



Chemotherapeutic Potential of AgNP Orchestrated *Semecarpus anacardium* Nut Extracts Against Ovarian Cancer Cell Line, PA-1

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Abstract

Several plants have been studied to find their efficacy and anti-cancer activity in various cancers by synthesizing organic metal nanoparticles. However, usage of *Semecarpus anacardium* (SA) and production of green synthesized nanoparticles have not been exposed. In our study we have focused on synthesizing silver nanoparticles using the nut extracts from SA. Characterization studies including UV-Visible spectrophotometry have confirmed the silver nanoparticle formation at 412 nm using 0.1 mM and 427 nm using 0.2 mM AgNPs. Particle size was recorded at 1.4 nm confirming their effectivity and zeta potential studies confirmed the respective charge of -38.6 mV of the particle. Anti-microbial activity was shown against gram negative bacteria. MTT assay studies confirmed the anti-cancer activity against ovarian cancer cell line, PA-1. These results depict the excellent cytotoxic effect on the PA-1 ovarian cancer cell line, with an IC₅₀ value of 250 µg/ml. Flow cytometry studies confirmed that SA methanolic nut extracts inhibited cell cycle at G₀/G₁ phase and induced apoptosis. Taken together, we are confirming that SA methanolic extracts have anti-cancer properties against ovarian cancer cell line, PA-1.

Introduction

One of the newest areas of study in the contemporary realm of materials science, nanotechnology, is a multidisciplinary field that is centered on the diverse properties of nanoparticles. "Nanotechnology is an emerging and multidisciplinary science, which is based on the design and application of nanostructures or nanomaterials that are usually in the range of 1-100 nm, which is used in different fields as medical, food, health, among others. The interest in the use of nanomaterials is due to their different properties, such as morphology, size and their area-volume relationship [1-4]. Nanoparticles are finding new applications in various scientific fields like clinical and industrial

pharmacy, medicine, diagnostics, and drug delivery [5,49-50]. High specific surface area and distinctive size and form are the characteristic features of nanoparticles. Due to their unique physicochemical properties, which include anticancer, antidiabetic, antibacterial, and magnetic capabilities, researchers are interested in developing novel techniques for synthesis [6-8].

The creation of metal nanoparticles has become a significant area of study in nanomedicine over the past ten years. The primary fascinating element that occurs naturally is silver, which is also incredibly malleable and ductile and is a little harder than gold. Of all metals, it exhibits the maximum electrical and thermal

More Information

How to cite this article: Lavudi K, Kokkanti RR, Patnaik S, Penchalaneni J. Chemotherapeutic Potential of AgNP Orchestrated *Semecarpus anacardium* Nut Extracts Against Ovarian Cancer Cell Line, PA-1. Eur J Med Health Res, 2024;2(2):51-62.

DOI: 10.59324/ejmhr.2024.2(2).05

Keywords:

Semecarpus anacardium,
Anti-bacterial activity,
Ovarian cancer,
PA-1,
Flow cytometry,
Green synthesis,
Anti-oxidant studies,
Anti-microbial studies.



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conductivity with the lowest contact resistance [9]. The therapeutic benefits of silver (Ag) have been known since ancient times, and current medical research is looking at the function and potential uses of silver nanoparticles (AgNPs) and have been playing a significant function in herbal nanotechnology to prevent chronic diseases including cancer, neurological disorders, and diabetes [10-11]. The difficulties posed by using harmful chemical reagents may be resolved by the synthesis of AgNPs using phyto molecules. Other than silver, several metal nanoparticles have been used for synthesizing nanoparticles which are used in different cancers to prove their efficacy in chemotherapeutics as well as immunotherapy [51-61,65-70]. Apart from metal nanoparticles, synthesis of photonic nanoparticles has also been tremendously increased in the recent years for cancer therapeutics [62]. Combinational treatments using photo therapy along with radiotherapy has gained popularity specifically in colorectal cancers both invitro and in-vivo [63]. Combination of both metal and non-metallic nanoparticles together are also being used for cancer treatment [64].

In recent years, ovarian cancer (OC), the seventh most common malignancy in women globally, has become one of the most serious diseases [12]. Epithelial ovarian carcinoma constitutes one of the most dangerous malignant types among women [43-45]. Family history, age, reproductive history, breast cancer, hormone therapy, obesity, gynecologic surgery, and human papillomavirus are the key risk factors for ovarian cancer [13]. Pressure in the pelvis, back or stomach pain, constipation, vaginal bleeding, bloating, frequent urination, nausea and indigestion, weight loss, dyspnea, fatigue, and anorexia, are signs of ovarian cancer. Ovarian malignancies can be diagnosed at several phases of the disease, including localized and systemic blood tests, imaging tests, laparoscopies, and biopsies [14]. Chemotherapy, radiation treatment, and immunotherapy are possible options to treat ovarian cancer [15]. Long term treatment using chemotherapy often results in tumor recurrence, which results in the combinational therapy treatments in recent years [46-48]. In traditional medicine, ovarian cancer is treated with a variety of herbs and plants, including *Boerhavia erecta*, *Allium sativum*, *Fumaria officinalis*, *Zingiber officinale*, *Camellia sinensis*, *Ginkgo biloba*, *Quercus tinctoria*, *Nigella Sativa*, *Taxus brevifolia*, *Azadirachta indica*, *Malva sylvestris*, *Melia azedarach* and *Macrotyloma Uniflorum* [16-19]. It is anticipated that the antiproliferative effects of metal nanoparticles on ovarian cancer cells will be substantially stronger if they are synthesized using these plants [19-22, 71].

Semecarpus anacardium Linn. (Family: Anacardiaceae), popularly known as Ballataka, is a well-known plant for its medicinal benefits that can be found in moist

deciduous forests throughout the world [23-24]. Bioactive components like biflavonoids, phenolics, bhilwanols, anacardic acid, sterols, and glycosides are present in *Semecardium* nut extract [25-26]. According to several reports, the extract exhibits anti-inflammatory, hepatoprotective, anti-cancer, antioxidant, anti-arthritis, anthelmintic, and hypoglycemic activities as well as acting as a cardiogenic agent and beneficial towards a wide range of illnesses, including infections and tumors [27-30]. As a result, the nut extract has been found to have potent anticancer properties in the current experiment. There have been no reports of AgNPs produced by *Semecarpus anacardium* having anti-human ovarian cancer capabilities to date. The obtained results demonstrate the potential antibacterial and free radical scavenging of green synthesized *S. anacardium* Linn. nut methanolic extract. To investigate the anticancer capabilities of AgNPs created by *S. anacardium* methanol extract against the human ovarian cancer cell line, PA-1, the current study examines these properties for the first time.

Materials and Methods

Collection of Plant Materials

Semecarpus anacardium (SA) (here mentioned as SA) nuts were collected from the trees in field region at Salur, Vizianagaram District, Andhra Pradesh, India. The collected nuts were authenticated as the same by the Dr. B. Nagaraj, Professor, Department of Botany, Sri Venkateswara University, Tirupati, India.

Preparation of Methanolic Extracts

100 g of fresh nuts were initially washed with tap water and then with distilled water to remove dirt and then were air and shade dried for 3 weeks at room temperature. The nuts were crushed and minced into fine powder using an electric blender. 15 g of SA nut sample was taken, and extraction was performed using 250 ml of methanol solvent by Soxhlet method for 8 hours at 50°C. Solvent in the siphon tube of an extract turned colorless. Finally, the nut extract in liquid solution (brown in color) was stored in a glass vial in a refrigerator at 4°C and protected from sunlight for later use.

Synthesis of Silver Nanoparticles

Various ranges from 1.0 mM and 2.0 mM silver nitrate solutions were prepared and treated with methanolic seed extract. To 5 ml diluted methanol extract, 10 ml of AgNO₃ was added and the mixture was then left at room temperature until the solution's color changed from colorless to light yellow to brown. The solution containing AgNPs was confirmed by the dark brown color.

Characterization of Silver Nanoparticles

Nanoparticles, by their name have attained the characteristic feature in terms of physicochemical properties, behavior, efficacy, safety, and their



distribution. In this study, we have performed the characterization studies including UV-vis spectroscopy, particle size distribution, zeta potential, Fourier transform infrared spectroscopy (FTIR). UV-VIS Spectrophotometer: UV-Visible characterization was performed by using Nanodrop (Thermo Scientific NanoDrop 8000 Spectrophotometer, Wilmington, DE, USA) between 200-600 ranges to study the wavelength range of biosynthesized AgNPs. Average size of the nanoparticles, their stability, poly dispersity index(PDI), and hydro dynamic diameter were measured using dynamic light scattering and zeta potential analyzer (Horiba nanopartica sz-100 Nanoparticle analyzer, Bunsen Irvine, CA, USA). The sample was filtered through 0.2-micron filters before performing the calculations at a defined range. Potassium bromide (KBR) pellet was used to study the FTIR (Bruker FTIR Spectrometer, Billerica, MA, USA) and the crude and methanolic AgNP samples were analyzed using FTIR spectrum in the range of 500-4000 cm^{-1} with the resolution of 2 cm^{-1} .

Antimicrobial Activity

Using *Staphylococcus aureus* and *Bacillus subtilis* as gram +ve, and *Klebsiella pneumonia* and *Escherichia coli* as gram -ve ones, antimicrobial activity of AgNP mediated *Semecarpus anacardium* nut extracts (10-50 $\mu\text{g/ml}$) with Levofloxacin as standard drug was determined by agar disc diffusion method. The clear zone appeared after incubation was measured as an inhibitory zone [28].

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Antioxidant Assay

DPPH activity was performed to determine the free radical concentration using Braca et al. [29] method. Different concentrations of biosynthesized nano and crude samples (100-500 $\mu\text{g/ml}$) were taken, and the final volume was made up to 1000 μl using methanol. 1 ml of 0.004% methanol solution of DPPH was added and vortexed and incubated in the dark for 30 minutes. Absorbance was recorded at 517 nm using methanol as a blank and readings were measured using a spectrophotometer. Ascorbic acid was used as a standard. The values recorded were calculated using the below - mentioned formula and the percentage of inhibition was observed.

$$\text{Inhibition \%} = 1 - (A_{\text{sample}} / A_{\text{blank}}) \times 100$$

Where:

A_{sample} : Absorbance of the sample

A_{blank} : Absorbance of the DPPH

Cytotoxicity Assay

PA-1 (Ovarian Teratocarcinoma origin) cell lines were obtained from NCCS (National Centre for Cell Sciences), Pune, India to carry out this research work. PA-1 cells

were cultured in DMEM medium containing pen-strep and 10% FBS in a 60 mm dish (Thermo Fischer). When cells were 80% confluent, dissociation was performed using 0.25% trypsin-EDTA followed by resuspension in culture media. Cells were counted under a microscope using Neubauer chamber. 1×10^5 cells were seeded in 96 well plate and incubated in 5% CO_2 incubator overnight. Next day, cells were treated with SA-AgNPs at a wide range of concentrations ranging from 0.1 $\mu\text{g/ml}$ to 1000 $\mu\text{g/ml}$. Further steps were followed as mentioned earlier [18].

Flow Cytometry Study

PA-1 cells were cultured in 6-well plates at an initial density of 2×10^5 cells/2ml into FACS tubes. After 24 hrs of initial seeding, the cells were treated with 250 μg of SA-AGNP extract and Camptothecin (5 μM). The cells were ethanol-fixed (70% cold ethanol) after trypsinization and washed with PBS and incubated with 0.5 mg/ml RNase A for 10 min at 37°C. Cells were then stained with propidium iodide (PI) and subjected to flow cytometry using a FACS Calibur (Becton Dickinson, United States).

Statistical Analysis

The data was expressed as mean $\pm$ SEM and each experiment was carried out in triplicate, and the statistical analysis was performed using SPSS version 17.

Results and Discussion

Characterization Studies

Semecarpus anacardium, which belongs to the family Anacardiaceae has the potential capacity to act against several ailments. Nut extract from this plant have been in research investigations from the past 50 years. Many studies have been reported in relation to the excellent antidiabetic characteristics, yet the significance of this nut extract has very little exposure in the field of cancer.

In this study, methanolic SA nut AgNPs was synthesized (Figure 1) and characterized to confirm the green synthesized silver nano particle formation. Change in color confirmation from slightly colorless to brown is an indication for the SA-AgNP formation (Figure 2). We conclude that this change might be observed due to the reduction of Ag^{3+} [35]. Reduction of AgNPs occur due to the surface plasmon resonance phenomenon. SA-AgNPs has a peak absorbance at 412 nm for 0.1mM and 427 nm for 0.2 mM after 30 minutes of incubation (Figure 3). Similar wavelength was observed for other plants including *Macrotyloma uniflorum* and *Argyrea nervosa* [18, 37]. The average size of spherical nano particles within the SPR region in general is 410-450 nm. Thus, we confirm that these synthesized nanoparticles obtain a spherical shape. Particle size determination is pivotal in confirming the penetration of nanoparticles within the cell. The particle size of the synthesized SA nut AgNPs was measured to be 1.4 nm,



confirming their effective penetration capability into cells (Figure 4). Similar average size was observed in *Artemisia marschalliana* aerial extracts and was reported by Salehi et al. [30]. Negative charge of the silver nanoparticles helps them to possess repulsion capacity within themselves and thus increases the stability of the respective particle. By performing the zeta potential study, the negative charge was observed to be -38.6mV (Figure 5). This negative charge helps the particles in preventing the agglomeration among themselves, thus increasing their stability for longer period. FTIR studies revealed the presence of various functional groups in AgNP mediated SA nut extracts (Figure 6). The reduction of silver was occurred due to the presence of hydroxyl groups (O-H; 3332.25) as well as the carbonyl and alkene group stretch. (Table 1). Water soluble ingredients which were present in the extract were responsible for reduction of Ag^+ to Ag^0 .

Our results were in accordance with the reports obtained by Govindaraj and Venkatachalam [36].

The nut extracts from *Semecarpus anacardium* utilize hydroxyl (OH) and carbonyl (C=O) functional groups as reducing agents, leading to the formation of AgNPs. These groups also act as capping agents, ensuring the stability of AgNPs and preventing agglomeration. AgNPs formation involves the reduction of Ag^+ ions by phytochemicals in the extract. The optical properties, particularly the surface plasmon resonance (SPR) at 412 nm (0.1 mM) and 427 nm (0.2 mM), are characteristic of AgNPs. The small particle size (1.4 nm) enhances their optical efficacy. Size, shape, and surface charge influence the optical properties of AgNPs. The observed SPR peak in UV-Visible spectroscopy is size-dependent, with smaller particles exhibiting blue-shifted absorption. The zeta potential of -38.6 mV indicates nanoparticle stability.

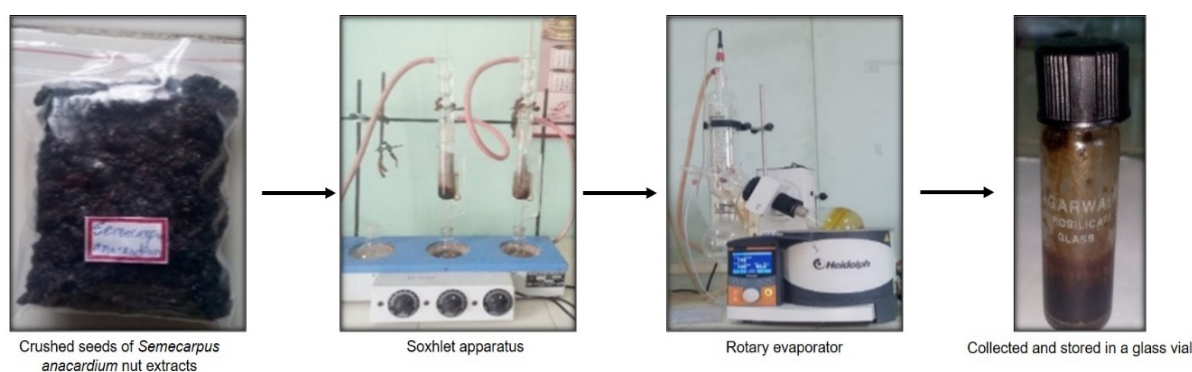


Figure 1: Collection and Extraction of *Semecarpus anacardium* Nut Extracts

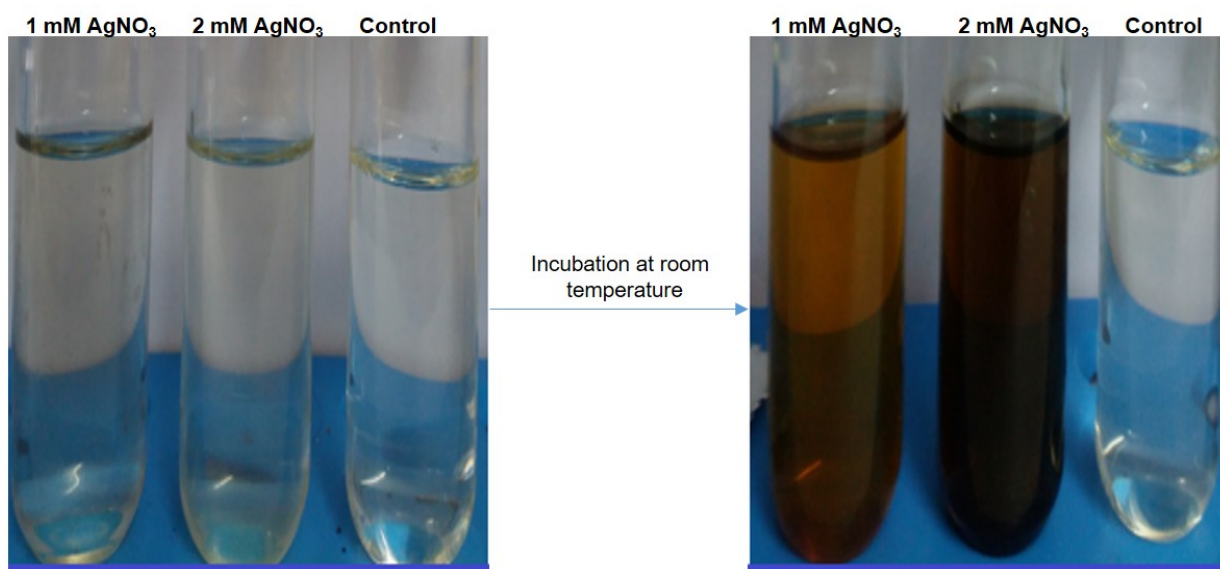


Figure 2: Synthesis of *Semecarpus anacardium* Silver Nanoparticles. Change in Colour to Light and Dark Brown after Exposure for 30 Minutes Confirms the Formation of AgNPs



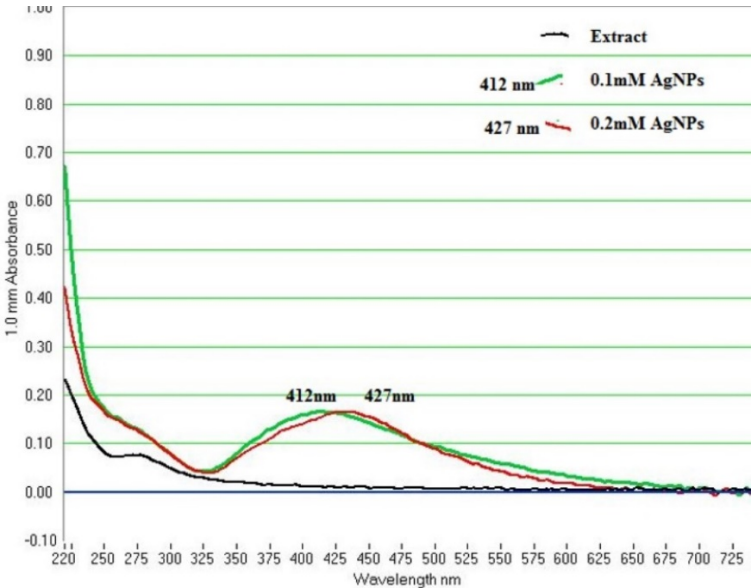


Figure 3: UV-Visible Absorption Spectra of AgNPs Synthesized from *Semecarpus* Nut Extracts. The Absorption Spectra of AgNPs Exhibited Strong Broad Peak at 412 nm and 427 nm with 0.1mM and 0.2mM Silver Nitrate

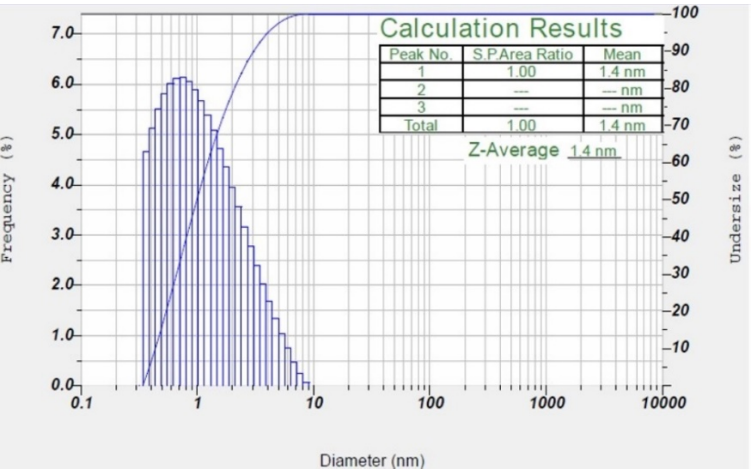


Figure 4: Size-Distribution Analysis by Particle Size Analyzer. The Particle Size Distribution Analysis Revealed that Particle Size was about 1.4 nm

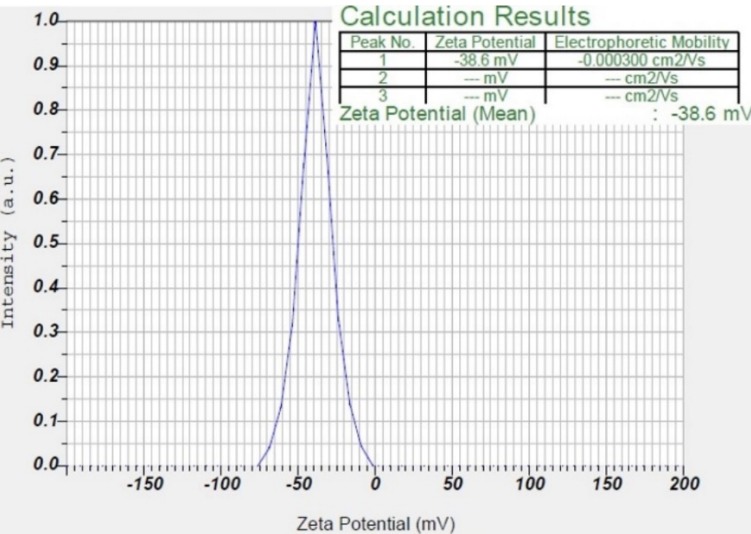


Figure 5: Zeta Potential Study of SA-AgNPs Nut Extracts



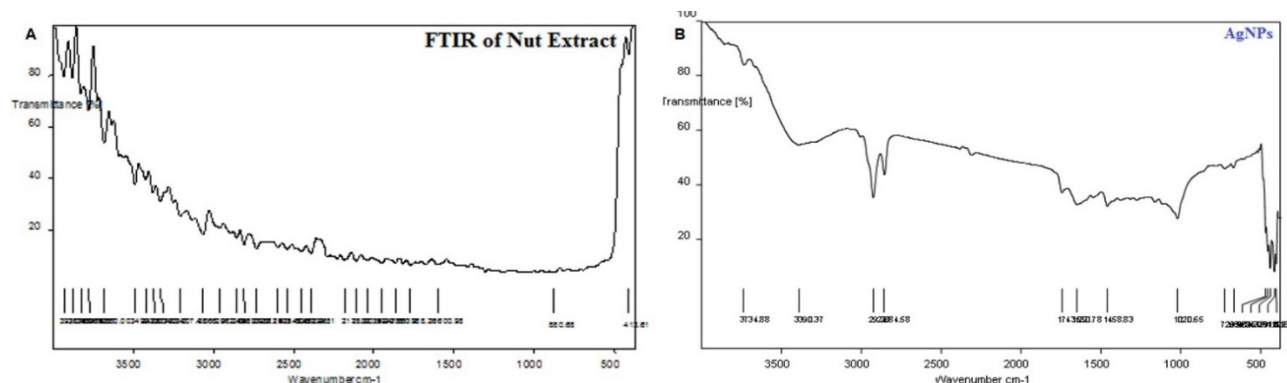


Figure 6: FTIR Analysis of A) Crude and B) AgNP Samples of *Semecarpus anacardium* Nut Extracts

Table 1: FTIR Spectra which Shows the Functional Groups of SA Crude and AgNP Extracts

Functional groups	Nut powder	AgNPs
Hydrogen bonded hydroxyl(OH)	3824.83, 3930.45 3680.00, 3490.08 3422.34, 3878.06 3377.01, 3207.48	3390.37 3734.88
C-H groups	2962.49, 2856.39 2810.86, 2733.79 2602.60, 2394.81 2452.05, 2107.19 2543.81, 2178.32, 2035.22	2924.84 2854.58
Alcohols	1947.61, 1860.94, 1768.26 1600.98	1743.22, 1020.65 1650.78, 1458.83
	880.68, 413.61	722.92, 669.24, 469.33
		454.31, 439.14, 412.16, 402.66

Antimicrobial Activity

All subcultures were freshly prepared prior use, and nutrient agar plates were used to maintain and culture both gram-positive and gram-negative bacterial strains. AgNP based *Semecarpus* nut extracts at concentrations of 10, 20, 30 and 50 µg/ml were treated, and levofloxacin was used as a positive control. Sterile paper discs of 5 mm in diameter were taken and used for treatment (Figure 7). Zone of inhibition was calculated after overnight culturing at 37°C and tabulated in Table 2.

Table 2: Anti-Bacterial Activity Assay Shows a Zone of Inhibition Against Both Gram-Positive and Gram-Negative Bacteria

	Inhibition Zone in mm			
	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>
Levofloxacin	13	14	15	14
10	7	8	6	7
20	4	8	8	5
30	7	8	7	8
50	8	7	6	7

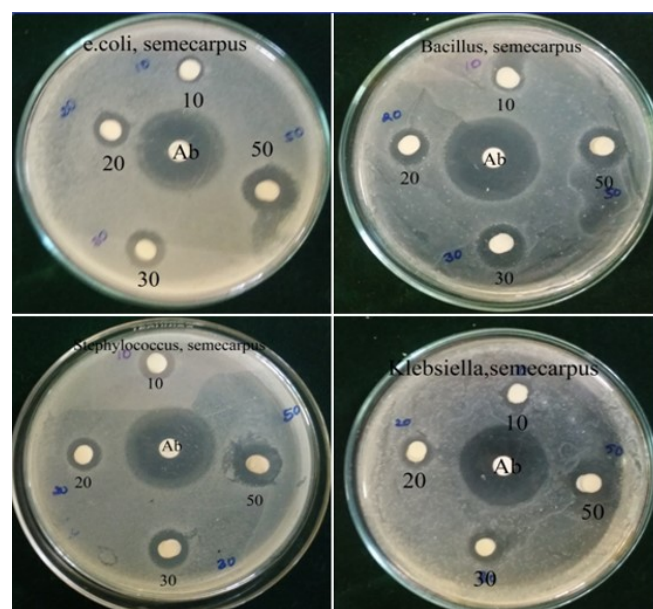


Figure 7: Anti-Bacterial Activity of SA-AgNPs (10-50 µg/ml) Against the Standard Antibiotic Levofloxacin and *E.coli*, *Bacillus subtilis*, *Streptococcus aureus* and *Klebsiella pneumoniae* strains



Free Radical Scavenging Assay

DPPH assay studies confirmed that AgNP mediated SA nanoparticles have the capacity to act as scavengers against the formed free radicals. The obtained data has shown that raising the concentration of SA-AgNPs

enhanced their ability to scavange radicals. AgNPs were shown to have 56.45% radical scavenging activity at a concentration of 100 $\mu\text{g/ml}$ (Figure 8). As a result, the biosynthesized SA-AgNPs were found to be very potent scavengers.

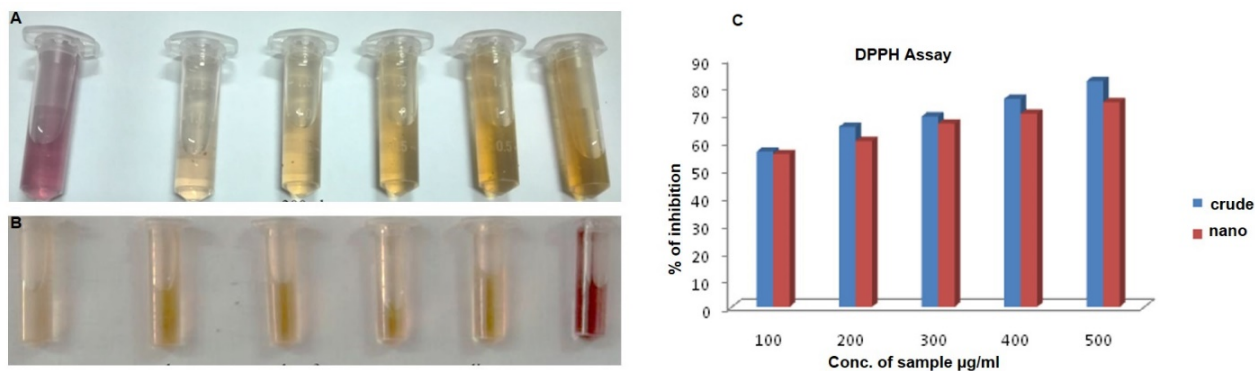


Figure 8: (A) and (B) Represent the Color Change in AgNP Synthesized and Crude Extracts of *Semecarpus anacardium* (100-500 $\mu\text{g/ml}$) (C) Represents the % of Free Radical Inhibition through DPPH Activity

MTT Assay

The proliferation of human ovarian cancer PA-1 cells treated with methanolic extract of AgNP SA nuts at different concentrations (0.1, 1, 10, and 100 $\mu\text{g/ml}$) for 24 h respectively, was determined by MTT assay (Figure 9). The growth of PA-1 cells was significantly inhibited by methanolic AgNP extract treatment (0.1, 1, 10, 100 $\mu\text{g/ml}$), and cell proliferation was also suppressed. Furthermore, the inhibitory effect of methanolic extract at the treatment (0.1, 1, 10, 100 $\mu\text{g/ml}$) of PA-1 cells shows a lesser effect on cell proliferation based on the dose. A dose-dependent rise in the inhibitory rate

of cell growth was observed. The IC_{50} value of methanolic SA nut extract was found to be 250 $\mu\text{g/ml}$. According to the investigation of Piktet et al. [37], biosynthesized nanoparticles triggered apoptosis and decreased the proliferation of ovarian cancer cell, supporting the idea that biosynthesized nanoparticles can induce apoptosis and impede the proliferation of ovarian cancer cells. The dose-response relationship emphasizes the potential for tailored therapeutic applications, where different concentrations may be strategically employed.

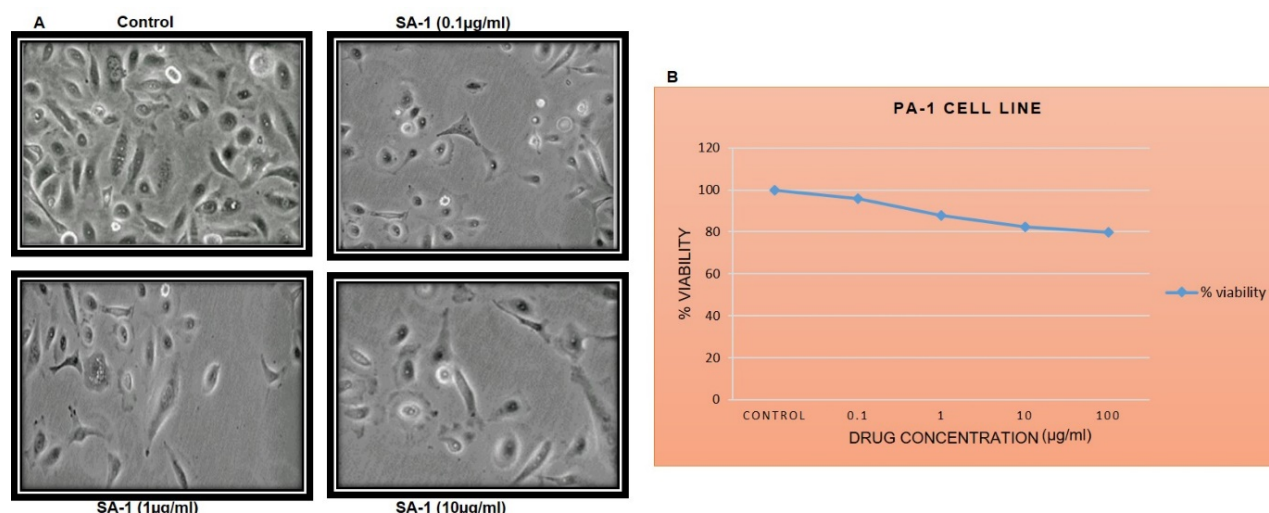


Figure 9: A) Effect of SA-AgNPs on the Proliferation of PA-1 Cell Line by MTT Assay B) Represents the Cytotoxic Effect of Silver Nanoparticles (AgNPs) on PA-1 Cells. Cells were Treated with AgNPs at Various Concentrations SA-AgNPs for 24 Hours, and Cytotoxicity was Determined by the MTT Assay



Flow Cytometry Study

By using IC_{50} value as a reference, flow cytometrical analysis was performed using SA methanolic nut extracts and taken camptothecin as a standard drug. Cells were arrested at the G₀/G₁ phase after treatment with methanolic SA nut extract, indicating that apoptosis had occurred. This might be due to the presence of several functional compounds in the nut extracts including flavanoids and phenols. There are similar results reporting the apoptotic effect of SA nut extracts on against various cancer cell lines [38-39].

Several reports also suggested the apoptotic role of nanosynthesized products against ovarian cancer [40-42]. Cells that were not treated experienced G₀/G₁ and G₂/M phase arrest (Figure 10). This is an unequivocal proof that the crude nut extract from *Semecarpus anacardium* has potential antiproliferative properties against the PA-1 cell line. G₀/G₁ inhibition of cell cycle which resulted in apoptosis might have a reduction of antiapoptotic factors, reinforcing the potential therapeutic impact of the nut extracts on ovarian cancer cells.

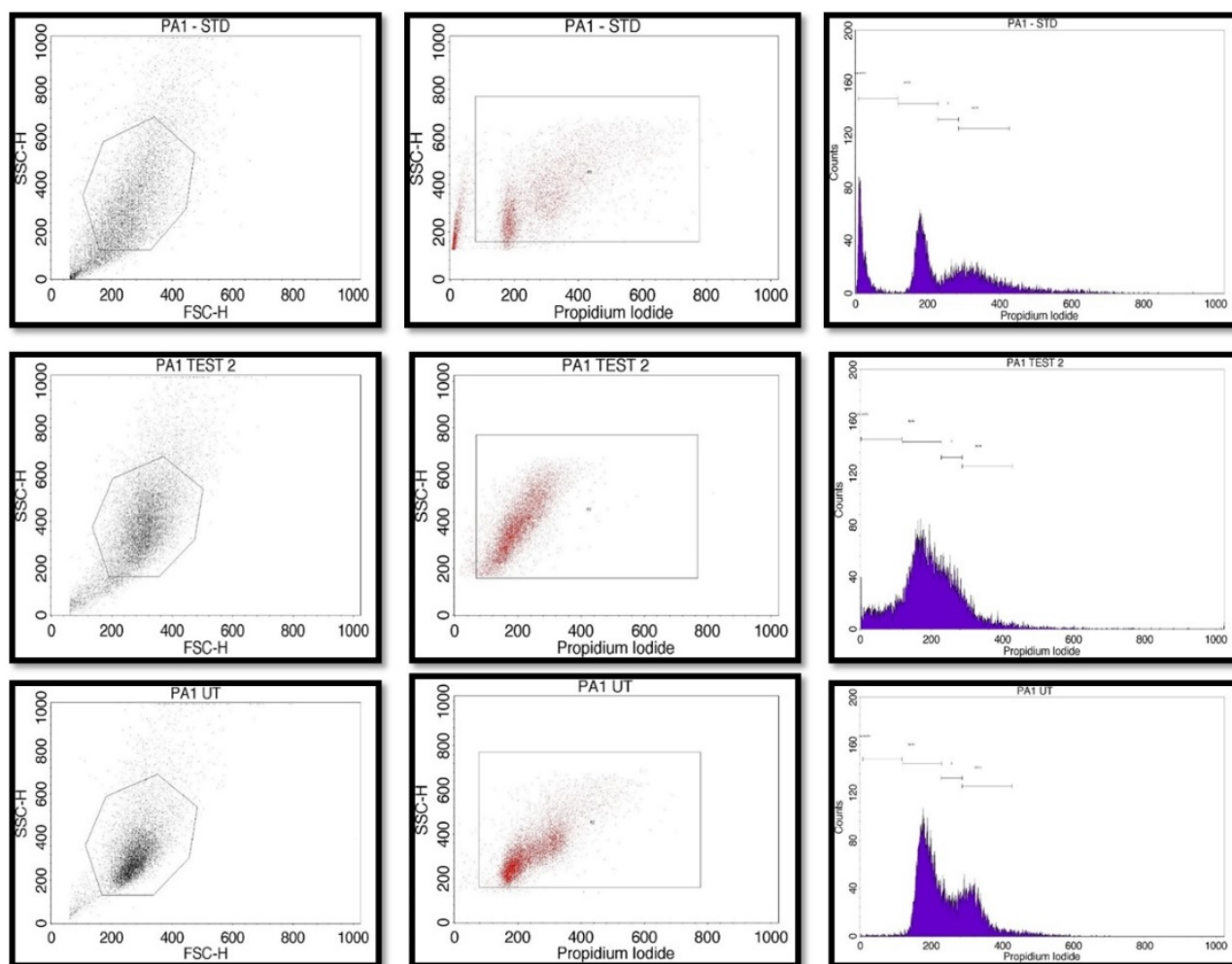


Figure 10: Represents the Cell Cycle Analysis of SA-AgNPs Treated with Control and Treated with Camptothecin as Standard

The use of silver nanoparticles (AgNPs) in nanomedicine for cancer treatment is underpinned by distinct advantages and poses associated challenges. AgNPs, owing to their unique physicochemical properties such as small size and high surface area, exhibit remarkable anticancer, antibacterial, and antioxidant capabilities [72-73]. These properties open avenues for targeted drug delivery, as AgNPs can selectively target cancer cells, enhancing the specificity and efficacy of therapeutic interventions [74]. The

green synthesis approach, utilizing plant extracts for AgNP production, not only harnesses the therapeutic properties of the botanicals but also presents an eco-friendly alternative to conventional chemical methods, aligning with sustainable and environmentally conscious practices [5].

However, the application of AgNPs in cancer treatment is not without challenges. Potential toxicity concerns, stability issues, and uncertainties regarding long-term effects necessitate comprehensive investigations and



risk assessments [75]. To progress toward clinical applications, it is imperative to delve into the molecular mechanisms underpinning the anticancer effects of AgNPs and address safety considerations. Rigorous in vivo studies employing animal models are pivotal for validating the efficacy and safety of AgNPs in a more complex biological milieu, providing crucial insights for translational research. Further research endeavors are essential to unlock the full therapeutic potential of AgNPs while mitigating associated risks, paving the way for their integration into cancer treatment strategies [76].

The utilization of *Semecarpus anacardium* in the synthesis of silver nanoparticles (AgNPs) showcases a remarkable synergy between nature and nanotechnology. The green synthesis approach not only capitalizes on the inherent therapeutic properties of SA but also aligns with eco-friendly practices. The AgNPs derived from SA exhibit distinct physicochemical properties that hold great promise for targeted cancer treatment.

Conclusions

Our research group was the first to report the anticancer activity of SA Methanolic AgNP extract against the ovarian cancer cell line-PA1. Characterization studies have confirmed the AgNP synthesis, and a zeta potential analysis has verified the nanoparticles' negative charge, which helps in maintaining their stability. FTIR studies has shown the presence of hydroxyl and carbonyl bonds. The synthesized nanoparticles have an average size of 4-10 nm which showed an excellent antibacterial capacity. Concluding the observations, our study showed evidence that AgNP derived methanolic SA nut extracts have the potential to inhibit ovarian cancer cell proliferation. Our findings demonstrate the unexplored anticancer properties of SA nut extracts and their AgNPs, offering promising prospects for nanomedicine applications. The observed antibacterial and anticancer activities highlight the significance of this study in the context of emerging nanotechnological approaches for cancer management. However, molecular mechanisms in depth must be explored using this extract to study the efficacy in ovarian cancer treatment. In vivo study using ovarian cancer tumor models are required to further extend the study.

Funding

This research received no external funding.

Acknowledgments

Authors are thankful to DST CURIE, SPMVV and DST PURSE, S.V. University, Tirupati, India for proving technical support to carry out the research work.

Conflicts of Interest

The authors declare no conflict of interest.

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