RESEARCH PAPER

Green synthesis of nanosilver using Ficus carica fruit extract - screening of its antioxidant, catalase, antibacterial and anticancerous activity

Sathya Priya Janaki Ram[1], Sangeetha Soundararajan[2*], Shanmuga Priya Venugopal[3]

[1,2,3]PG & Research Department of Zoology, Pachaiyappa's College, Chennai -600 030. Affiliated to University of Madras, Chennai, TamilNadu, India

ABSTRACT

The current work uses the aqueous fruit extract of Ficus cariaca as a reducing agent to explore the antibacterial, catalase, anticancerous, and antioxidant activities of silver nanoparticles synthesized by an environmentally friendly approach of green biosynthesis. This research uses visual color change to characterize the biosynthesised AgNPs; SEM analysis were carried out to characterize the average size of AgNPs, which ranged from 46.65 nm to 73.75 nm; and UV-Vis spectroscopic examination reveals a high absorbance band at 450 nm respectively. Antibacterial activity were determined against common bacterial strains using the agar disc diffusion method. DPPH, ABTS, and SOD assays were used to determine the antioxidant property. MTT assays were used to test the anticancerous property. The Ficus fruit may eventually be utilized for therapy or to prevent oxidative stress-related disorders, some types of cancer, and nanobiomedical applications due to its high concentration of plant-based phytoestrogens and/or natural antioxidants.

Keywords: Ficus cariaca , Anticancerous property, natural antioxidant, antibacterial activity, biosynthesis

How to cite this article: Ram SPJ, Soundararajan S, Venugopal SP. Green synthesis of nanosilver using Ficus carica fruit extract - screening of its antioxidant, catalase, antibacterial and anticancerous activity. Int J Drug Deliv Technol. 2026;16(7s): 704-715; DOI: 10.25258/ijddt.16.7s.75

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Since ancient times, silver products have been used to prevent and treat a wide range of illnesses, most notably infections, due to their widely recognized bactericidal and inhibitory properties as well as their broad spectrum of antimicrobial activities. The nanoparticles (NPs) and nanoproducts with their unique size-related physico-chemical properties that set them apart from larger matter made it be widely used in many fields where silver nanoparticles (AgNPs), among other nanomaterials, have become the industry standard for nanotechnology products (Reidy et al., 2013). Numerous uses for nanosilver have been found in consumer goods, ranging from soaps, food, water treatment to the disinfection of medical equipment and household appliances. A variety of synthesis techniques have been used to meet the need for AgNPs. Conventional physical and chemical techniques often appear to be highly costly and dangerous compared to biosynthesis of AgNPs (Gurunathan et al., 2015). Remarkably, biologically produced AgNPs exhibit high stability, solubility, and yield (Gurunathan et al., 2009) Reduction is the primary reaction that occurs during the process of nanoparticle biosynthesis. Therefore, scientists used plant extracts (phytochemicals)(Johnson and Prabu, 2015) and microbial enzymes to create nanoparticles at a lower cost. They are often in charge of reducing metal compounds into their corresponding nanoparticles due to their reducing or antioxidant properties (Veerasamy et al., 2011). Utilizing plants to synthesis nanoparticles can have advantages over other biological processes because it can be appropriately scaled up for large-scale production of nanoparticles in non-aseptic conditions. Numerous analytical techniques, such as X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), ultraviolet visible spectroscopy (UV-vis spectroscopy), dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and others, have been used to assess the properties of synthesized nanomaterials. (Sapsford et al., 2011). Here, we describe the green synthesis of silver nanoparticles using a cell-free aqueous extract of ficus fruit to lower the silver ions in a silver nitrate solution as it acts as a reducing and stabilizing agent. The synthesis of silver nanoparticles (AgNPs) was examined using SEM, UV absorption

techniques, visual examination (colour change), and catalase activity, antibacterial and anticancer properties were studied.

MATERIALS AND METHODS

Preparation of Ag NPs

The fruit from *Ficus carica* was obtained and silver nitrate (AgNO3) was acquired. All of the aqueous solutions were made with double-distilled deionized water. Fresh Ficus Carica fruits were thoroughly cleaned with distilled water before being finely ground into a wet paste using a mortar and pestle. This was mixed with 100 ml of DI water and allowed to stand for two days in a dark area at room temperature. Whatman paper No 1 was used to filter the light-yellow colored extract and used for Ag NPs synthesis and stored at 4 °C for further experiments

Synthesis of AgNPs

About 5 g of ficus fruit extract was diluted with 10 ml of dimethyl sulfoxide (DMSO) for the green biosynthesis of AgNPs. A magnetic stirrer was used to stir this with 200 ml of 1 mm AgNO3 until the color changes from yellow to dark orange to brown.

UV-Vis spectroscopy

The biogenic synthesis of AgNPs was monitored periodically by UV-Vis spectroscopy. All spectra were collected by scanning from 350 to 500 nm ranges. All spectra were corrected against the background spectrum of water as reference. The most useful method for evaluating metal ion reduction based on optical characteristics known as surface plasmon resonance (SPR) is UV Vis spectroscopy (Alzoubi et al., 2023).

Field emission scanning electron microscopy (FE-SEM) analysis

With the help of a field emission SEM, morphological and structural examinations were conducted to characterize the size and shape of the silver nano particles synthesized by the green biosynthesis from the ficus extract.

Antioxidant assay

DPPH Assay

Using the stable radical DPPH, the free radical scavenging activity of AgNPs was evaluated. For DPPH assay (Molyneux, 2004) Aliquot 3.7 ml of absolute methanol in all test tubes and 3.8ml of absolute methanol was added to blank.100µl of BHT was added to tube marked as standard and 100µl of respective samples were added to all other tubes marked as tests. 200µl of DPPH reagent was added to all the test tubes including blank. Test tubes were incubated at room temperature in dark condition for 30 minutes. The absorbance of all samples was read at 517nm. Using the following formula, the percentage of inhibition representing the free radical scavenging activity was calculated.

$$\% \text{ Antioxidant activity} = \frac{(\text{Absorbance at blank}) - (\text{Absorbance at test})}{(\text{Absorbance at blank})} \times 100$$

ABTS Assay

For ABTS Assay(Re et al., 1999) the reaction was initiated by the addition of 1.0 ml of diluted ABTS to 10 µl of different concentration of the sample and 10µl of methanol as control. BHT was used as standard. The absorbance was read at 734 nm and the percentage inhibition was calculated. The % inhibition was calculated according to the equation.

$$\text{ABTS scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

Assay Of Superoxide Dismutase(SOD) (Kakkar et al., 1984)

The assay mixture contained 1.2 ml of Sodium pyrophosphate buffer, 0.1ml of PMS, 0.3ml of NBT, 0.3 ml of the enzyme preparation and water in a total volume of 2.8ml. The reaction was initiated by the addition of 0.1ml of NADH. The mixture was incubated at 300c for 90 seconds and arrested by the addition of 0.1ml of glacial acetic acid. The reaction mixture was then shaken with 4.0ml of n-butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560nm in a spectrophotometer (Genesys 10-S, USA). One unit of enzyme activity is defined as the amount of enzyme that gave 50 % inhibition of NBT reduction on one minute.

Colorimetric Assay

The activity of Catalase was determined by the method of (Sinha, 1972). Dichromate in acetic acid was converted to per chromic acid and then to chromic acetate, when heated in the presence of H2O2. The chromic acetate formed was measured at 620nm. The Catalase preparation was allowed to spit H2O2 for various periods of time. The reaction was stopped at different time intervals by the addition of dichromate-acetic acid mixture and the remaining H2O2 as chromic acetate was determined calorimetrically.

Antibacterial Assay

The agar disc diffusion assay was used to evaluate AgNPs' antibacterial activity. Strains used in this study were gram positive bacteria's such as *Staphylococcus aureus, Bacillus subtilis*, gram negative bacteria's such as *Escherichia Coli, Klebsiella pneumoniae* and *Salmonella enterica*. Using sterile cotton swabs, the above said bacterial species were inoculated on Mueller Hinton Agar medium, and different AgNPs concentrations were loaded onto the sterile plate. For comparison, ampicillin was used as a positive standard. The inoculated plates were covered with dried discs, which were then incubated at 37°C overnight. The diameter of the zone of inhibition surrounding the disc was measured to determine the antibacterial activity.

Cell culture

MCF 7 cell line was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in DMEM supplemented with 10% FBS, penicillin (100 µg /ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO2 at 37 °C.

Cytotoxic Assay (MTT)

The fruit extract mediated silver nanoparticles was tested for its cytotoxic effect using dimethyl thiazolyl tetrazolium bromide (MTT)assay(Mosmann, 1983) on MCF 7 cell line . Approximately (1 × 105/well) cells that were incubated in 24-well culture plate for 24 hours of incubation at 37°c in 5% CO2 was treated with AgNPs of varying concentrations while the control culture was treated with DMSO and incubated for 24 hours and then 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added to each well and further incubated for 4 h. Later 1 ml of DMSO was added to each well. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

% Cell viability = A570 of treated cells / A570 of control cells × 100

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

The silver nanoparticles were efficiently synthesized from F. fruit extract with 1mm Ag NPs containing solution at room temperature. About 5 g of ficus fruit extract was diluted with 10 ml of dimethyl sulfoxide (DMSO) for the green biosynthesis of AgNPs. A magnetic stirrer was used to stir this with 200 ml of 1 mm AgNO3 until the color changes from yellow to dark orange to brown. AgNPs formation was indicated when the reaction mixture's color changed from light yellow to orange-brown (Fig. 1). The control solution without FC extract didn't show this color change.

Characterization of silver nanoparticles

The silver nanoparticles synthesized using green bio synthesis was confirmed by uv vis spectroscopy which show a peak at 450nm, demonstrates that the bioactive substances found in the ficus fruit extract are more effective in producing silver nanoparticles(Fig. 2).

SEM Analysis

The morphology of the silver nanoparticles was examined using scanning electron microscopy (Vega 3 Tescan), and pictures were produced at 30 kV. The generated silver nanoparticles had a spherical shape, which ranged in size from 46.65 nm to 73.75 nm, were distributed uniformly throughout the colloidal mixture, based on SEM images (Fig 4).

Antioxidant activity

DPPH assay

The results of DPPH assay confirm the antioxidant property of AgNP and the percentage of DPPH free radicle scavenging activity of the synthesized AgNPs of varying concentration were studied and given in (table.1; Fig.8)

Table.1 DPPH Activity of synthesised AgNP

S.NO	Concentration (µg/ml)	O.D	DPPH %
1	200	0.551	9.37
2	400	0.441	27.46
3	600	0.346	43.09
4	800	0.245	59.70
5	1000	0.146	75.98

Control: 0.608

ABTS assay

ABTS decolorization assay involves the generation of the ABTS+ chromophore by the oxidation of ABTS with ammonium persulphate. It is applicable for both

hydrophilic and lipophilic compounds. The scavenging activity of the synthesized AgNPs on ABTS radical action were measured and given in the following table.2; Fig.7.

Table.2 ABTS Activity of synthesised AgNP

S.NO	Concentration (µg/ml)	O.D	ABTS activity (%)
1	200	0.419	15.18
2	400	0.361	26.92
3	600	0.294	40.48
4	800	0.224	54.65
5	1000	0.156	68.42

Control : 0.494

ASSAY OF SUPEROXIDE DISMUTASE

The assay of SOD is based on the inhibition of the formation of NADH-phenazine methosulphate-nitrobue tetrazolium formazon. The colour formed at the end of the reaction can be extracted into butanol and measured at 560nm.The results are calculated and given in table 3 and Fig.10.

$$\%INHIBITION = A_{560nm} \text{ of blank} - A_{560nm} \text{ of test} / A_{560nm} \text{ of blank } X100$$

Table.3 SOD Activity of synthesised AgNP

S.NO.	Concentration (µg/ml)	OD value	AVERAGE %
1	**200**	**0.213**	**17.76**
2	**400**	**0.182**	**29.72**
3	**600**	**0.144**	**44.40**
4	**800**	**0.111**	**57.14**
5	**1000**	**0.076**	**70.65**

Control: 0.259

Colorimetric assay

To 0.9ml of phosphate buffer, 0.1mL of supernatant of 20% homogenate of tissue extract and 0.4mL of hydrogen peroxide was added. The reaction was arrested after 60 seconds by adding 2.0mL of dichromate-acetic acid mixture. The tubes were kept in boiling water bath for 10 min, cooled and the colour developed was read at 620nm.Catalase activity was expressed as mol of H_2O_2consumed/min/mg of protein.(table 4, fig 9)

Table.4 Catalase Activity of synthesised AgNP

S.NO.	Concentration	OD VALUE	CATALASE (H_2O_2consumed/min)
1	**200**	**0.189**	**1.488**
2	**400**	**0.240**	**1.889**
3	**600**	**0.301**	**2.370**
4	**800**	**0.346**	**2.724**
5	**1000**	**0.401**	**3.157**

Antibacterial Activity

It has been shown that Ag-NPs work well as a broad-spectrum antibiotic against both Gram-positive and Gram-negative bacteria (Marambio-Jones and Hoek, 2010).Furthermore the nanoparticle syntheses by green route are found to be highly effective against multi-drug resistant human pathogenic bacteria. Antibacterial activity of silver nanoparticles against the earlier

mentioned gram positive and negative bacteria were investigated and compared with the standard drug. The zone of inhibition was observed and given in table 5.

S.No	STRAIN	CONTROL	SILVER NANOPARTICLE		
	Culture	Standard (20µl)	1000 µg (20µl)	750 µg (20µl)	500 µg (20µl)
1	*Staphylococcus aureus*	15	10	7	-
2	*Bacillus subtilis*	51	33	27	27
3	*Escherichia Coli*	15	10	7	7
4	*Klebsiella pneumoniae*	18	17	17	9
5	*Salmonella enterica*	11	7	-	-

Table.5 Antibacterial Activity of control and AgNP at different concentrations.

Anticancerous Activity

The silver nanoparticles induced numerous morphological alterations against MCF 7 cells at 36 h incubation, however there is no such alterations were seen in untreated cells (Fig.5). The cytotoxic capability of silver nanoparticles by green synthesis was assessed at varying concentrations (7.8 to 1000 µg/ml) against the triple positive breast cancer cells. According to our findings, MCF 7 cells showed increased cytotoxicity when exposed to varying concentrations of silver nanoparticles in a dose dependent manner (Table 6)

S.No	Concentration (µg/ml)	Absorbance (O.D)			Average	Cell Viability (%)
1	1000	0.151	0.152	0.151	0.151	18.55
2	500	0.210	0.213	0.211	0.211	25.92
3	250	0.272	0.270	0.274	0.272	33.41
4	125	0.334	0.336	0.332	0.334	41.03
5	62.5	0.396	0.399	0.393	0.396	48.64
6	31.2	0.458	0.459	0.458	0.458	56.26
7	15.6	0.519	0.519	0.518	0.518	63.63
8	7.8	0.578	0.575	0.579	0.577	70.88

9	**Cell control**	**0.814**	**100**

Table.6 Anticancerous Activity of synthesized AgNP against MCF 7 cells

CONCLUSION

The silver nanoparticles were successfully produced by green biosynthesis using ficus fruit extract and its synthesis is confirmed by uv vis spectroscopy where the peak was observed at 450nm. The antioxidant property of the green biosynthesized AgNPs were measured by DPPH , ABTS , FRAP analysis and colorimetric assay and SOD assay and their results collectively conclude that the silver nanoparticle is a brilliant antioxidant. The shape and size of the nanoparticles produced was recorded ranges between 46-74 nm using SEM analysis. this study concludes that green biosynthesis of silver nanoparticles is a effective and ecofriendly method for pharmaceutical and medical applications as these AgNPs show antibacterial against *Klebsiella pneumoniae* and *Bacillus subtilis* and was very much effective against the triple positive breast cancer (MCF7) cells as it exhibits anticancerous activity. These nanosilver can be used in therapy after critical examination of toxicity of the AgNPs

FIGURE CAPTIONS:

Fig.1. visual confirmation – color change from yellow to brown indicating formation of silver nanoparticles

Fig.2. UV-Vis spectrum analysis of AgNPs

Fig.3. Antibacterial activity of AgNPs

Fig.4. SEM image of AgNPs reduced by the fruit extract of Ficus

Fig.5. MTT Assay of AgNPs.

Fig. 6. Image of MCF7 Cells (Control and treatment with varying concentration of AgNPs.

Fig. 7. ABTS Assay

Fig. 8. DPPH Assay

Fig. 9. Colorimetric Assay

Fig. 10. SOD Assay

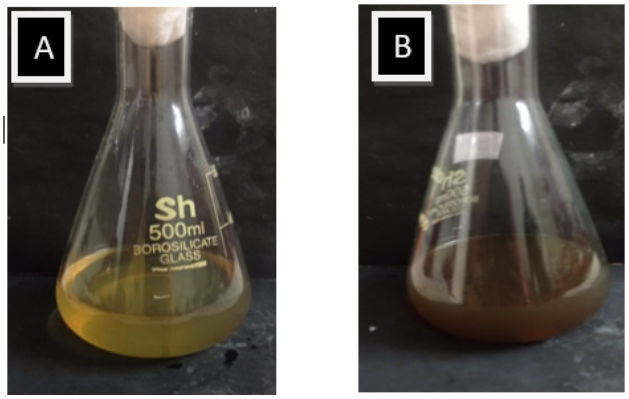


FIG -1 visual confirmation (A) aqueous fruit extract (B) 1 mm silver nitrate solution after reaction with ficus fruit extract for 24 hours

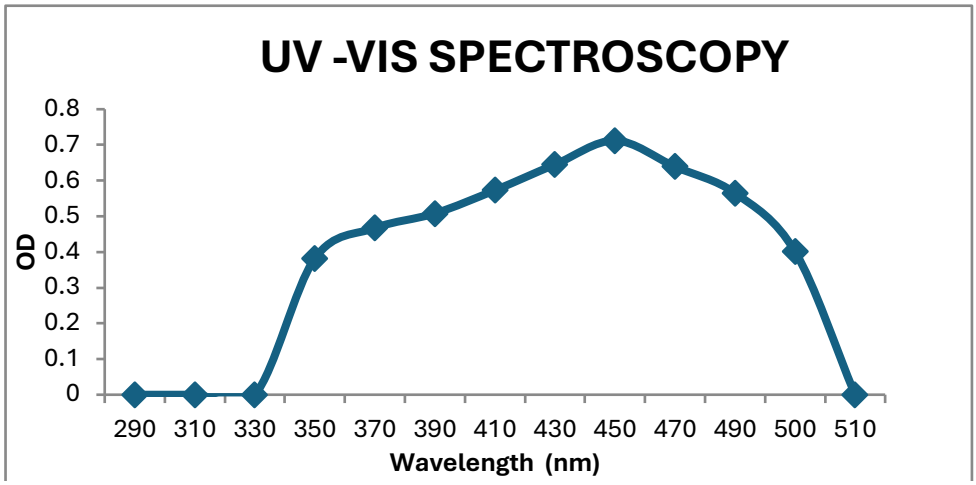
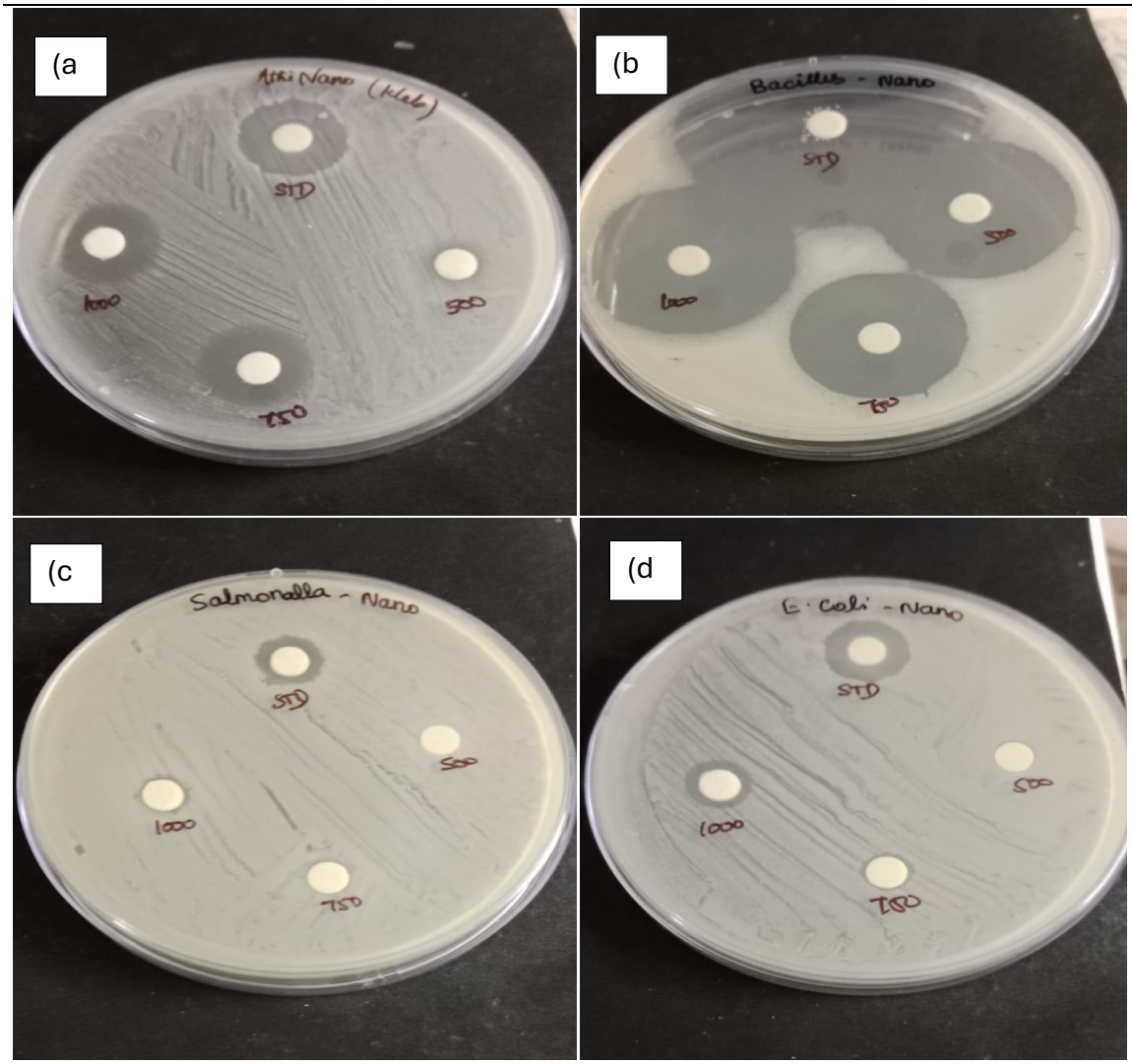


FIG – 2 The synthesized AgNPs showed absorbance spectra at 450 nm in UV -VIS spectroscopy



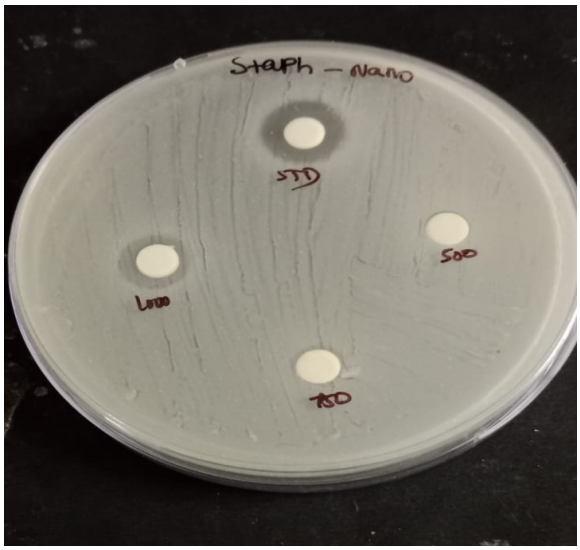


FIG – 3 Antibacterial activity of biosynthesized silver nanoparticle against (a) *Klebsiella pneumoniae* (b) *Bacillus subtilis* (c) *Salmonella enterica* (d) *Escherichia Coli* and (e) *Staphylococcus aureus*

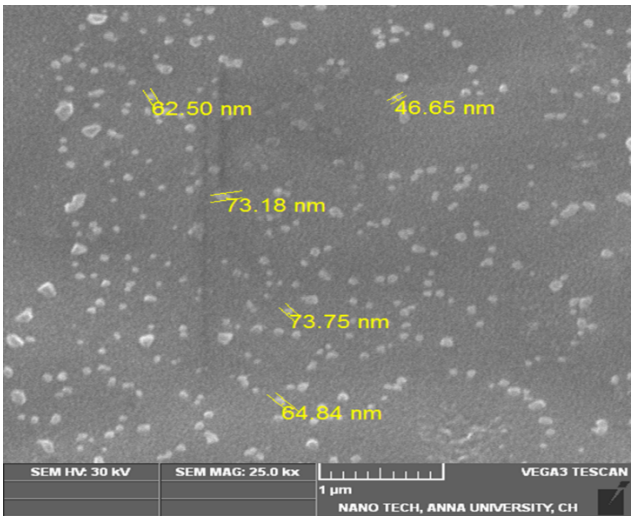


FIG – 4 SEM analysis of green biosynthesized AgNPs showing spherical morphology

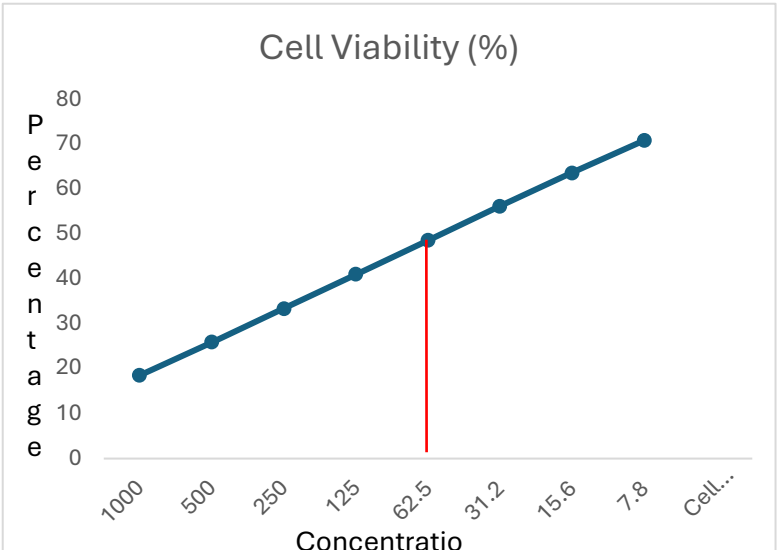


FIG - 5 MTT Assay of AgNPs

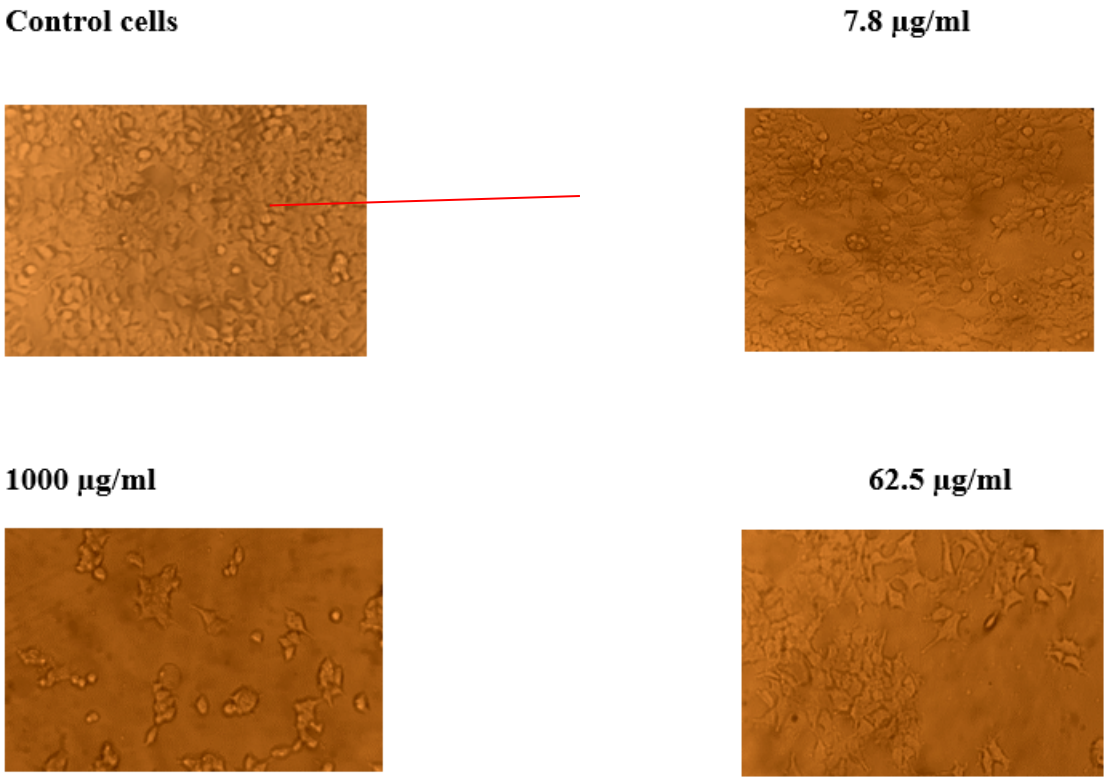


FIG – 6 MCF7 cells upon treatment with different concentrations of sample

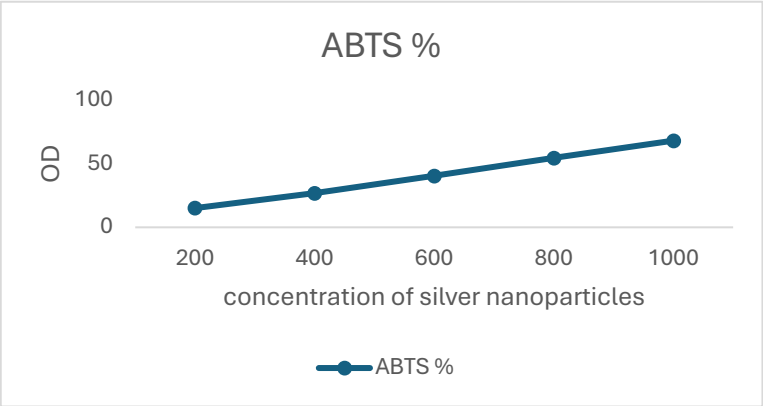


FIG – 7 ABTS activity of synthesized AgNP

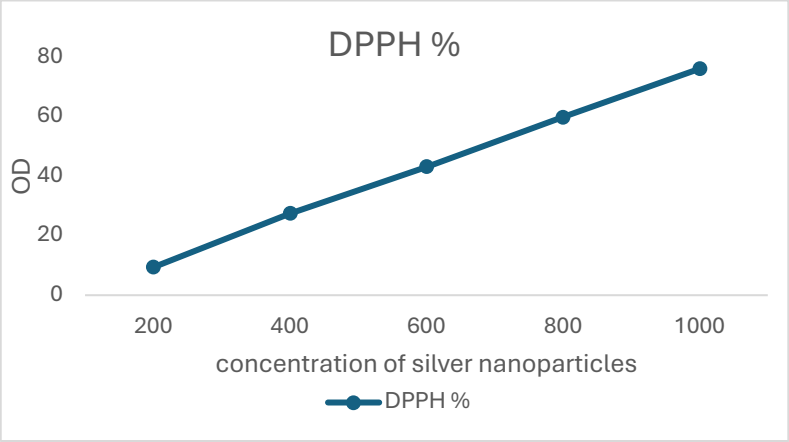


FIG – 8 DPPH activity of synthesized AgNP

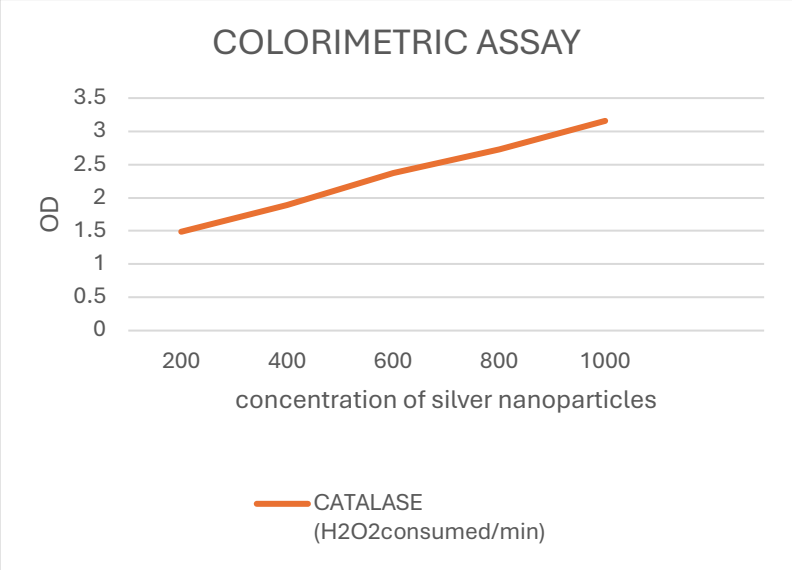


FIG – 9 Catalase activity of synthesized AgNP on increasing concentration

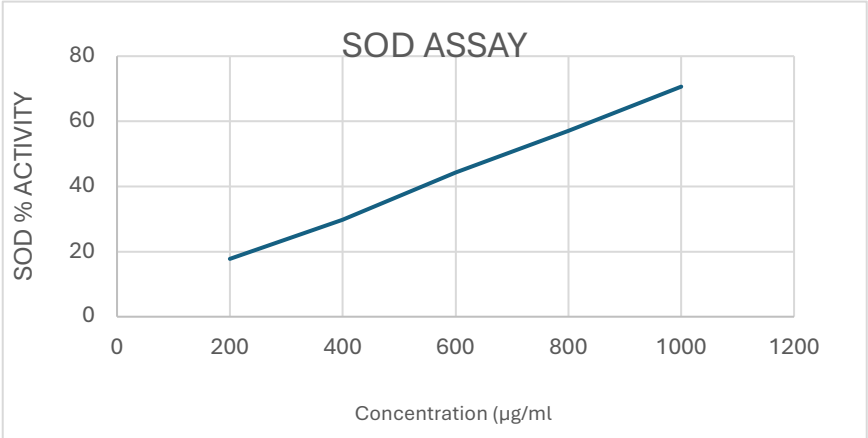


FIG – 10 SOD activity of synthesized AgNP

Acknowledgement

The authors would like to thank the Principal of Pachaiyappa's College, Chennai, for providing the necessary facilities to conduct this work.

Funding Sources

This project did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

Authors do not have any conflict of interests to declare

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Author Contributions

Sathya Priya Janaki Ram: Conceptualization, Methodology, Writing – Original Draft.
Sangeetha Soundararajan: Visualization, Supervision, Project Administration.
Shanmuga Priya Venugopal: Data Collection, Analysis, Writing – Review & Editing.

REFERENCES

1. Alzoubi, F.Y., Ahmad, A.A., Aljarrah, I.A., Migdadi, A.B., Al-Bataineh, Q.M., 2023. Localize surface plasmon resonance of silver nanoparticles using Mie theory. J Mater Sci: Mater Electron34, 2128. https://doi.org/10.1007/s10854-023-11304-x
2. Gurunathan, S., Kalishwaralal, K., Vaidyanathan, R., Venkataraman, D., Pandian, S.R.K., Muniyandi, J., Hariharan, N., Eom, S.H., 2009. Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli. Colloids and Surfaces B: Biointerfaces 74, 328–335. https://doi.org/10.1016/j.colsurfb.2009.07.048
3. Gurunathan, S., Park, J.H., Han, J.W., Kim, J.-H., 2015. Comparative assessment of the apoptotic potential of silver nanoparticles synthesized by Bacillus tequilensis and Calocybe indica in MDA-MB-231 human breast cancer cells: targeting p53 for anticancer therapy. Int J Nanomedicine 10, 4203–4222. https://doi.org/10.2147/IJN.S83953
4. Halvorsen, B.L., Holte, K., Myhrstad, M.C.W., Barikmo, I., Hvattum, E., Remberg, S.F., Wold, A.-B., Haffner, K., Baugerød, H., Andersen, L.F., Moskaug, Ø., Jacobs, D.R., Blomhoff, R., 2002. A systematic screening of total antioxidants in dietary plants. J Nutr 132, 461–471. https://doi.org/10.1093/jn/132.3.461
5. Johnson, I., Prabu, H.J., 2015. Green synthesis and characterization of silver nanoparticles by leaf extracts of Cycas circinalis, Ficus amplissima, Commelina benghalensis and Lippia nodiflora. Int Nano Lett 5, 43–51. https://doi.org/10.1007/s40089-014-0136-1
6. Kakkar, P., Das, B., Viswanathan, P.N., 1984. A modified spectrophotometric assay of

superoxidedismutase. Indian J Biochem Biophys 21, 130–132.
7. Marambio-Jones, C., Hoek, E.M.V., 2010. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. J Nanopart Res 12, 1531–1551. https://doi.org/10.1007/s11051-010-9900-y
8. Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65, 55–63. https://doi.org/10.1016/0022-1759(83)90303-4
9. Reidy, B., Haase, A., Luch, A., Dawson, K.A., Lynch, I., 2013. Mechanisms of Silver Nanoparticle Release, Transformation and Toxicity: A Critical Review of Current Knowledge and Recommendations for Future Studies and Applications. Materials (Basel) 6, 2295–2350. https://doi.org/10.3390/ma6062295
10. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26, 1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3
11. Sapsford, K.E., Tyner, K.M., Dair, B.J., Deschamps, J.R., Medintz, I.L., 2011. Analyzing Nanomaterial Bioconjugates: A Review of Current and Emerging Purification and Characterization Techniques. Anal. Chem. 83, 4453–4488. https://doi.org/10.1021/ac200853a
12. Sinha, A.K., 1972. Colorimetric assay of catalase. Anal Biochem 47, 389–394. https://doi.org/10.1016/0003-2697(72)90132-7
13. Veerasamy, R., Xin, T.Z., Gunasagaran, S., Xiang, T.F.W., Yang, E.F.C., Jeyakumar, N., Dhanaraj, S.A., 2011. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. Journal of Saudi Chemical Society 15, 113–120. https://doi.org/10.1016/j.jscs.2010.06.004