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Research Article

Eco-friendly silver nanoparticles from *Stereospermum colais*: A novel therapeutic strategy for breast cancer and antimicrobial applications

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Abstract

Stereospermum colais (Patala) is an ingredient of the reputed dasamula (ten roots) used in many important ayurvedic formulations. The most important constituents of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, saponins, tannins and glycosides. Their intense surface plasmon resonance peaks at 431 and 585 nm in UV-visible spectroscopy confirm the formation of the nascent silver and gold nanoparticles. The polyphenolic and alcoholic functional groups present in the aqueous leaf extract that performed the bioreduction processes, detected by Fourier transform infrared spectroscopy, powder X-ray diffraction and selected area electron diffraction patterns settled face-centred cubic crystal structures. The synthesized silver nanoparticles have accomplished well with physical and therapeutic properties. The antiproliferative in-vitro effect of the synthesized silver nanoparticles against MCF7 was studied by MTT assay. The study showed significant effect and markable cytotoxicity on human breast cancer cell lines (MCF-7). The antibacterial activity of silver nanoparticles was evaluated against *Streptococci* and *Pseudomonas* by measuring the percentage of inhibition at various concentrations (0-320 µg/ml). A dose-dependent inhibition observed for both the bacterial strains. *Streptococci* exhibited a stronger response, with a maximum inhibition of 76.6% at 320 µg/ml, while *Pseudomonas* showed a peak inhibition of 53.2% at the same concentration. These findings suggest that ScAgNP₃ are more effective against *Streptococci* than *Pseudomonas*, with concentration playing a critical role in enhancing antibacterial activity. In summary, this ecologically benign approach to producing biological silver nanoparticles offers rates of synthesis that are quicker than, or equivalent to, those of chemical methods. It has the potential to be used in various human-contact applications, including skincare products, food items, and therapeutic applications

Key words : ScAgNP₃ , MCF-7, MTT assay and MIC

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Introduction

India is home to a diverse array of medicinal plants. Herbal medicine is one of the most prominent branches of traditional medicine worldwide. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have a folklore reputation, in a more intensified way. *Stereospermum colais* L. (Patala or Padri) is a well-known medicinal plant mentioned in the "Glossary of Indian Medicinal Plants" by Chopra in 1952. The root is one of the important ingredients in *Dasamula*, an Ayurvedic formulation. The important preparations using the plant are *Dasamularishtam*, *Dhanvantaram tailam*, *Chyavanaprasham*, and *Agastyarasayanam*. *Saligrama Nighantu* recognizes three types of *Patala*: *Bhumipatala*, *Ksudrapatala*, and *Vallipatala*, but it has only mentioned two: the white-flowered one as *Patala* and the copper-red flowered one as *Tamrapatala*. Thus, it is considered an efficient medicinal plant [1,2,3].

Herbal medicines are thought to be safer than contemporary conventional medicines and more affordable. They are becoming more popular around the world and show promise for treating patients, preserving and improving health, and preventing and treating numerous diseases [4,5]. However, most of these physiologically active phytochemical ingredients have certain drawbacks, including poor absorption and distribution, a lack of target selectivity, reduced bioavailability, and diminished biological activity. Additionally, these phytochemical substances must be administered in high concentrations to have any effect, and some are unstable and sensitive to acidic environments [5,6,7,8,9]. These limitations prevent their use in clinical settings.

We are currently in the era of a "nano revolution," which will ultimately define all human endeavours on our planet. Nanoparticles are microscopic molecular assemblages with at least one dimension between 1 and 100 nm in size. They have the potential to significantly advance several fields in modern society, such as electronics, telecommunications, transportation, and medicine [10,11]. However, due to their long-term use, exposure, and environmental accumulation in the air and water, nanomaterials are also poised to cause detectable alterations to our ecosystem. The bottom-up approach to nanomaterial synthesis is more versatile than the top-down methods like chemical and physical processes.

Environmentally safe precursors and solvents were used throughout the synthetic process in green processes, including green chemistry and green nanotechnology. These procedures seek to eliminate harmful compounds from reaction media as well as toxic byproducts that harm our environment [12]. Green nanotechnology aims to eradicate all harmful materials from production processes [13]. The use of plant-derived phytochemicals as reducing and stabilizing agents for the conversion of metal ions into

metallic nanoparticles is a unique aspect of green nanotechnology [14]. 12 widely recognized principles underpin green chemistry and serve as a foundation for creating or enhancing materials, procedures, products, and systems. These principles include: (i) preventing hazardous waste and maximizing the atom economy of the synthetic method, (ii) atom economy of the synthetic route to maximize the final product, (iii) avoiding hazardous chemicals in synthetic routes, (iv) designing biocompatible and safe materials, (v) using environmentally friendly solvents like water, (vi) applying minimal energy to achieve high yields with minimal environmental and economic impacts, (vii) using renewable raw materials or feedstock for sustainability, and (viii) reducing derivatives to limit further stearic acid production. Other principles include designing processes that produce harmless degradation products, conducting life cycle analyses to reduce and prevent environmental contamination, improving production, storage, and transportation efficiency, and increasing overall process effectiveness [15].

Silver nanoparticles (AgNPs) are used extensively in the medical, pharmaceutical, agricultural, and water purification fields. Both chemical and biological methods are used to create these nanoparticles. However, the more complex chemical processes used to create silver nanoparticles often have toxic byproducts, which are harmful to the ecosystem. In contrast, biological synthesis of silver nanoparticles is less time-consuming, less expensive, and more environmentally friendly. As a result, scientists are increasingly exploring potential biological methods for the synthesis of silver nanoparticles [16]. AgNPs are being used in many of devices, from photovoltaics to chemical and biological sensors, due to their unique optical, electrical, and thermal properties. Examples include the use of silver nanoparticles in conductive inks, pastes, and fillers because of their excellent electrical conductivity, stability, and low sintering temperatures. AgNPs are also used in molecular diagnostics and photonic devices. Many textiles, keyboards, wound dressings, and biomedical devices now incorporate silver nanoparticles, which continually release small amounts of silver ions to defend against bacteria. This application of silver nanoparticles is becoming increasingly widespread.

Silver nanoparticles and their compounds have long been recognized for their potent antimicrobial properties, making them widely used in various medical and industrial applications. The bactericidal effects of silver nanoparticles are attributed to their ability to disrupt microbial cellular processes, including membrane integrity, protein function, and DNA replication. With the rise of antibiotic resistance, silver-based antimicrobials have gained renewed interest as potential alternatives or adjuncts to conventional antibiotics.

In the current study, *Stereospermum colais* leaf extract was used to carry out the green synthesis of silver

nanoparticles, which were then examined using UV-Vis spectroscopy, SEM with EDX, and FTIR analysis. The cytotoxicity of the synthesized AgNPs against the MCF-7 breast cancer cell line was determined. Additionally, the nanoparticles were evaluated for antimicrobial properties against *Pseudomonas aeruginosa* and *Streptococcus aureus*.

Sample Collection

The plant samples were collected from IIHR in Hesaraghatta, Bengaluru district, in the months of November and December. The plant was identified as *Stereospermum colais* Mabb and further authenticated by the National Ayurveda Dietetics Research Institute, Bangalore, with a voucher specimen number RRCBI-3840.

Sample Processing and Extraction

The collected leaves were washed thoroughly in running water and shade-dried for 15 days. The dried leaves were pulverized using an electric blender, and the powdered material was stored in airtight containers for further analysis. Approximately 200g of dry, coarsely powdered *Stereospermum colais* samples were subjected to exhaustive extraction in a Soxhlet apparatus using methanol as the solvent. The methanol extracts were concentrated under vacuum using a rotary evaporator, and the solvent-free methanol extracts were stored at 4°C for future use.

Preparation of Bioinspired Silver Nanoparticles

Different concentrations of leaf extract and silver nitrate (20:80, 30:70, 50:50) were monitored for optimum precipitation and absorbance. The best results were observed with a 20:80 ratio of leaf extract to 1 mM silver nitrate solution after 24 hours at room temperature. Absorbance was measured within the UV-visible range (200-800 nm) using UV spectrophotometry. The reduction of silver ions and the formation of silver nanoparticles occurred within one hour of reaction. A control was maintained using AgNO₃ alone. The solution was centrifuged at 5000 rpm for 20 minutes. The resulting brown pellet was washed three times with distilled water and dried at 40°C in an incubator. The novel nanocomposite (ScAgNPs) was further studied for physical and surface characteristics using SEM-EDX, FTIR, DLS, and XRD analysis.

Characterization of ScAgNPs

UV-visible analysis was conducted using the UV-1800 spectrophotometer from Shimadzu, with distilled water as the blank. For morphological examination, the nanoparticles were diluted in distilled water before SEM analysis with the Hitachi S3400N. X-ray diffraction (XRD) analysis was performed on the purified silver nanoparticles using an advanced powder X-ray diffractometer (Bruker, Germany, Type D8) with a scanning range between 10° and 90°. FTIR spectroscopy was employed to characterize the powdered, purified AgNPs. Particle size and stability were determined using dynamic light scattering (DLS)

and zeta potential analysis with a Microtrac, USA model.

Antiproliferative Study

Cell Culture: The MCF-7 cell line was procured from ATCC. Stock cells were cultured in DMEM supplemented with 10% inactivated fetal bovine serum (FBS), penicillin (100 IU/mL), and streptomycin (100 µg/mL) in a humidified atmosphere of 5% CO₂ at 37°C until confluent. The cells were dissociated using 0.05% trypsin and centrifuged at 1000 rpm for 5 minutes. The culture media was discarded, and the cell pellet was gently suspended in 2 mL of complete DMEM media. Cell viability was checked, and a single-cell suspension of 5.0×10⁵ cells/mL was prepared.

For cytotoxicity studies, 32 mg/mL stock solutions were prepared using DMSO. Serial two-fold dilutions were made, ranging from 320 µg/mL to 10 µg/mL, using plain DMEM media for treatment. Vehicle controls were treated with plain media containing 1% DMSO.

Source of Reagents

DMEM, FBS, PenStrep, and trypsin were procured from Invitrogen.

Methodology

To each well of the pre-labelled 96-well microtiter plate, 100 µL of the prepared cell suspension (50,000 cells/well) was added and incubated at 37°C with 5% CO₂. After 24h of incubation, the supernatant was removed and the monolayer was rinsed with plain media. To each pre-designated well, 100 µL of test drugs at various concentrations were added and incubated for 24hrs. After incubation, the test solutions in the wells were discarded and 100 µL of MTT reagent (5 mg/10 mL of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37°C in 5% CO₂. The supernatant was removed and 100 µL of DMSO was added and the plates were gently shaken to solubilize the formazan crystals. The absorbance was measured using a microplate reader at 590 nm wavelength using a multimode plate reader, Spectramax i3X, Molecular devices. The percentage growth inhibition was calculated using the following formula and the concentration of test drug to inhibit cell growth by 50% (IC₅₀) values is generated from the dose-response curves for each cell line using GraphPadPrism 5.0 software. The inhibition was calculated by the formula % Inhibition = ((OD of Control – OD of sample)/OD of Control) x 100. The half maximal inhibitory concentration (IC₅₀) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. IC₅₀ values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist.

Anti microbial study: The organisms were procured from ATCC. The bacterial inoculum was prepared in accordance with the CSLI recommendation where the OD₆₀₀ value was adjusted to the equivalent of

McFarland equivalence turbidity of 0.5 (10^8 CFU /mL), which was confirmed from a calibration curve for each microorganism. Cell suspension prepared from bacterial cultures grown on NB broth and cells were adjusted to $1-2 \times 10^8$ cells/ml. Stocks $320 \mu\text{g}$ was two fold diluted in broth to give $160, 80, 40, 20, 10 \mu\text{g/ml}$ with final concentration DMSO 1%

$90 \mu\text{L}$ of NB broth with the drug was mixed with $100 \mu\text{L}$ of inoculum, the control tube was prepared without the drug. The plates were incubated at 37°C for 24 hours, after which $30 \mu\text{L}$ of 0.015% resazurin was added to each well and further incubated for 2-4 hours to observe color changes.

Results and Discussions

UV- Visible spectrophotometry: The UV-1800 spectrophotometer from Shimadzu was used for the UV-visible analysis. distilled water was used as blank. It was done to obtain the transmission electron diffraction (SAED) pattern. Extract is added to a 1 mM AgNO_3 solution. changed about 15 minutes, Previous reports make the presence more clear AgNPs go from

colourless to yellowish brown. due to the stimulation of surface plasmon vibrations, AgNPs exhibit a yellowish brown colour in solution [25]. At room temperature, it can take up to 75 minutes to 24 hours for the reaction to finish. The rise in temperature may be to blame for this. Smaller nanoparticles eventually form because of the reactants being reacted quickly. The amount of time and total silver reduction in this procedure is less than in past studies. It might be because the synthesised AgNPs' Surface Plasmon Resonance (SPR) was excited [26]. The green synthesis of AgNPs with leaf extract was confirmed by the Surface Plasmon Resonance peak at 430 nm [27]. UV-visible spectrum analysis was used to confirm that AgNPs were formed using a 1 mM solution of AgNO_3 .

For reactions conducted at room temperature, the distinctive Surface Plasmon Resonance band of biogenic AgNPs appears at 430 nm .

With increasing temperature, it is seen that the blue shifts in the peak from 430 nm to 420 nm . This shows that the particle size is reduced at room temperature. (22)

FTIR analysis

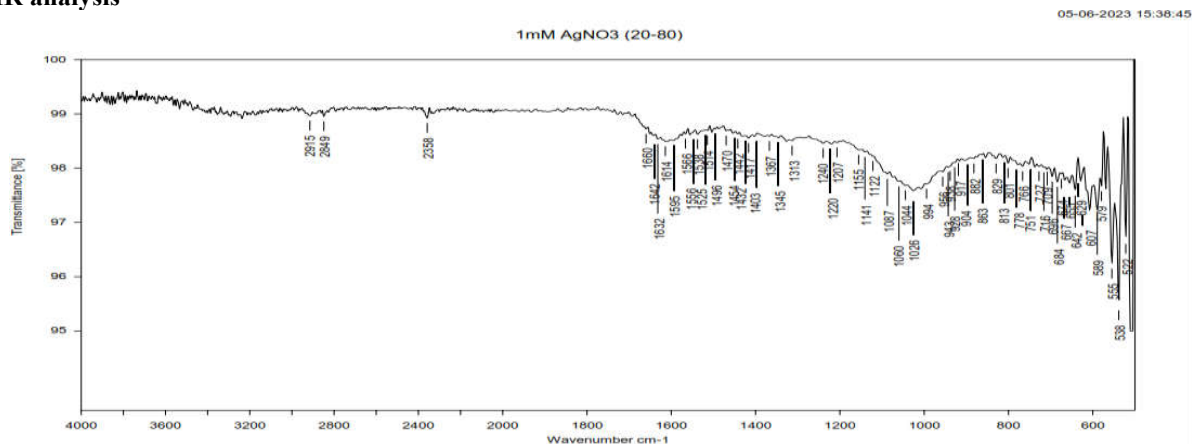


Fig1:FTIR spectrum of the silver NPs

Fig. 1 displays the FTIR measurements that were performed to determine the potential biomolecules in leaf extract that are capping the silver nanoparticles and effectively stabilising them [28]. No peaks at 3470 cm^{-1} and 1640 cm^{-1} in amines from plant proteins, respectively, correlate to N-H stretching and bending vibrations. While the stretching vibrations of the O-H bonds at 3280 cm^{-1} corresponds to alcohols and phenols and the C-H bonds at 2920 cm^{-1} are caused by plant metabolites, the stretching vibrations of the O-H at 2400 cm^{-1} peak and the C=O at 1680 cm^{-1} are caused

by carboxylic acid. Two peaks, 1600 cm^{-1} and 1500 cm^{-1} which are all derived from plant metabolites, are related to C=C stretching vibrations from aromatic rings [29–30]. After bioreduction, the absorption band between 1601 and 1595 cm^{-1} shifts, indicating the creation of AgNPs, which are thereafter sealed off by biomolecules. It demonstrates that the methanol extract of plant played complex roles in the bioreduction of the precursors and the evolution of the nanoparticles' shapes.

XRD

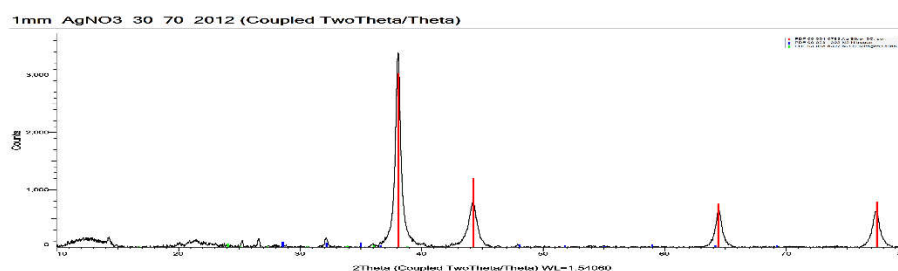


Fig2: XRD pattern of the silver NPs

A typical XRD pattern of the silver NPs as they were created as displayed in Figure 3. The XRD spectrum showed four distinct diffraction peaks at 38.28°, 44.33°, 64.33° and 77.53° corresponding lattice plane value was indexed at (111), (200), (220) and (311) planes of face centered cubic (fcc) silver with a lattice parameter of $a = 4.08 \text{ \AA}$. According to JCPDS's LE no. 4-0783, the XRD pattern's peaks can all be classified as face-centered cubic (fcc) structures. The (111), (200), and

(220) planes' diffraction peaks may be seen in the XRD pattern. Particle size on average The major (111) diffraction peak of the X-ray diffraction pattern was measured using the Scherrer method, and its value was found to be $20 \pm 2 \text{ nm}$. Presence of these peaks was due to seed extract which contains organic compounds and is responsible for the reduction of silver ions and stabilization of resultant nanoparticles.

SEM Analysis of ScAgNPs

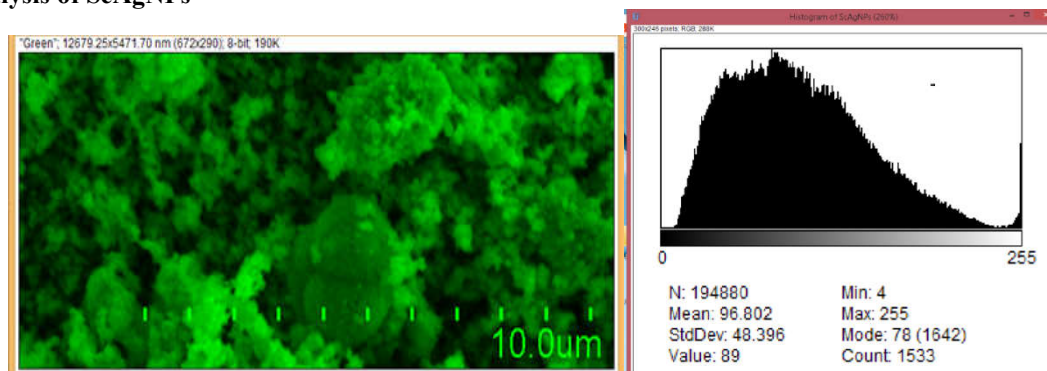


Fig3: SEM image and histogram of ScAgNPs

SEM images of ScAgNPs shows relatively spherical and high density nanoparticles. The size ranges from the diameter of 4-78 nm. The size and shape was analysed by Fiji software. The silver nanoparticles are formed due to the interaction between hydrogen bonds and electrostatic force of attraction of bioorganic capping molecules of *Stereospermum* extract which are bound to Ag molecules and are resulted with ScAgNPs. The aggregated nanoparticles indicates the stabilization of by capping agent(31). Structural studies done by SEM showed the presence of phenolic compounds, amino acids, waxes, antioxidants vitamins, and flavonoids. SEM image and its particular magnified image along with histogram (32)

Cytotoxicity

The MTT assay was used to assess the cytotoxic activity of AgNPs produced using *Stereospermum* leaf extract (Figure 5). The current investigation determined that, after 24 hours, the minimum inhibitory concentration (IC) of AgNPs on MCF-7 cells was $155.4 \mu\text{g/ml}$. On MCF-7 cells, exposure to escalating

AgNP concentrations exhibits dose-dependent cytotoxicity. The findings of a previous study [33] that found that sapium leaves had the greatest cytotoxic effect against the HeLa cell line are consistent with our findings. Antioxidant activity in *Stereospermum* leaves has also been found; given the complicated role antioxidants play in cancer prevention, this could contribute to the observed activity in cancer cell lines [33]. This implies that as the concentration of AgNPs increased, the cytotoxic effects on MCF-7 cells became more pronounced. This dose-response relationship is a common observation in toxicity studies.

IC_{50} values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on a sigmoid dose-response curve (variable) and computed using Graph Pad Prism 5 (Graph pad, SanDiego, CA, USA). The observational data are modelled by a function which is a nonlinear combination of the model parameters and depends on one or more independent variables. The data are fitted by a method of successive approximations.

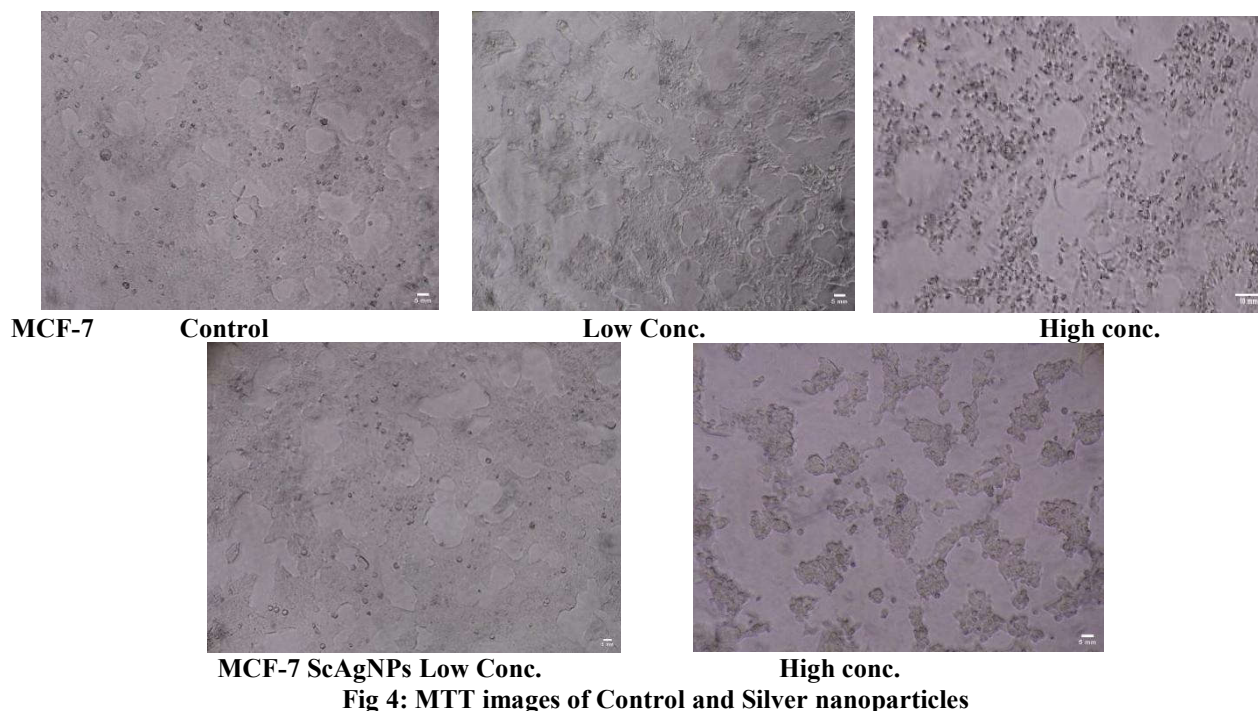


Fig 4: MTT images of Control and Silver nanoparticles

IC50 plots

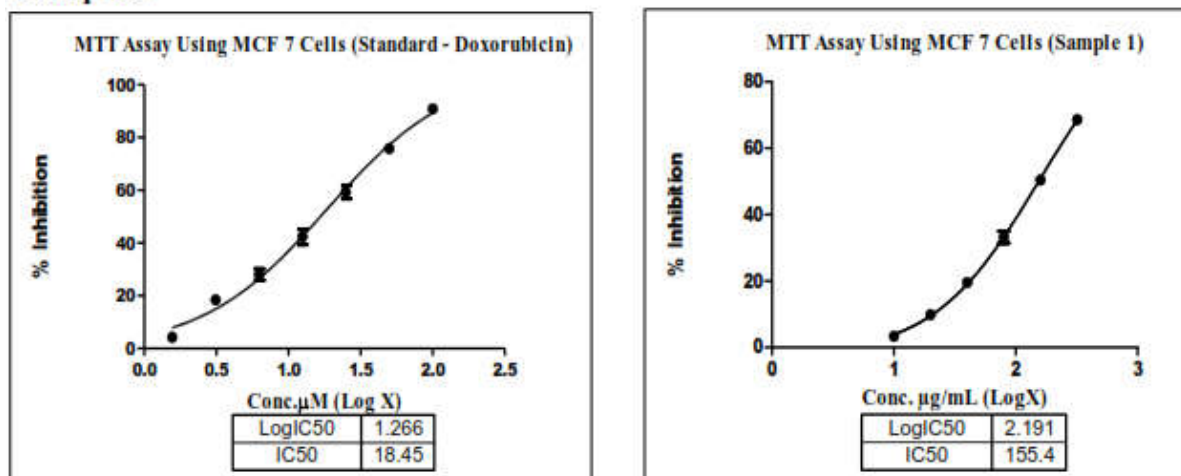


Fig 5: MTT assay showing IC50 values

Antimicrobial results: Both bacterial strains show increasing inhibition with higher AgNO_3 concentrations, *Streptococci* is inhibited more effectively than *Pseudomonas* across the tested concentration range. show a significant positive correlation between concentration and inhibition, with *Streptococci* having a stronger correlation and higher inhibition rate per concentration unit. Statistical analysis revealed a strong

positive correlation between AgNO_3 concentration and % inhibition for both bacteria, with correlation coefficients of 0.92 for *Streptococci* and 0.86 for *Pseudomonas*. Linear regression analysis further demonstrated that *Streptococci* has a higher inhibition rate per unit increase in concentration than *Pseudomonas*, emphasizing its greater sensitivity to AgNO_3 .

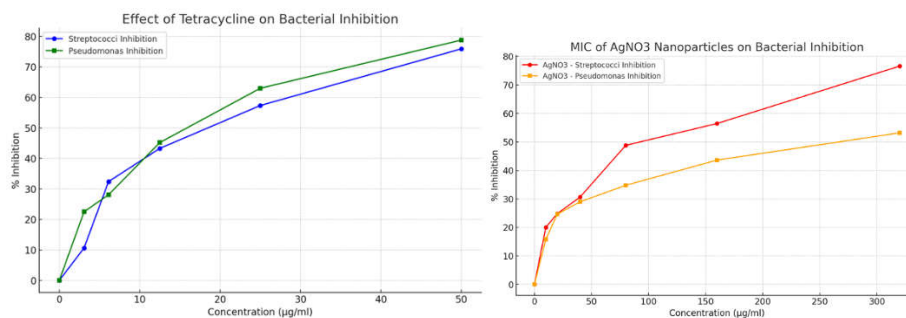


Fig 6: MIC of tetracycline and silver nanoparticles against Pathogens

In summary, the results suggest that AgNPs produced using *Stereospermum* leaf extract exhibit dose-dependent cytotoxicity on MCF-7 cells, with a minimum inhibitory concentration determined. The consistency with a previous study and the mention of antioxidant activity add complexity to the interpretation, prompting further investigation into the specific mechanisms and potential implications for cancer prevention or treatment. The findings suggest that AgNO₃ nanoparticles are more effective antimicrobial agent against gram-positive *Streptococci* compared to gram-negative *Pseudomonas*. These results underscore the potential for AgNO₃ in targeted antibacterial therapies, particularly against pathogens that are resistant to conventional antibiotics.

Conclusions

The biosynthesis of silver nanoparticles using extract from *Stereospermum colais* has proven to be a facile and environmentally friendly process, employing the plant extract as stabilizing, capping, and reducing agents. The characterization of the synthesized nanoparticles through various techniques, including UV/VIS, FTIR, SEM edx, and XRD, has provided comprehensive insights into their nature.

The UV/VIS analysis revealed a distinct surface plasmon resonance (SPR) band at approximately 421 nm, confirming the presence of silver nanoparticles. The FESEM micrograph illustrated the morphology of ScAgNPs, showcasing a mean diameter ranging from 25 to 80 nm.

Importantly, the synthesized ScAgNPs exhibited robust anticancer activity against breast cancer cell lines. The observed cytotoxicity was dose-dependent, indicating the potential for further enhancement of the nanoparticles' effectiveness as a therapeutic agent. ScAgNPs also showed significant potential as antimicrobial agents, especially in combating *Streptococci*. Further research could explore the optimization of nanoparticle formulations and investigate their efficacy against a broader spectrum of pathogens, particularly those resistant to conventional antibiotics. This research highlights the promising application of green synthesis methods for silver nanoparticles and underscores their potential role in the development of effective and eco-friendly anticancer treatments. Further investigations and optimizations can pave the way for the practical implementation of these findings in the field of nanomedicine.

NOTE: The authors have no conflict of interest

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