Chemotherapeutic potential of AgNP orchestrated Semecarpus anacardium nut extracts against Ovarian cancer cell line, PA-1.

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Research Article

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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 material science, nanotechnology is a multidisciplinary field that is centered on the diverse properties of nanoparticles. Nanoparticles are finding new applications in various scientific fields like clinical and industrial pharmacy, medicine, diagnostics, and drug delivery (Njud et al. 2022). High specific surface area and distinctive size and form are the characteristic features of nanoparticles. Due to their unique physicochemical properties, which include anticancer, anti-diabetic, antibacterial, and magnetic capabilities, researchers are interested in developing novel techniques for synthesis (Balachandar et al. 2019).

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 little harder than gold. Of all metals, it exhibits the maximum electrical and thermal conductivity with lowest contact resistance (Mohanta et al. 2020). 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 (Helena et al. 2021). The difficulties posed using harmful chemical reagents may be resolved by the synthesis of AgNPs using phyto molecules.

In recent years, ovarian cancer (OC), the seventh most common malignancy in women globally, has become one of the most serious diseases. (Bruna et al. 2019). Family history, age, reproductive history, breast cancer, hormone therapy, obesity, gynecologic surgery and human papillomavirus are the key risk factors for ovarian cancer (Grossman et al. 2018). 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 using blood tests, imaging tests, laparoscopies, and biopsies. Chemotherapy, radiation treatment, and immunotherapy are the possible options to treat ovarian cancer (Gibson et al. 2016). In traditional medicine, ovarian cancer is treated with a variety of herbs and plants, including *Allium sativum, Boerhavia erecta, Fumaria officinalis, Zingiber officinale, Camellia sinensis, Ginkgo biloba, Quercus tinctoria, Nigella Sativa, Taxus brevifolia Azadirachta indica, and Macrotyloma Uniflorum* (Lavudi et al. 2022; Huang et al. 2021; Ruan et al. 2021; Sandhya et al. 2020; Shaneza et al. 2018). It is anticipated that the anti-proliferative effects of metal nanoparticles against ovarian cancer cells will be substantially stronger if they are synthesized using these plants.

Semecarpus anacardium Linn. (Family: Anacardiaceae), popularly known as Bhallatakah, is a well-known plant for its medicinal benefits that can be found in moist deciduous forests throughout the world (Nikam 2022). Bioactive components like biflavonoids, phenolics, bhilawanols, anacardic acid, sterols and glycosides are present in *Semecardium* nut extract (Sahu 2017). According to several reports, the extract exhibits anti-inflammatory, hepatoprotective, antioxidant, anti-arthritic, anthelmintic, and hypoglycemic activity as well as acting as a cardiotonic agent (Singh et al. 2020; Salehi et al. 2016) and beneficial towards a wide range of illnesses, including infections and tumors (Premalatha 2000). As a result, the nut extract has been found to have potent anticancer property 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 after mentioned as SA) nuts were collected from the trees in field regions 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 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 hrs at 50°C. Solvent in siphon tube of an extract turned colorless. Finally, the nut extract in liquid solution (brown in color) was stored in a glass vial in 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 $AgNO_3$ 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 property, 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 8000 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. The sample was filtered through 0.2-micron filters before performing the calculations at defined range. Potassium bromide (KBR) pellet was used to study the FTIR 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, anti-microbial activity of AgNP mediated *Semecarpus anacardium* nut extracts (10-50 µg/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 (Cos et al. 2006).

DPPH (2, 2-diphenyl-1-pycrylhydrazyl) antioxidant assay

DPPH activity was performed to determine the free radical concentration using Braca et al. (2001) method. Different concentrations of biosynthesized nano and crude samples (100-500 µg/ml) were taken, and final volume was made upto 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 spectrophotometer. Ascorbic acid was used as a standard. The values recorded were calculated using the below mentioned formula and percentage of inhibition was observed.

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

A_{sample}: Absorbance of the sample

A_{blank} : Absorbance of the DPPH.

Cytotoxicity assay

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 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 of concentrations ranging from 0.1 µg/ml to 1000 µg/ml. Further steps were followed as mentioned earlier (Lavudi et al. 2022).

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 Campothecin (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 ± SEM and each experiment was carried out in triplicates and the statistical analysis was performed using SPSS version 17.

Results And Discussion

Characterization studies

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

In this study, methanolic SA nut AgNPs was synthesized (Fig. 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 (Fig. 2). We conclude that this change might be observed due to the reduction of Ag^{3+} (Prasanna raj and Venkatachalam, 2016). 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 (Fig. 3). Similar wavelength was observed for other plants including *Macrotyloma uniflorum* and *Argyreia Nervosa* (Lavudi et al. 2022; Subramanyam et al. 2021). 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. An average diameter of 1.4 nm was found for the synthesized nanoparticles (Fig. 4). This confirms the penetration capacity of the synthesized SA nut AgNPs into the cells. Similar average size was observed in *Artemisia marschalliana* aerial extracts and was reported by Soheil Saheli et al. 2016. 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, negative charge was observed to be -38.6mV (Fig. 5). This negative charge helps the particles from 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 (Fig. 6). The reduction of silver was taken place 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 (2016).

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	3390.37
	3680.00, 3490.08	3734.88
	3422.34, 3878.06	
	3377.01, 3207.48	
C-H groups	2962.49, 2856.39	2924.84
	2810.86, 2733.79	2854.58
	2602.60, 2394.81	
	2452.05, 2107.19	
	2543.81, 2178.32, 2035.22	
Alcohols	1947.61, 1860.94, 1768.26	1743.22, 1020.65
	1600.98	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 the 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 extract 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 (Fig. 7). Zone of inhibition was calculated after overnight culturing at 37°C and tabulated at Table 2.

Table 2
Anti-bacterial activity assay shows zone of inhibition against both gram positive and gram negative bacteria

	Inhibition Zone in mm			
	E. coli	*B. subtilis*	*S. aureus*	*K. pneumoneae*
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

Free radical scavenging assay

DPPH assay studies confirmed that AgNP mediated SA nanoparticles have the capacity to act as scavengers against formed free radicals. The obtained data has shown that raising the concentration of SA-AgNPs enhanced their ability to scavenge radicals. AgNPs were shown to have a 56.45% radical scavenging activity at a concentration of 100 µg/ml (Fig. 8). As a result, the biosynthesized SA-AgNPs were found to be very potent scavengers.

MTT assay

The proliferation of human ovarian cancer PA-1 cells treated with methanolic extract of AgNP SA nuts at different concentration (0.1, 1, 10, 100 µg/ml) for 24 h respectively, was determined by MTT assay (Fig. 9). The growth of PA-1 cells was significantly inhibited by methanolic AgNP extract treatment (0.1, 1, 10, 100 µg/ml), and cell proliferation was also suppressed. Furthermore, the inhibitory effect of methanolic extract at the treatment (0.1, 1, 10, 100 µg/ml) of PA-1 cell shows lesser effect of cell proliferation based on the dose. A dose-dependent rise in the inhibitory rate of cell growth was observed. IC_{50} value of methanolic SA nut extracts was found to be 250 µg/ml.

Flow cytometry study

By using IC_{50} value as a reference, flow cytometrical anaylsis was performed using SA methanolic nut extarcts and taken Campothecin as a standard drug. Cells were arrested at the G0/G1 phase after treatment with methanolic SA nut extract, indicating that apoptosis had taken place.This might be due to the presence of several functional compounds in the nut extracts including flavanoids and phenols. Cells that were not treated experienced G0/G1 and G2/M phase arrest (Fig. 10). This is unequivocal proof that the crude nut extract from Semecarpus anacardium has potential anti-proliferative properties against the

PA-1 cell line. G0/G1 inhibition of cell cycle which resulted in apoptosis might have a reduction of anti-apoptotic factors.

Conclusions

Our research group was the first to report the anti-cancer activity of SA Methanolic AgNP extracts against 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 study have 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 potential to inhibit the ovarian cancer cell proliferation. However, molecular mechanisms in depth must be explored using this extract to study the efficacy in ovarian cancer treatment. In vivo studies using ovarian cancer tumor models is required to further extend the studies.

Declarations

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Author Contributions

Conceptualization, Josthna Penchalaneni, Kousalya Lavudi ; methodology, Kousalya Lavudi ; analysis, Harika G V S; investigation, Kousalya Lavudi and Harika G V S; writing, Rekha Rani Kokkanti, Kousalya Lavudi; supervision, Srinivas Patnaik . All authors have read and agreed to the published version of the manuscript.

Funding

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Braca A, De Tommasi N, Di Bari L, Pizza C, Politi M, Morelli I (2001) Antioxidant principles from *Bauhinia tarapotensis*. J Nat Prod 64(7):892–895. https://doi.org/10.1021/np0100845
2. Bruna C, Simone P, Robert SC, Mauro F (2019) Nanotechnology and Immunotherapy in Ovarian Cancer: Tracing New Landscapes. J Pharmacol Exp Ther 370:636–646

3. Cos P, Vlietinck AJ, Berghe DV, Maes L (2006) Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. J Ethnopharmacol 106(3):290–302. https://doi.org/10.1016/j.jep.2006.04.003
4. Grossman DC, Curry SJ, Owens DK, Barry MJ, Davidson KW, Doubeni CA, Epling JW (2018) Screening for ovarian cancer: US preventive services task force recommendation statement. JAMA 319(6):588–594. https://doi.org/10.1001/jama.2017.21926
5. Gibson SJ, Fleming GF, Temkin SM, Chase DM (2016) The application and outcome of standard of care treatment in elderly women with ovarian cancer: A literature review over the last 10 years. Front Oncol 6:63
6. Ruan H, Wang L, Wang M, Mo W, Jin J, Chinnathambi A, Alahmadi TA, Meganathan V, Zhang L (2021) Anti-human ovarian cancer, cytotoxicity, and antioxidant effects of Nigella Sativa green-formulated Au nanoparticles: Describing a new chemotherapeutic supplement. Arch Med Sci. https://doi.org/10.5114/aoms/140492
7. Premalatha B (2000) Semecarpus anacardium Linn. nuts–a boon in alternative medicine. Indian J Exp Biol 38(12):1177–1182
8. Huang Y, Zhu X, Xie R, Ni M (2021) Green synthesis of nickel nanoparticles using Fumaria officinalis as a novel chemotherapeutic drug for the treatment of ovarian cancer. J Exp Nanosci 16(1):368–381. https://doi.org/10.1080/17458080.2021.1975037
9. Sandhya P, Josthna P, Kousalya L, Susmila G, Subbaiah VK, Varadarajulu C (2020) Green synthesis of silver nanoparticles by leaf extracts of *Boerhavia erecta* and spectral characterization and their antimicrobial, antioxidant ad cytotoxic studies on ovarian cancer cell lines. Lett Appl NanoBioScience 9(3):1165–1176. https://doi.org/10.33263/LIANBS93.11651176
10. Sahu PK (2017) Phytochemical analysis of nuts of *Semecarpus anacardium* using gas chromatography-mass spectrometry and high performance thin layer chromatography: Effect of Shodhana. Int. J. Green Pharm. 11 (1) | S100. https://doi.org/10.22377/IJGP.V11I01.879
11. Nikam YP (2022) Potent ethanomedicinal plant *Semecarpus anacardium* Linn: A Review. Sys Rev Pharm 13(3):349–355. https://doi.org/10.31858/0975-8453.13.3.349-355
12. Singh RK, Ranjan A, Tripathi R, Verma SS, Sharma V, Singh M, Gupta SC, Singh AK, Srivastava AK (2020) *Semecarpus anacardium* Linn. leaf extract exhibits activities against breast cancer and prolongs the survival of tumor bearing mice. https://doi.org/10.1101/2020.01.08.898940. BioRxiv
13. Helena GIO, Martins CSM, Prior JAV (2021) Silver nanoparticles as carriers of anticancer drugs for efficient target treatment of cancer cells. Nanomaterials 11(4):964
14. Njud SA, Nehad SA, Afnan IF (2022) Green synthesis of silver nanoparticles using medicinal plants: Characterization and application. J Radiat Res Appl Sci 15(3):109–124
15. Balachandar R, Gurumoorthy P, Karmegam N, Natchimuthu K, Hamed B, Ramasamy S et al (2019) Plant-mediated synthesis, characterization and bactericidal potential of emerging silver nanoparticles using stem extract of Phyllanthus pinnatus: A recent advance in phytonanotechnology. J Clust Sci 30:1481–1488. https://doi.org/10.1007/s10876-019-01591-y

16. Mohanta YK, Biswas K, Jena SK, Hashem A, Abd_Allah EF, Mohanta TK (2020) Anti-biofilm and antibacterial activities of nilver nanoparticles synthesized by the reducing activity of phytoconstituents present in the Indian medicinal plants. Front Microbiol 11:1143. https://doi.org/10.3389/fmicb.2020.01143
17. Shaneza A, Umesh KG, Deepa S, Tahseen K (2018) Herbal treatment for the ovarian cancer. SGVU J Pharm Res Educ 3(2):325–329
18. Lavudi K, Harika GVS, Kokkanti RR, Patchigolla S, Sinha A, Patnaik S, Penchalaneni J (2022) 2-Dimensional in vitro culture assessment of ovarian cancer cell line using cost effective silver nanoparticles from *Macrotyloma uniflorum* seed extracts. Front Bioeng Biotechnol 10:978846. https://doi.org/10.3389/fbioe.2022.978846
19. Govindaraj P, Venkatachalam P (2017) Hepatoprotective effect of engineered silver nanoparticles coated bioactive compounds against diethylnitrosamine induced hepatocarcinogenesis in experimental mice. J Photochem Photobiol B Biol 167:309–320,ISSN1011-1344https://doi.org/10.1016/j.jphotobiol.2017.01.009
20. Subramanyam GK, Gaddam SA, Kotakadi VS, Palithya S, Penchalaneni J, Challagundla VN (2021) *Argyreia nervosa (Samudra pala)* leaf extract mediated silver nanoparticles and evaluation of their antioxidant, antibacterial activity, *in vitro* anticancer and apoptotic studies in KB oral cancer cell lines. Artif Cells Nanomed Biotechnol 49(1):635–650. https://doi.org/10.1080/21691401.2021.1996384
21. Salehi S, Shandiz SA, Ghanbar F, Darvish MR, Ardestani MS, Mirzaie A, Jafari M (2016) Phytosynthesis of silver nanoparticles using *Artemisia marschalliana* Sprengel aerial part extract and assessment of their antioxidant, anticancer, and antibacterial properties. Int J Nanomedicine 11:1835–1846. https://doi.org/10.2147/IJN.S99882
22. Mathivadhani P, Shanthi P, Sachdanandam P (2007) Apoptotic effect of *Semecarpus anacardium* nut extract on T47D breast cancer cell line. Cell Biol Int 31(10):1198–1206. https://doi.org/10.1016/j.cellbi.2007.04.004
23. Nair PK, Melnick SJ, Wnuk SF, Rapp M, Escalon E, Ramachandran C (2009) Isolation and characterization of an anticancer catechol compound from *Semecarpus anacardium*. J Ethnopharmacol 122(3):450–456. https://doi.org/10.1016/j.jep.2009.02.001

Figures

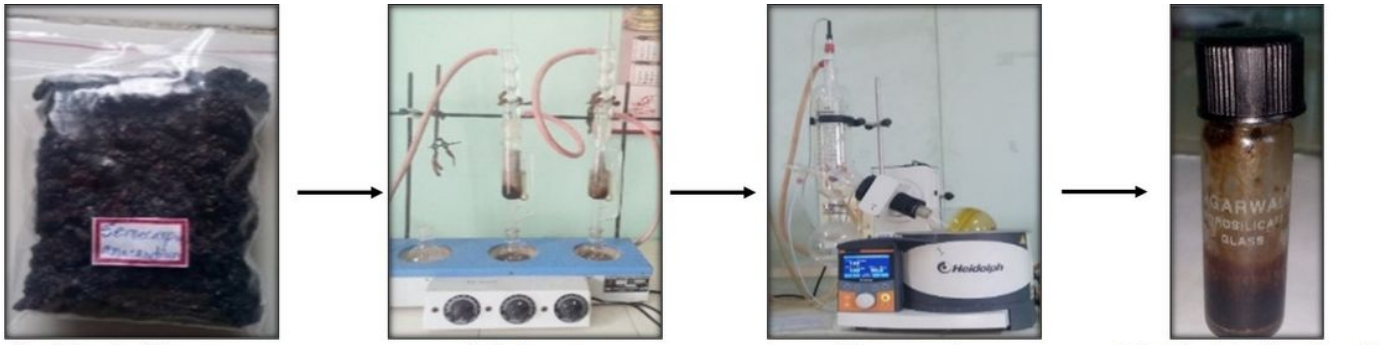

Fig. 1 Collection and extraction of *Semecarpus anacardium* nut extracts.

Figure 1

Collection and extraction of *Semecarpus anacardium* nut extracts.

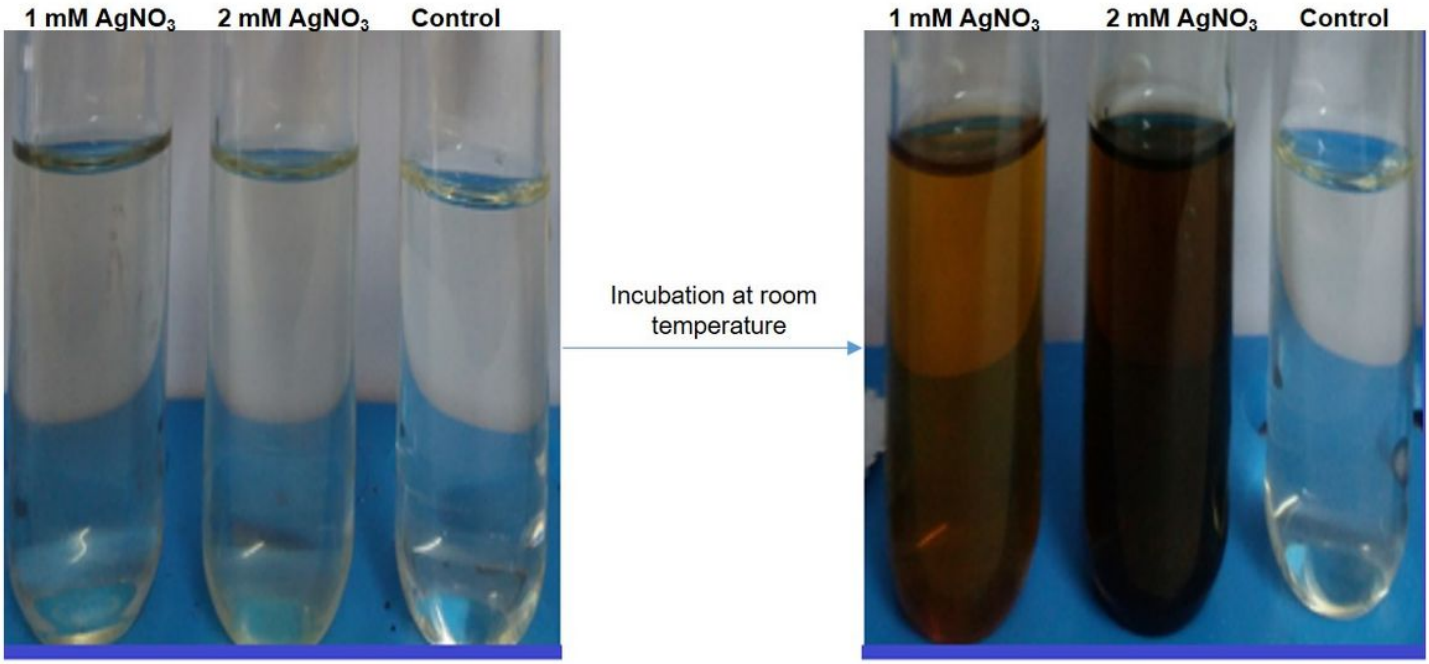

Fig. 2 Synthesis of *Semecarpus anacardium* silver nanoparticles. Change in colour to light and dark brown after exposure for 30 minutes confirms the formation of AgNPs

Figure 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

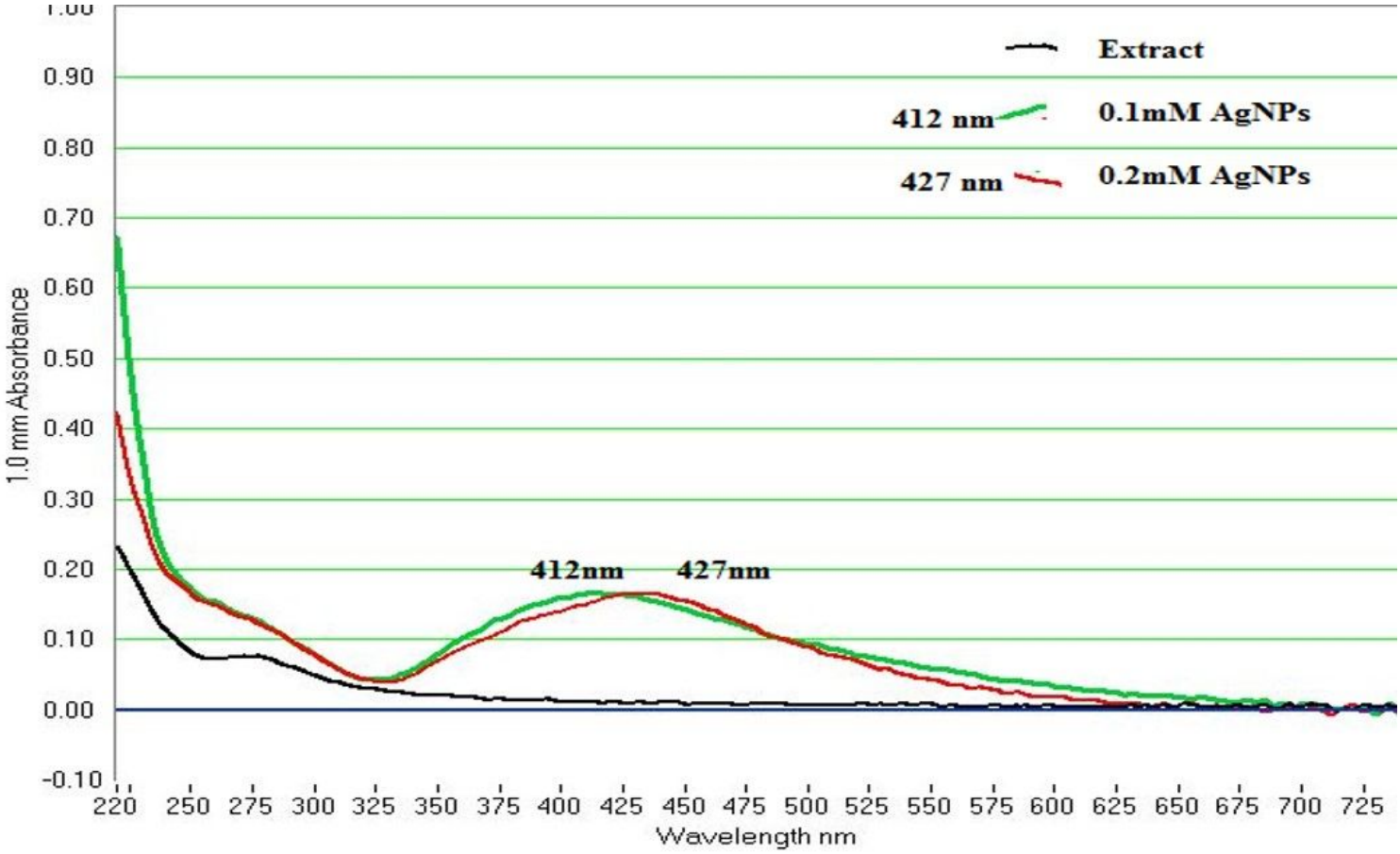

Fig. 3 UV-Visible absorption spectra of AgNPs synthesized from S*emecarpus* nut extracts. The absorption spectra of AgNPs exhibited a strong broad peaks at 412 nm and 427 nm with 0.1mM and 0.2mM silver nitrate.

Figure 3

UV-Visible absorption spectra of AgNPs synthesized from *Semecarpus* nut extracts. The absorption spectra of AgNPs exhibited a strong broad peaks at 412 nm and 427 nm with 0.1mM and 0.2mM silver nitrate

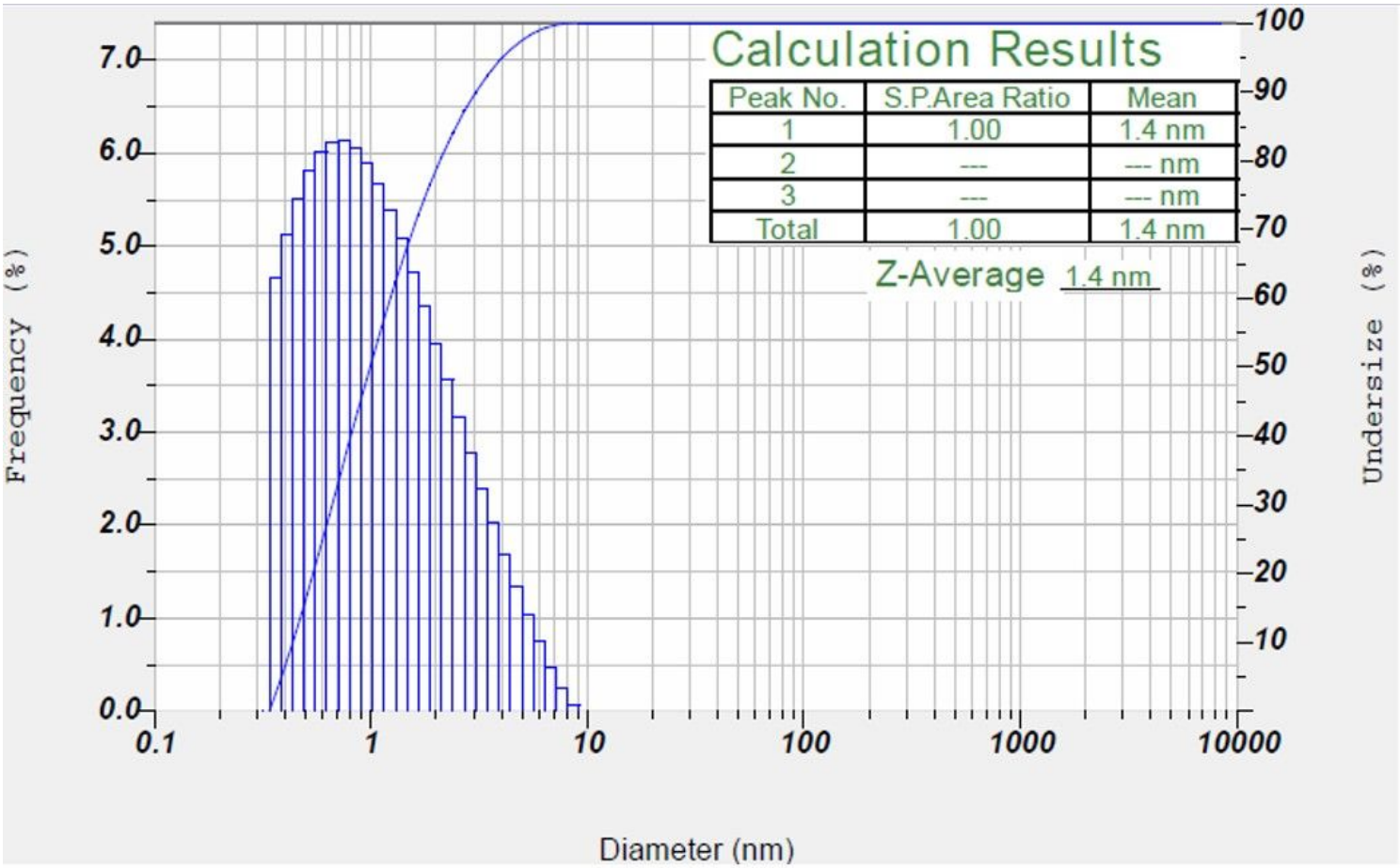

Fig. 4 Size-distribution analysis by Particle size analyzer. The particle size Distribution analysis revealed that particle size was about 1.4 nm

Figure 4

Size-distribution analysis by Particle size analyzer. The particle size Distribution

analysis revealed that particle size was about 1.4 nm

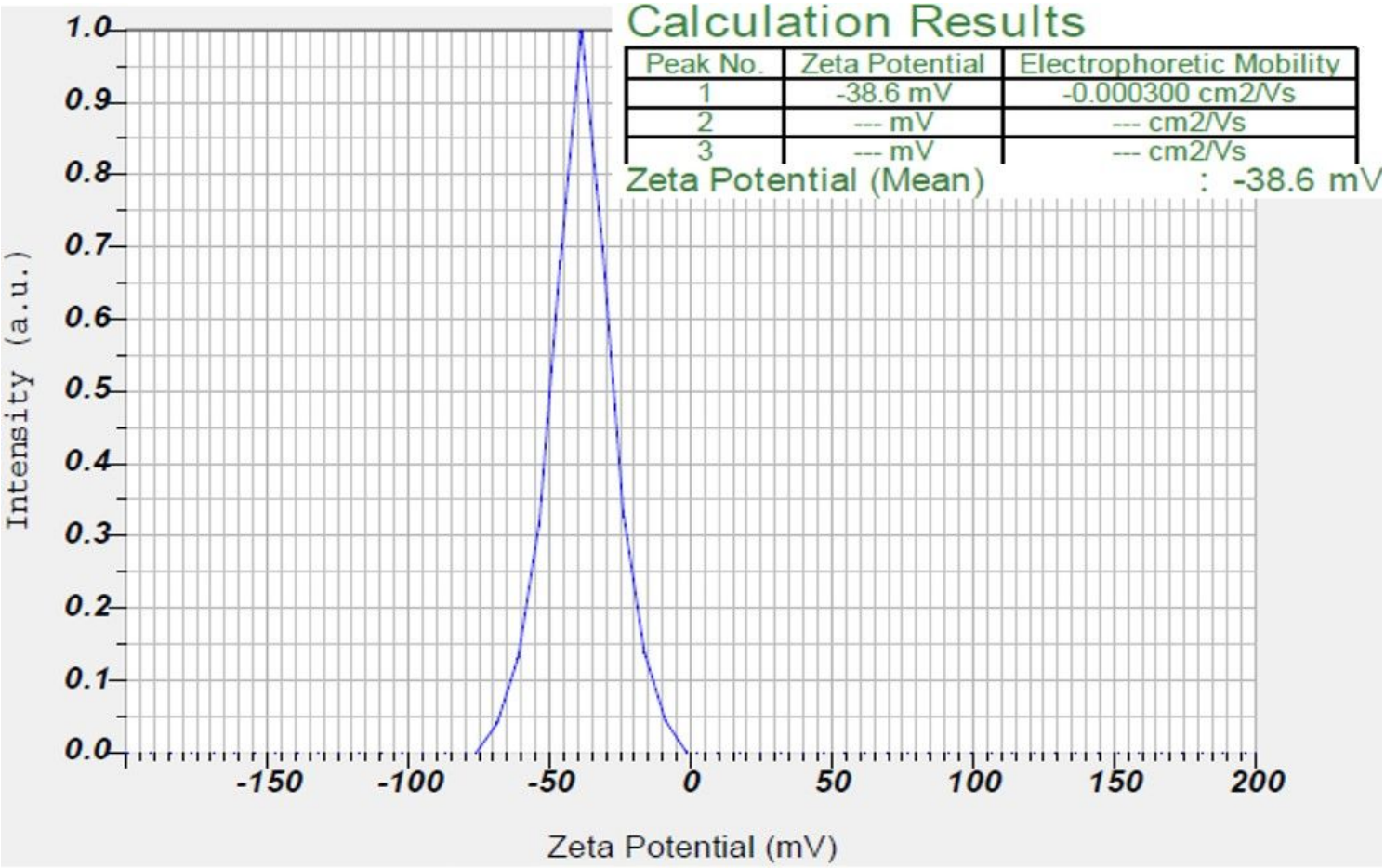

Fig. 5 Zeta potential study of SA-AgNPs nut extracts

Figure 5

Zeta potential study of SA-AgNPs nut extracts

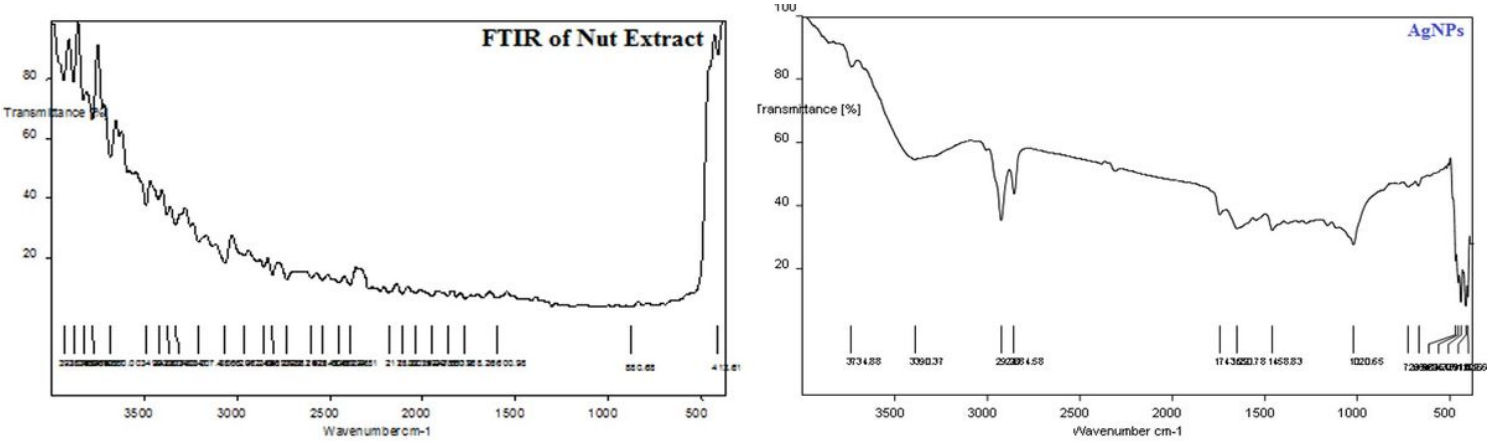

Fig. 6 FTIR analysis of **A)** crude and **B)** AgNP samples of *Semecarpus anacardium* nut extracts

Figure 6

FTIR analysis of A) crude and B) AgNP samples of *Semecarpus anacardium* nut

Extracts

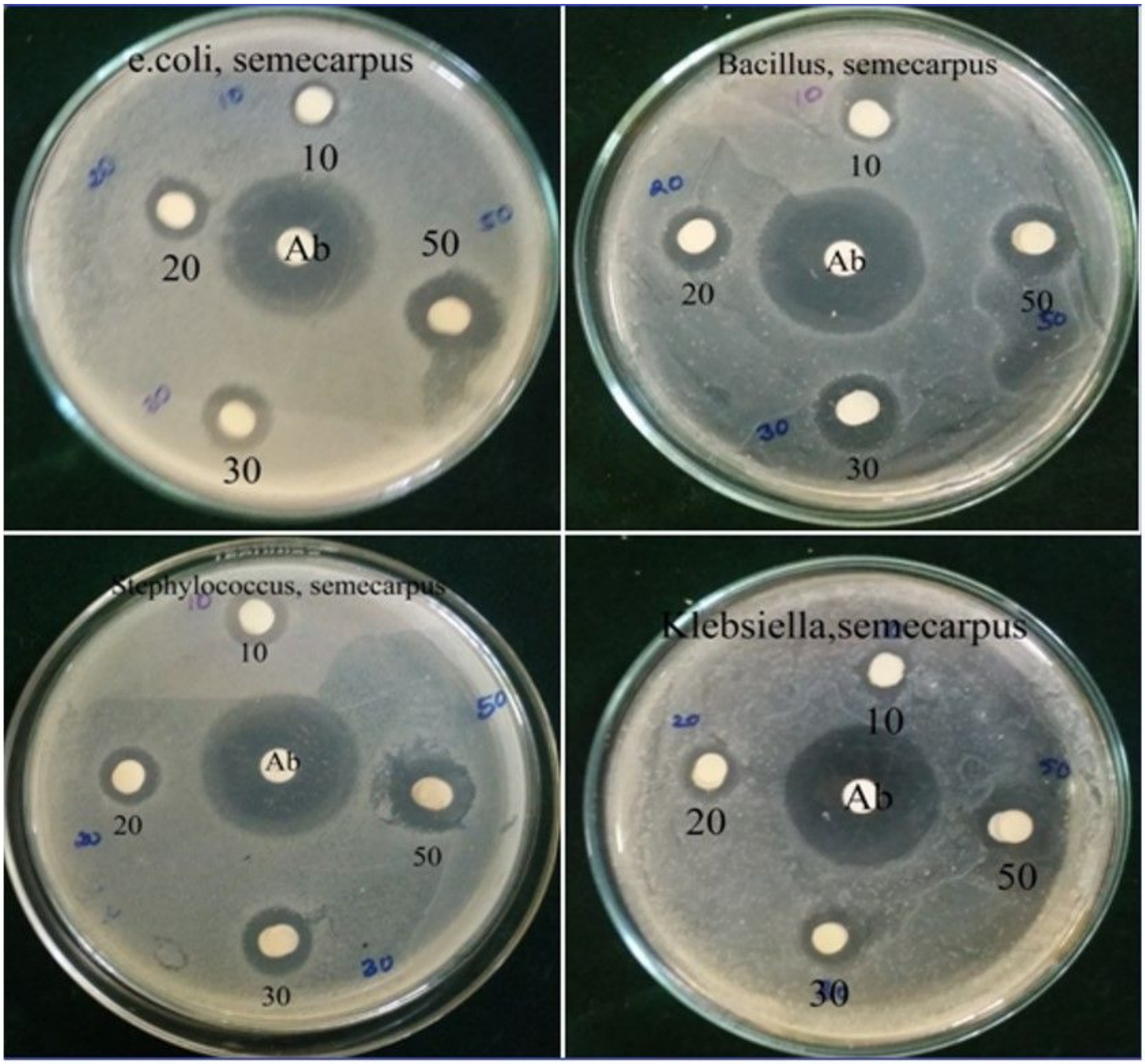

Fig. 7 Anti-bacterial activity of SA-AgNPs (10-50 µg/ml) against the standard antibiotic Levofloxacin and *E.coli, Bacillus subtilis, Streptococcus aureus* and *Klebsiella pnemoniae* strains

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*

pnemoniae strains

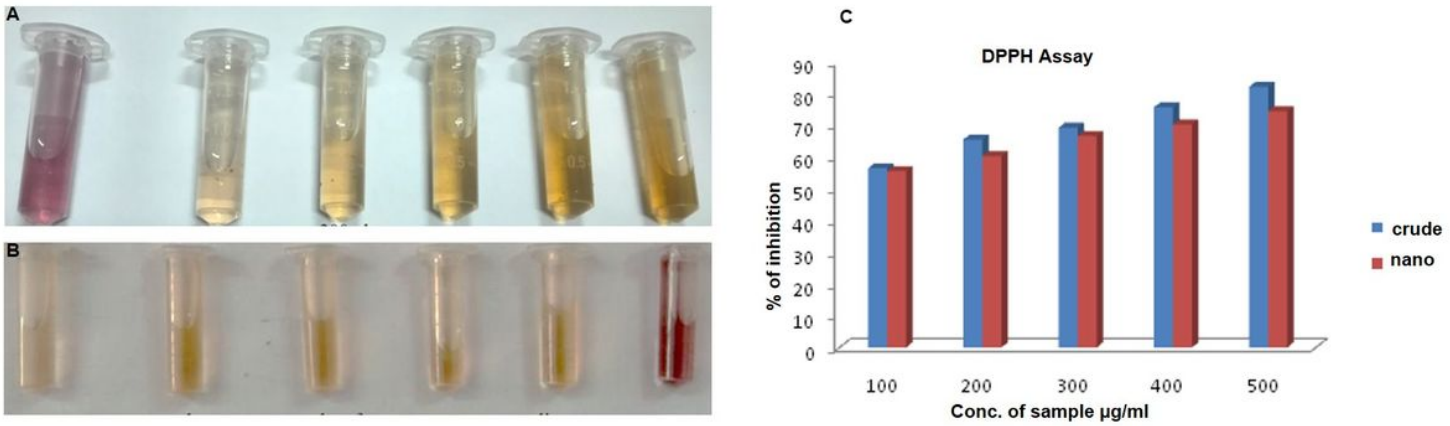

Fig. 8 (A) and (**B**) represents the color change in AgNP synthezised and crude extracts of *Semecarpus anacardium* (100-500 µg/ml). (**C**) represents the % of free radical inhibition through DPPH activity

Figure 8

(A)and (**B**) represents the color change in AgNP synthezised and crude extracts of *Semecarpus anacardium* (100-500 µg/ml). (**C**) represents the % of free radical inhibition through DPPH activity\

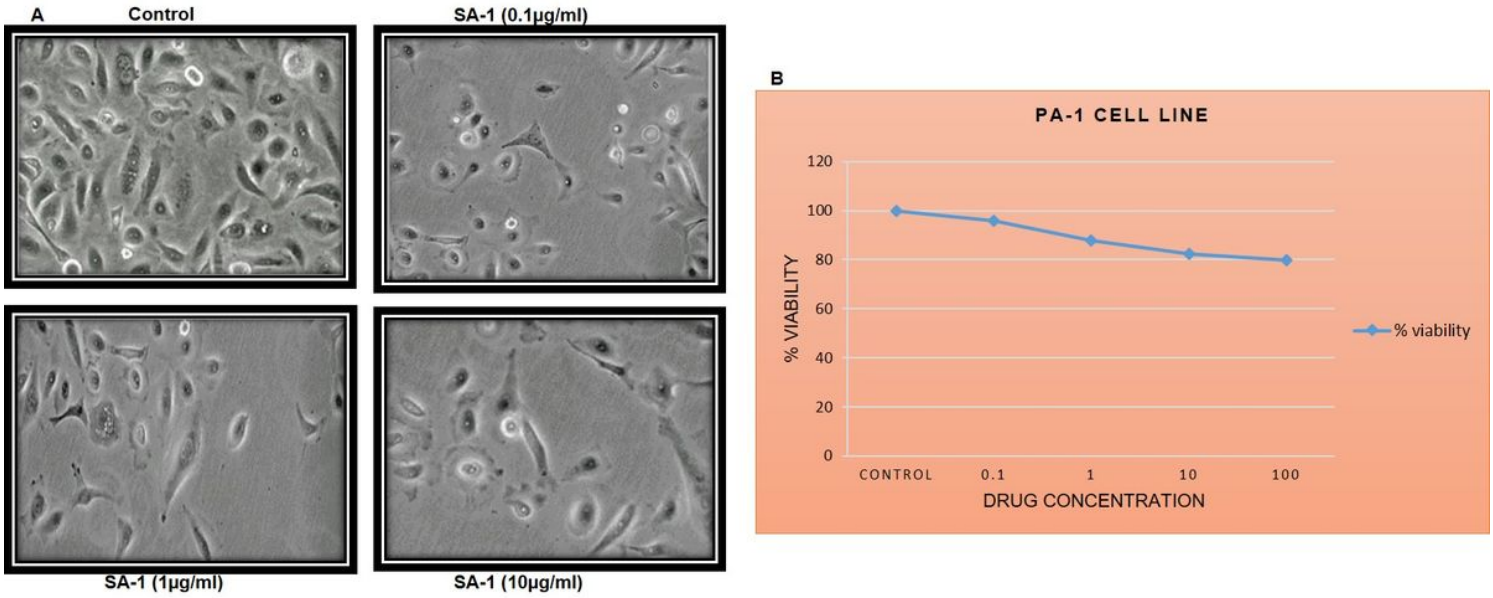

Fig. 9 A) Effect of SA-AgNPs on the proliferation of PA-1 cell line by MTT assay **B)** Represents the Cytotoxic effect of silver nanoparticles (AgNPs) on PA-1 cells. Cells were treated with AgNPs at various concentrations SA-AgNPs for 24 hours, and cytotoxicity was determined by the MTT assay

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

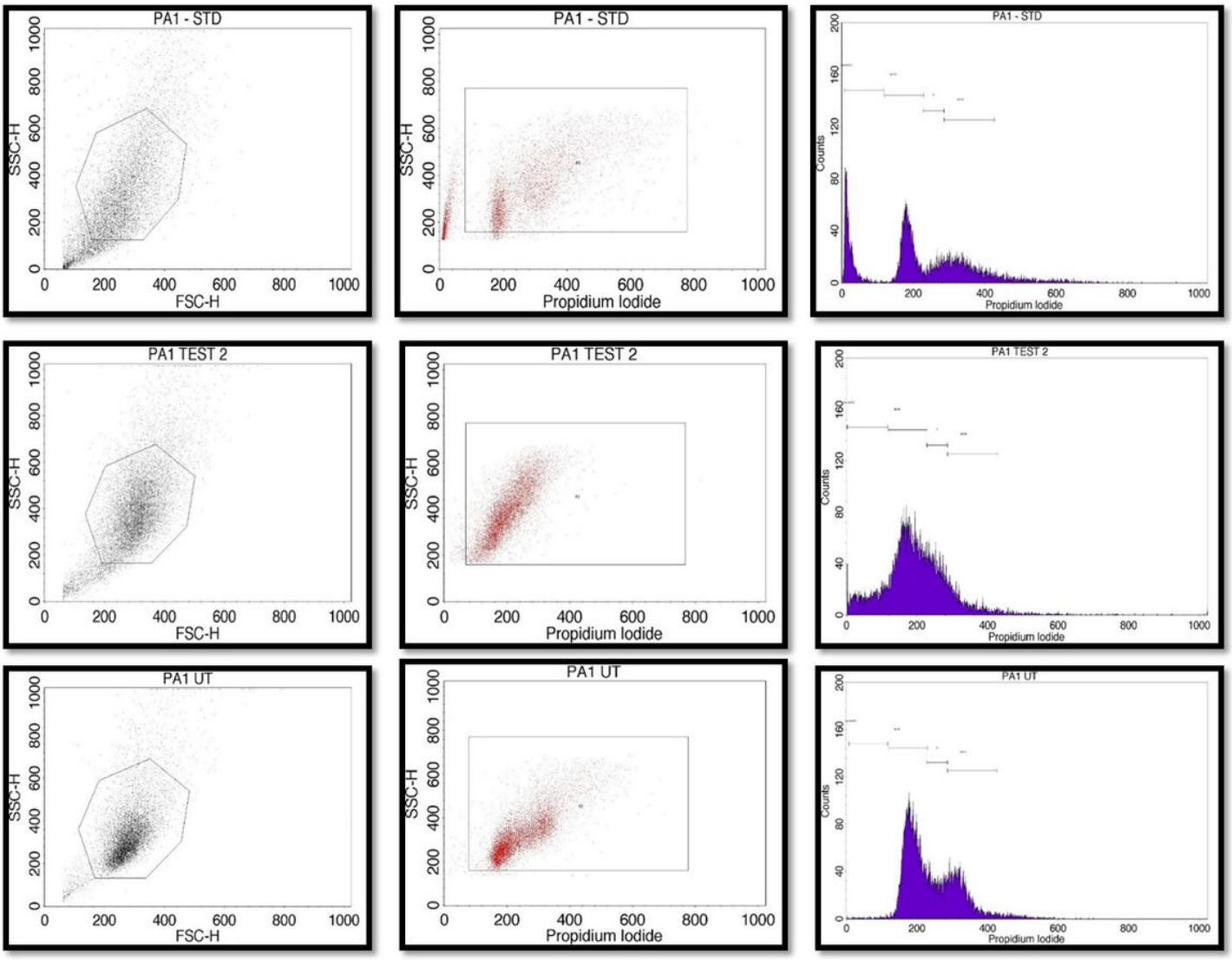

Fig. 10 Represents the cell cycle analysis of SA-AgNPs treated with control and treated with Campothecin as standard

Figure 10

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