VOLUME 10 ISSUE 1 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 1, 355-363 (2024)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Green Synthesis and Characterization of Silver Nanoparticles from *Santalum album* and their Antimicrobial Antioxidant and Anticancer Activity

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Received: 26th November, 2023; **Accepted:** 26th December, 2023; **Published online:** 4th March, 2024

https://doi.org/10.33745/ijzi.2024.v10i01.040

Abstract: The Earth is adorned with a rich and varied array of flora. It has been estimated that the botanical diversity in India contributes significantly, comprising about 11.4% of the total global species diversity. In a paradigm shift towards safer and more diverse alternatives, plants, known for their absence/least side effects, are emerging as readily available substitutes for commercial drugs. Traditional pharmaceuticals, often laden with toxicity, high costs, and adverse reactions, are being challenged by the exploration of plant-derived alternatives. This study centers on the synthesis and characterization of silver nanoparticles (AgNPs) derived from the methanol leaf extract of *Santalum album*. Silver nitrate and leaf methanol extracts are employed separately as the precursor and capping reducing agent. The characterization of the synthesized silver nanoparticles is conducted through various techniques, including UV-Visible Spectrophotometry (UV), Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), and Fourier-transform Infrared Spectroscopy (FT-IR). Antimicrobial assessments involve testing against both gram-positive bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) and gram-negative bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), as well as two fungal species (*Aspergillus niger* and *Candida albicans*) using the agar well diffusion method. Furthermore, the AgNPs from *Santalum album* methanol leaf extract exhibit *in vitro* antioxidant activity, assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Their potential in combating cancer is explored through the evaluation of anticancer properties against MCF-7 cell lines (Breast Cancer cell lines) using the MTT assay and the determination of nuclear morphology via AO/PI staining. The findings of this study suggest that silver nanoparticles from *Santalum album* methanol leaf extract may serve as a promising natural drug for both the treatment and prevention of cancer progression.

Keywords: *Santalum album,* Fourier-transform infrared spectroscopy, Particle size analyzer, Scanning electron microscope, UV- Visible Spectrophotometer

Citation: Asha Monica Alex Rajarathinam, Kumar Sathish, Swabna Vivekanadam and Arockiasamy Edward: Green synthesis and characterization of silver nanoparticles from *Santalum album* and their antimicrobial antioxidant and anticancer activity. Intern. J. Zool. Invest. 10(1): 355-363, 2024.

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Introduction

The branch green chemistry involves utilization of the phytoconstituents bestowed within the plant species, thereby reducing the emission of hazardous substances that are drastic to the environment (Asha and Senthilkumar, 2020). Furthermore, overuse of the antibiotics without proper prescription has created a threat in modern times due to the development of MDR wherein the bacteria continue to survive and cause deadly disease irrespective of the dosage of antibiotics (Adedeji, 2016). Plant species have been implemented in orthodox medical practice and is used to cure a wide range of diseases including malaria, epilepsy, infantile convulsion, diarrhea and dysentery, fungal and bacterial infections (Gangoue-Pieboji *et al*., 2016).

The rich and diverse flora found in nature has long been a source of inspiration for scientific exploration, with plants serving as a reservoir of potential therapeutic compounds. India, boasting approximately 11.4% of the world's species diversity, stands as a significant contributor to the global botanical landscape (Sen and Samanta, 2015). In recent times, there has been a burgeoning interest in harnessing the medicinal properties of plants, particularly those devoid of adverse side effects, as alternatives to conventional drugs known for their toxicity and high costs (Tomlinson and Akerele, 2015). This study focuses on the synthesis and characterization of silver nanoparticles (AgNPs) utilizing the methanol leaf extract of *Santalum album* (Ozturk and Hakeem, 2018). The silver nitrate and methanol leaf extracts serve as the precursor and capping reducing agent, respectively (Sureshbabu *et al*., 2012). Characterization techniques such as UV-Visible Spectrophotometry (UV), Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), and Fourier-transform Infrared Spectroscopy (FT-IR) are employed to analyze and identify the synthesized silver nanoparticles (Singla *et al*., 2022). The antimicrobial efficacy of these AgNPs was assessed against both gram-positive bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) and gram-negative bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), as well as two fungal species (Aspergillus niger and Candida albicans) using the agar well diffusion method (Al Masoud *et al*., 2021). Furthermore, the *in vitro* antioxidant activity of AgNPs from *Santalum album* methanol leaf extract was evaluated through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The research extends to explore the potential anticancer properties of these nanoparticles against MCF-7 cell lines (Breast Cancer cell lines) using the MTT assay and determining nuclear morphology through AO/PI staining (Salari *et al*., 2019). The outcomes of this study suggest that AgNPs from *Santalum album* methanol leaf extract may emerge as promising candidates for natural drug development, particularly in the treatment and prevention of cancer progression. This multi-dimensional approach not only underscores the versatility of plant-based nanoparticles but also highlights their potential in addressing complex biomedical challenges.

Materials and Methods

Materials required:

High-purity AR grade Silver Nitrate was procured from Sigma Aldrich chemicals. Fresh leaves of *Santalum album* were meticulously washed with distilled water. Subsequently, 20 g of the selected leaves were subjected to boiling in distilled water at 60°C for a duration of 5 to 10 min, after which the mixture was filtered. The resulting leaf broth was carefully stored at 4°C, ready for utilization in the subsequent stages of the study.

Plant Material and Extract Preparation:

Fresh leaves of *Santalum album* were collected from Salem, Tamil Nadu, India. The collected plant material underwent a thorough identification process, which was duly confirmed at the Department of Botany, St. Joseph's College, Tiruchirappalli. Following identification, the leaves were carefully shade-dried for a period of approximately fourteen days. Subsequently, the dried leaves were finely powdered and stored,

ready for further examination and analysis.

Synthesis of Nanoparticles:

A 10 ml solution of 0.1 mM $AgNO_3$ was prepared under dark conditions. To this solution, 0.5 ml of the leaf broth was added, and the mixture was allowed to rest for 30 min at a temperature of 37°C. This step in the process likely involves the interaction and synthesis of silver nanoparticles from the *Santalum album* leaf extract with the silver nitrate solution under controlled conditions (Mashau *et al*., 2023).

Characterization of Silver Nanoparticles:

Synthesis of silver nanoparticles was determined by the following spectroscopic and analytical methods:

UV- Visible Spectrophotometer:

The reduction of silver ions was confirmed by measuring the absorbance of the mixture at regular intervals at a wavelength of 300- 800 nm.

Particle size analyser:

Particle size was measured. The example particles in the cell experience Brownian movement. A light source is brought into the cell, and the dispersed light is gathered. In response to this, the system selects the optimum scattering angle and cell position depending on the sample concentration and intensity (Jiu *et al*., 2023).

SEM:

Scanning electron microscope analysis, the size and shape of the synthesized nanoparticles was determined by observing the mixture under SEM.

FTIR:

FTIR analysis determined the functional groups present in the synthesized nanoparticles. The solution containing nanoparticles was centrifuged at 60,000 rpm and the pellet obtained was employed for the study (Latha *et al*., 2023)

Antimicrobial activity:

The antimicrobial activity (Bauer, 1966) of the synthesized nanoparticles was evaluated by the standard protocol against 4 bacterial strains viz., *E. coli, Klebsiella sp, Staphylococcus aureus, Bacillus sp* and 2 fungal strains viz., *A. Niger* and *Candida albicans*.

Antioxidant activity:

The radical scavenging potential (Alothman *et al*., 2009) of the nanoparticles was determined by using DPPH reagent. Solvent served as blank, ascorbic acid was used as control and the test sample were taken at five different concentrations. DPPH was added to all the tubes and their absorbance was measured at 517nm.

Anticancer Activity:

Breast cancer cell lines were grown in T75 flasks containing DMEM media. It was maintained at 37°C in a humidified atmosphere having 5% carbon dioxide (McIntosh *et al*., 2010).

In vitro anticancer activity Assay:

Inhibitory capacity of synthesized nanoparticle was tested on breast cancer cell line MCF-7 by MTT assay (Mosmann and Tim, 1983). Synthesized nanoparticles were first dissolved in dimethyl sulfoxide (DMSO) to make a stock. These stock solutions were diluted separately with media to get various concentrations of the complex. 200 µl of these samples were added to wells containing 5 X 103 MCF^{-7} cells per well. DMSO solution was used as the solvent control. After 24 h, 20 µl of MTT solution (5 mg/ml in PBS) was added to each well and the plate was wrapped with aluminum foil and incubated for 4 h at 37°C (Nordin *et al*., 2017). The purple formazan product was dissolved by addition of 100 µl of DMSO to each well. The absorbance was monitored at 570 nm (measurement) and 630 nm (reference) using a 96-well plate reader (Bio-Rad, iMark, USA) (Mohamed *et al*., 2022). Data were collected for three replicates each and used to calculate the respective mean. The percentage inhibition was calculated, from this data, using the formula:

[Mean of absorbance of untreated cells (Control) – Mean of absorbance of treated cells]/ [Mean of absorbance of treated cells -Mean of absorbance of untreated cells (control)] X 100

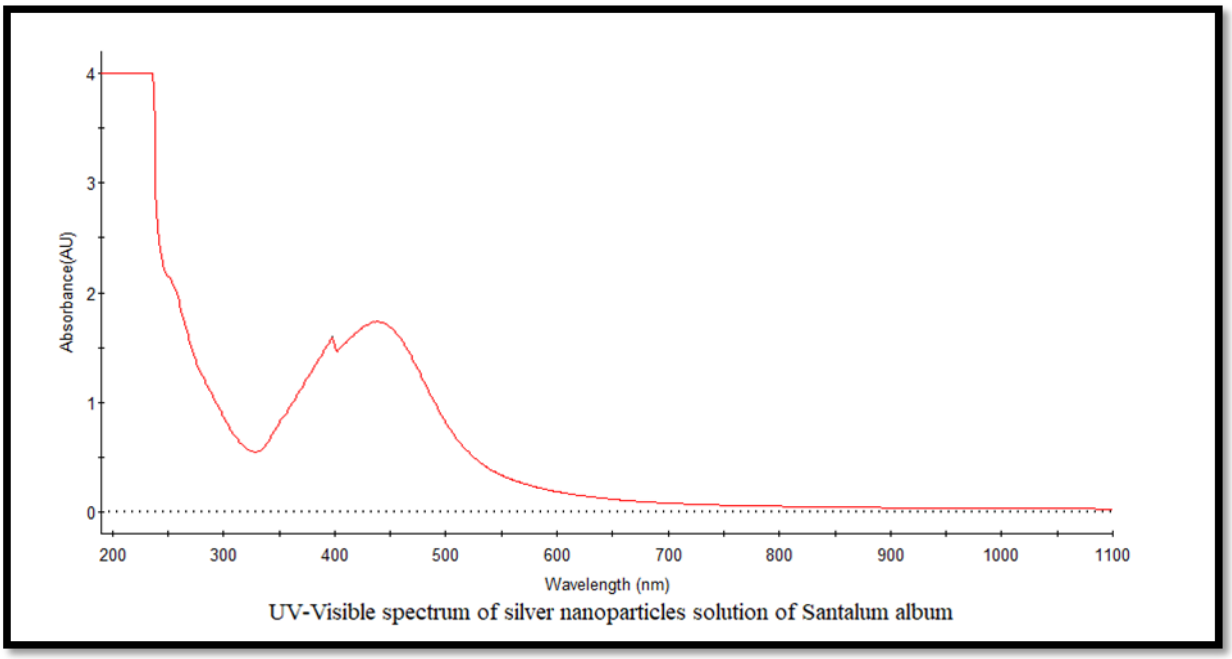

Fig. 1: UV-Visible spectrophotometry.

Determination of nuclear morphology (*AO/PI*):

Characteristic morphological changes upon induction of apoptosis were surveyed by fluorescence microscopy utilizing acridine orange/propidium iodide (AO/EB) double recoloring (Liu *et al*., 2015). Suitable cells with an unblemished cell layer were recolored by AO alone and showed up brilliant green while apoptotic cells recolored by both AO and PI showed a red-orange shaded appearance with chromatin buildup. Information introduced are illustrative of at least three autonomous analyses.

Results and Discussion

Green synthesis of Santalum album silver nanoparticles:

The color of the methanol extract to reddish brown due to surface plasmon resonance indicating that silver ions have been reduced to metallic silver nanoparticles. The original aqueous extract was yellowish in color. However, the addition of *Santalum album* $AgNO_3$ followed by incubation at room temperature has changed the color to reddish brown reflecting the synthesis of silver nanoparticles.

The synthesized nanoparticles were continuously assessed by employing various characterization techniques as follows:

UV-Visible spectrophotometry:

UV- Visible spectrophotometer serves as an important tool to determine if the plant extract has the ability to reduce and stabilize the nanoparticles. Following the blending of the extract and silver nitrate solution, UV absorption spectra was measured in 30 min. A stable bell-shaped curve was obtained at 454.60 nm indicating that surface plasmon resonance has occurred (Fig. 1).

SEM:

The size and appearance of the synthesized nanoparticles were determined by Scanning Electron Microscopy. This showed that the particles were spherical shaped and flake surfaced spherical and whiskers shape with an average size of 40-60 nm (Fig. 2).

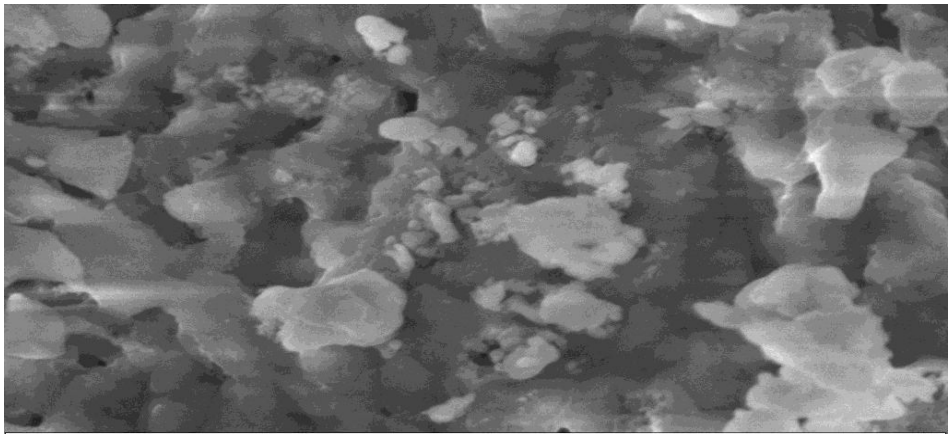

Fig. 2: SEM analysis.

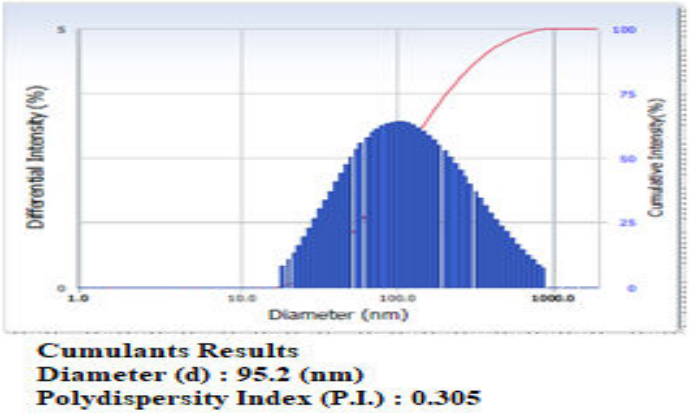

Fig. 3: Particle Size Analyzer.

Particle Size Analyzer:

The average size distribution of the AgNPs was determined by the particle size analyzer and the result is displayed in Figure 3. It shows that the Zeta average diameter of the particle is 95.2 nm with P.I.:0.305.

FTIR:

To elucidate the functional groups and the metabolites present in the plant extract that has reduced silver ions, FTIR was performed by comparing the peaks for the synthesized nanoparticles from plant extract. It was apparent that strong peaks were obtained at 3350 cm^{-1} corresponds to O-H 185 stretching H-bonded alcohols and phenols. The peak at 2967 cm^{-1} corresponds to Carboxylic Acid O-H Stretch (Fig. 4). The assignment at 1636 cm^{-1} corresponds to N-H bend primary amines. The bands observed at 1168 cm^{-1} corresponds to C-N stretching alcohols. Accordingly, the incorporated nanoparticles were encompassed by proteins and metabolites.

Antimicrobial activity of the synthesized AgNPs:

The inhibitory effect of the synthesized AgNPs on microbes was assessed. Methanol extract of *Santalum album* and the biosynthesis AgNPs were analysed against the chosen clinical isolates. It was found that among the bacterial species tested, the most susceptible bacterium to AgNPs was

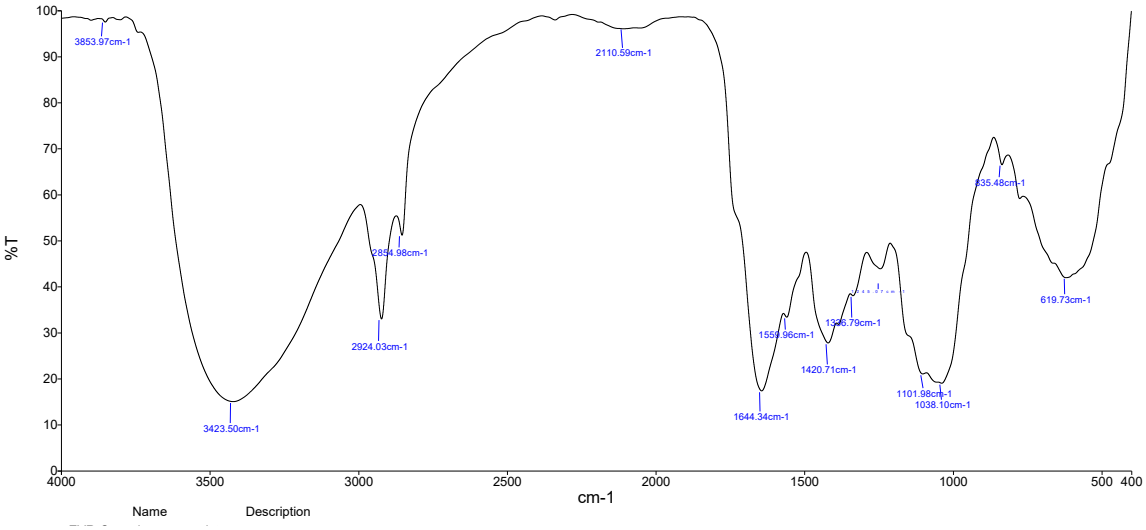

Fig. 4: FTIR analysis.

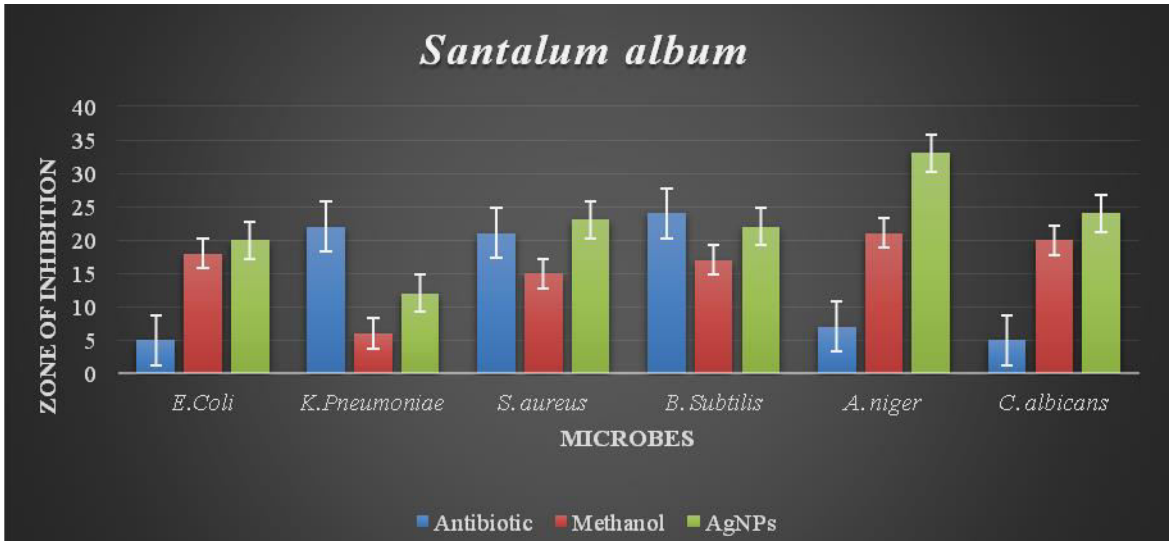

Fig. 5: Antimicrobial activity of the synthesized AgNPs.

Staphylococcus aureus and Bacillus sp with a zone of inhibition of 23 mm and 22 mm (Fig. 5). *E. coli* and *Klebsiella pneumoniae* showed zones of diameters 20 mm and 12 mm, respectively. With reference to their activity of fungi, *Aspergillus niger* was most sensitive with a zone of inhibition measuring 33 mm whereas *Candida albicans* with 24 mm zone of inhibition was less susceptible to the synthesized nanoparticles (Fig. 5).

Antioxidant Activity of the synthesized AgNPs:

The assay for evaluating the radicals scavenging potential of AgNPs were carried out by the reported method. The antioxidant activity of AgNPs at various concentrations was compared with standard Ascorbic acid. It was found that the maximum scavenging potential of AgNPs was at a 500 µl concentration with 83 % activity, whereas ascorbic acid displayed 97% activity at that concentration (Fig. 6).

Anticancer activity:

The cytotoxic activities of AgNPs synthesized from *Santalum album* have been investigated against

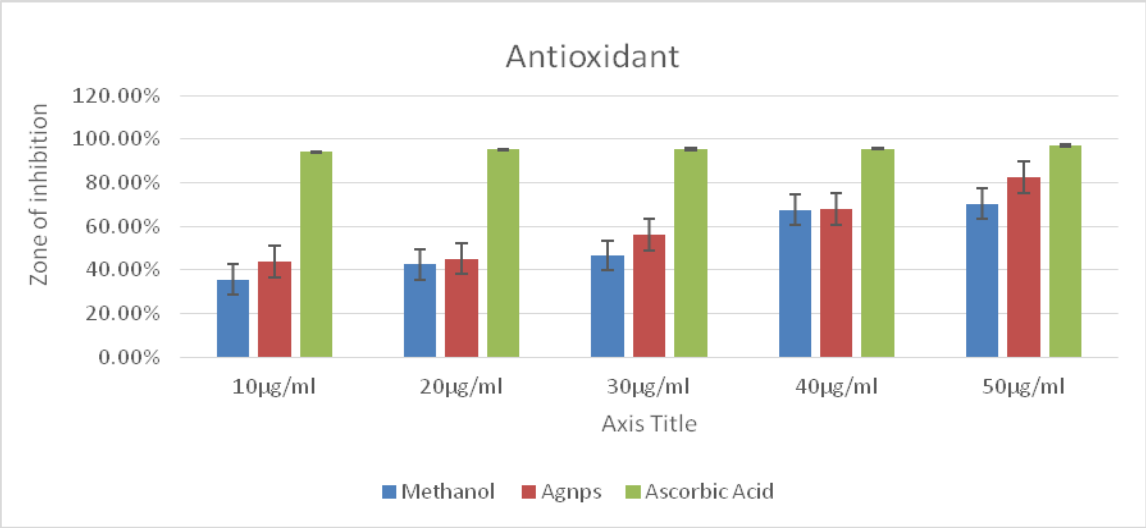

Fig. 6: Antioxidant Activity of the synthesized AgNPs.

Table 1: MTT assay of breast cancer cell line

Stock concentration	*Methanol*	*AgNPs*
25	54.32	78.56%
50	61.75	82.87%
100	70.65	89.76%
150	82.654	92.68%
200	85.687	93.56%

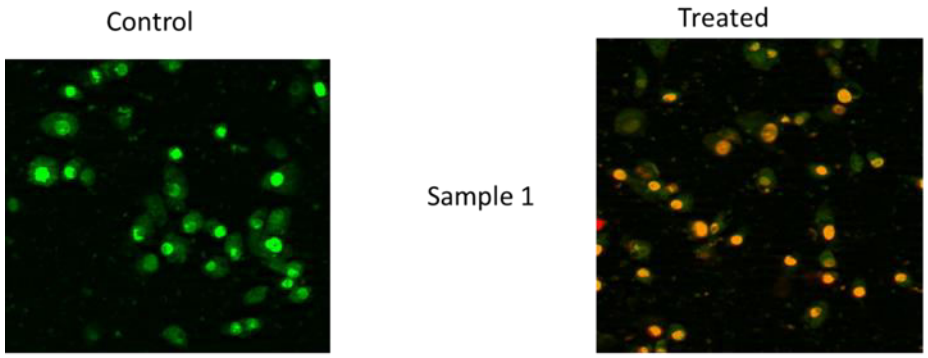

Fig.7: Determination of nuclear morphology (AO/PI).

the human breast cancer cell line MCF-7 by using MTT assay. The cytotoxic activity was determined according to the dose values of the exposure of the *Santalum album* AgNPs synthesized. Our results from this MTT assay indicated reduced cell viability of MCF-7 cells in concentration dependent manner. *Santalum album* showed more pronounced cytotoxic activity and inhibited the tumor cell growth for 85.687% in 200 µg/ml concentration of MCF-7 Cells. The reduction in cell viability increased significantly for higher AgNPs synthesized from *Santalum album* concentration (Table 1).

Determination of nuclear morphology (AO/PI):

AO/PI staining used for determination of live and

dead cells. All live nucleated cells are appeared in fluoresce green and all dead nucleated cells are appeared in fluoresce red (Fig. 7).

Conclusion

The nanoparticles derived from the plant extract demonstrated notable antimicrobial, antioxidant, and cytotoxic activities. The robust bioactive properties observed in the synthesized nanoparticles suggest that the plant's inherent compounds play a pivotal role in the reduction of silver ions. Consequently, it is evident that the *Santalum album* methanol leaf extract, enriched with bioactive constituents, holds significant promise as a natural drug for both the treatment and prevention of cancer progression. This underscores the potential of plant-based nanoparticles in contributing to innovative and effective therapeutic strategies in the field of medicine.

Acknowledgements

Asha Monica wish to thank Rev. Dr. M. Pavulraj Michael SJ, Rev. Dr. K.Amal SJ, Secretary, Rev. Dr. M. Arockiasamy Xavier SJ, Principal, St. Joseph's College, Tiruchirappalli for providing laboratory facilities to carry out this research work.

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