Particular surface area increases as particle size decreases. If the particle contains pores, the particular surface area as well. Chemical reactions and industrial processes both depend on the particular surface area[29].

UV-Spectroscopy: Silver nitrate is colorless before AgNP production, whereas herbal extract has a distinct color, after interacting with the orange peel extract, AgNPs synthesize silver solution that takes on a yellowish brown color,demonstrated by the UV visible absorption in 400-475 nm wavelength range.

X-Ray Diffraction (XRD): This is a method thatis primarily used to determine the condition of matter at various radiation angles, whether it is crystalline or amorphous[31]. Cell dimension and phase (crystalline/amorphous) are determined by this method. This is a versatile technique used extensively in nanotechnology to precisely determine the composition,

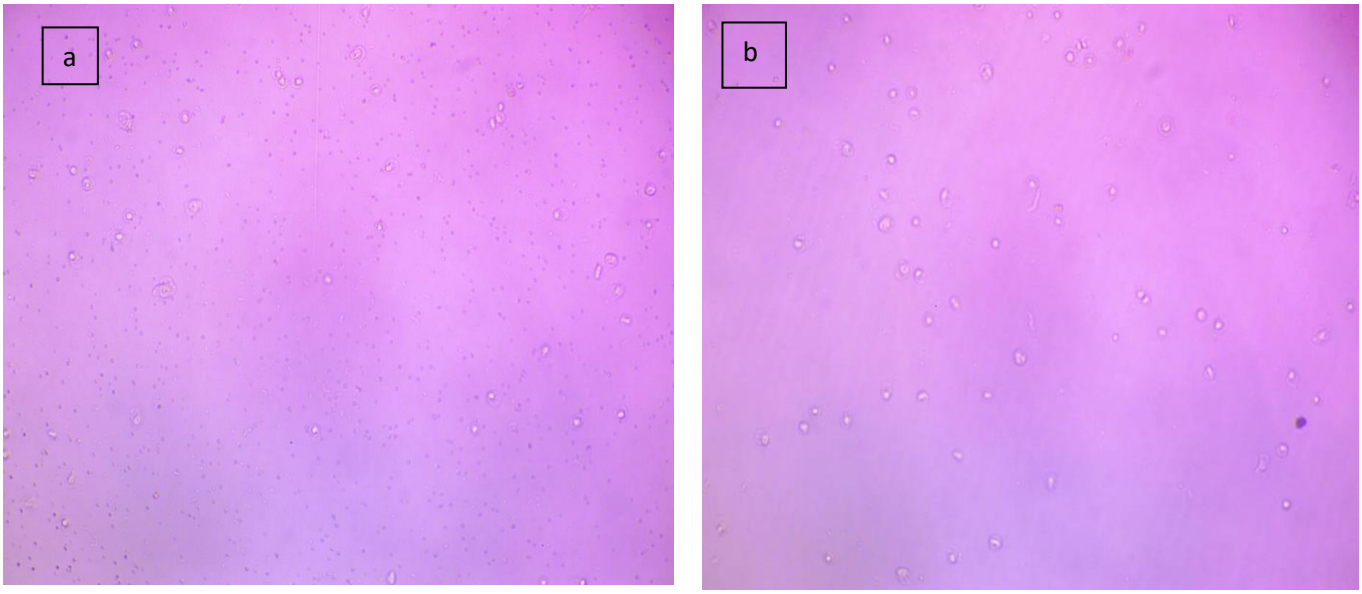


Figure 13: (a)and(b) Anti cancer activity A1 formulation

crystal structure, and crystalline grain size of nanoparticles. For XRD analysis, thin films of nanoparticles are created by drop-casting the nanoparticle onto an appropriate low-background substrate[30]. Samples of nanoparticles with the desired orientation are positioned on an appropriate goniometer, and multiple reflections are assessed

*Scanning Electron Microscopy (SEM)***:** Scanning electron microscopy was primarily utilised to verify the nanometre ranges of nanoparticle sizes [27]. surface morphology of produced AgNPs. Silver nitrate was added to SEM plates to create a solution smear on slides. By creating a thin layer of platinum and coating slides in it, conductivity was added to the system[(28)].High-quality images were obtained by scanning the prepared slides at a speeding voltage of 20 KV. An excellent instrument for determining the dimensions and configuration of nanoparticles is the excellent quality scanning electron microscopy (SEM).

Fourier Transfer Infrared Spectrophotometer (FTIR): An investigative method for identifying or confirming the OH group known as the functional group present in the moiety indicative of that molecule is FTIR spectroscopy. The primary functional components found in AgNPs and the herbal extract were detected by scanning the samples between 4000 and 400 cm-[1]. It gives the capacity to identify and examine nanoparticles, as well as macro-flaws such as secondary phases, porosity, fissures, and microscopic imperfections. A decrease in particle size results in an increase in specific surface area. If the particle contains pores, the specific surface area increases as well. Chemical reactions and industrial processes both depend on the particular surface area[29]

MTT Assay

The MTT Assay was used to assess the cytotoxicity of the supplied materials on the A549 cell line (purchased from NCCS Pune). The 96-well plate containing 10,000 cells per well was grown for 24 hours at 37°C with 5% CO2 in DMEM medium (Dulbecco's Modified Eagle Medium-AT149-1L) supplemented with 10% FBS (Foetal Bovine

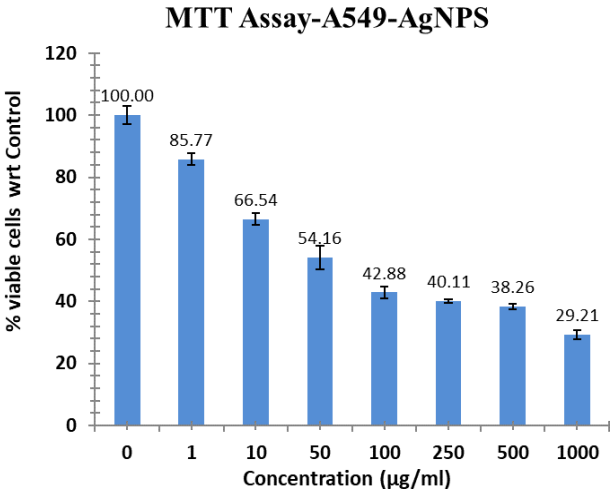


Figure 12: MTT Assay

Serum - HIMEDIA-RM 10432) and 1% antibiotic solution. Cells were treated the following day at varying concentrations (as indicated in the excel sheet).Untreated cells were referred to as Control, and MTT-free cells were referred to as Blank. Following the 24-hour incubation period, the cell culture was supplemented with MTT Solution (concentration as specified in the excel sheet) and incubated for an additional two hours. After removing the culture supernatant at the end of the experiment, the cell layer matrix was dissolved in 100 µl of Dimethyl Sulfoxide (DMSO–SRL-Cat no. 67685), and the results were read at 540 nm using an Elisa plate reader (iMark, Biorad, USA). GraphPad Prism-6 was the program used to calculate the IC50. The AmScope digital camera 10MPAptimaCMOS was used to take pictures under an inverted microscope (Olympus ek2). Presenting the 50% inhibitory concentration (IC50) as Mean±SEM (StandardErrorofMean).

RESULTS & DISCUSSION

AgNPs were created by combining orange peel extract with AgNo3.The development of Ag nanoparticles is

Table 3: IC_{50} value

Sample code	*IC50*value(µg/ml)
AgNPS1	79.32±0.06

indicated by the existence of an absorption peak at 430 nm.Findings from studies using UV-visible spectroscopy to evaluate the effect of pH on AgNPsLa formation. The investigations conducted on AgNPs are listed below, displaying the results that are consistent. According to the MTT assay results, cytotoxic activity was determined for sample AgNPS and 50% inhibitory concentration when the A549 cell line was expose to varying concentration of the sample, as shown in table 1. The amount of an inhibitor, sample or formulation at which the number of viable cells is cut in half is known as the IC50. In this present work, formulation, and evaluation of $AgNo_3$ nanoparticles were carried out in different work formulations (A1-A3) and have been prepared separately. From this above formulation, the best formulations were selected for the SEM,FTIR,XRD, and UV analysis.

In this research work, the orange peel extract was used for the anti-cancer activity. Orange peel extract has various chemical constituents. The alkaloids and glycoside components posseing can be shown in the anti-cancer activity which is used for further drug supplements. In this formulation of nanoparticles, A1 shows the best formulation in all the evaluation parameters.

CONCLUSION

This work demonstrated the green manufacture of silver nanoparticles utilizing extract from Citrus sinensis peels at increasing concentrations (1%, 2%, and 3%). It was discovered that the peels of Citrus sinensis contained a notable quantity of flavonoids and phenols, two phyto-constituents that stabilize and cap silver nanoparticles. The current research study aimed to create silver nanoparticles through Orange peel extract which shows the current aspects of lung cancer. Then silver nanoparticles through orange peel extract therefore determined having good compatibility and effectiveness in the cancer cells and being sustainable for the growth of cancer.

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