JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2025/49695
RESEARCH ARTICLE

Optimisation of Photosynthesised Silver Nanoparticles Using Leaf Extract of *Desmostachya bipinnata* for Oral Mucosal Applications

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Abstract

Background: *Desmostachya bipinnata* (Db), a perennial grass of the Poaceae family, is esteemed in traditional medicine for its sacred and therapeutic properties. Rich in bioactive phytochemicals with anti-inflammatory, antimicrobial, and antioxidant activities, Db offers significant potential for biomedical applications. Green synthesis of silver nanoparticles (AgNPs) using plant extracts is a sustainable and eco-friendly method that enhances biocompatibility while reducing toxic chemical use. This study focused on optimising Db-mediated AgNP synthesis using various solvents to enhance phytochemical extraction and nanoparticle efficacy, particularly for oral mucosal applications. **Aim:** To synthesise, characterise, and evaluate the antimicrobial, antioxidant, cytotoxic, and biocompatibility profiles of *Desmostachya bipinnata* silver nanoparticles (Db-AgNPs) synthesised using five different solvents—water, ethanol, methanol, acetone, and chloroform—and to identify the most effective solvent system for biomedical application, especially in oral mucosa. **Methods:** Db-AgNPs were synthesised via green reduction of silver nitrate using Db leaf extracts prepared in each of the five solvents. Nanoparticles were characterised through UV–Visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy (SEM-EDS), and zeta potential analysis. Antimicrobial efficacy was tested using the Minimum Inhibitory Concentration (MIC) assay. Antioxidant potential was assessed using DPPH and nitric oxide radical scavenging assays. Cytotoxicity was evaluated against HeLa cells via MTT assay. Biocompatibility was assessed through hemolysis testing and zebrafish embryo toxicity assays. **Results:** All Db-AgNPs were spherical, averaging ~28 nm in size. Among the tested solvents, ethanol-based Db-AgNPs displayed the highest colloidal stability, strongest antibacterial effect (notably against *E. coli*), and superior antioxidant potential. Ethanol-synthesised Db-AgNPs demonstrated potent cytotoxic effects against HeLa cells at lower concentrations, minimal hemolysis (<2.5%), and no significant embryotoxicity in zebrafish models. **Conclusion:** Ethanol emerged as the optimal solvent for synthesising stable, bioactive, and biocompatible Db-AgNPs. These findings support the development of ethanol-based Db-AgNPs for oral mucosal drug delivery targeting conditions such as oral mucositis, candidiasis, leukoplakia, and Oral Squamous Cell Carcinoma (OSCC).

Major Findings: This study successfully synthesized and characterized silver nanoparticles (Db-AgNPs) from *Desmostachya bipinnata* using five solvents, identifying ethanol as the most effective. Ethanol-based Db-AgNPs showed superior physicochemical stability (28 nm, spherical), antibacterial efficacy, and antioxidant activity. They exhibited significant cytotoxicity against HeLa cells at low concentrations, with minimal hemolysis and no zebrafish embryotoxicity. Characterization confirmed their stability via UV–Vis, FTIR, SEM-EDS, and zeta potential analyses. These findings support ethanol-extracted Db-AgNPs as a promising agent for oral mucosal therapies targeting OSCC and related disorders.

Keywords: Cytotoxicity, Desmostachya bipinnata, Green Synthesis, Hemocompatibility, Oral Mucosa, Silver Nanoparticles

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Article Received on: 04.05.2025 Revised on: 23.06.2025 Accepted on: 05.07.2025

1. Introduction

Introduction of nanoparticles in the field of medical sciences has witnessed a prodigious research development and has produced promising results by their distinct physicochemical, biological, optical and catalytic properties through commercialised drug delivery systems[1,2]. Over the years, metal nanoparticles have been widely recommended for their diagnostic and therapeutic properties, while these nanoparticles, along with potent herbal extract, are extensively used in treating various terminal illnesses, including cancers[3,4]. One such nanoparticles of potent medical application used efficiently are the Silver Nanoparticles (AgNPs) that exhibit remarkable biological activities due to their enhanced anti-microbial, anti-fungal, anti-inflammatory, anti-viral and anti-oncogenic properties. Though there are various methods in the preparation or synthesis of Silver NP's as Ultraviolet radiation, laser ablation, lithography, aerosol technologies and photochemical reductions, the area of focus has largely shifted towards green production of nanoparticles using plants, bacteria, yeast and fungi to reduce environmental hazards[4,5].

Green separation of nanoparticles using whole plant crude extracts or from parts such as rhizomes, roots, leaves, flowers of *Acalypha indica, Allium sativum, Azadirachta indica, Curcuma longa, Hibiscus rosasinensis, Imperata cylindrica, Mentha piperita, Psidium guava, Shorea robusta, Withania somnifera,* and several other herbs has shown promising results owing to their anti-microbial, anti-fungal, anti-inflammatory, anti-oncogenic and anti-viral properties. It has been documented that proteins, phenolic compounds, nitro compounds, carbonyls, aromatics, alkane and alkyl halides, hydroxyl/amines, tannins, and lipid molecules present in plant extract could play a significant role in the reduction and stabilisation of AgNPs. This process of green synthesis of NP's assisted by plant extracts are eco-friendly, clean, cost-effective method and is highly appreciated for its lower bio-hazardous and non-toxic nature as well as for human beings[4-8].

In the cancers of the maxillofacial region, the most common cancer in India remains the Head and Neck Squamous Cell Carcinoma (HNSCC), related to the use of tobacco, alcohol consumption, Human Papillomavirus infections (HPV), radiation, and genetics. Though recent advances in diagnosis and treatment of oral cancer are emerging, but still the quality of life of the patients remains challenging[9,10].

Conventional therapies such as surgery, chemotherapy and radiotherapy are beneficial to some extent, but at times they also exert adverse effects which affect the quality of life of the patients. So, an alternate approach to managing cancer using nanoparticles with phytochemical agents has been carried out ever since the introduction of silver nanoparticles. Traditional medicines using biologically modified active AgNPs can be one of the treatment options to reduce the adverse effects of conventional therapies in the management of oral cancers[11-14].

Studies have revealed that the roots of *Erythrina indica,* leaves of *Eclipta alba, Taraxacum officinale, Musa paradisiaca, Madhuca longifolia, Punica granatum, Vitex negundo,* seeds of *Elettaria cardamomum,* and fruit extract of *Solanum trilobatum* are widely used in human colon cancers, breast cancers and liver cancers for their cytotoxicity towards various experimental cell lines, cell growth inhibition, induction of apoptosis, cell death triggering activity, inhibiting mitochondrial damage and several biologically active or mediated pathways[14-16].

Kusha Grass is known for its medicinal properties and can be used to synthesise silver nanoparticles. Its bioactive compounds, such as polyphenols, flavonoids, terpenoids, and alkaloids, make it an excellent reducing agent for silver ions. *Desmostachya bipinnata* (Poaceae) has been reported to possess phytochemicals with reducing properties, which can effectively facilitate the reduction of silver ions and stabilisation of the resulting nanoparticles. These phytochemicals act as reducing agents by donating electrons to the silver ions, leading to the formation of silver nanoparticles. Additionally, biomolecules such as proteins and polysaccharides present in the plant extract can act as capping agents, prevent the agglomeration and stabilise the synthesised nanoparticles. The use of *Desmostachya bipinnata* as a reducing agent for silver nanoparticles offers several advantages, including its abundance, cost-effectiveness, and eco-friendly nature. Moreover, utilising plant extracts for nanoparticle synthesis aligns with the principles of green chemistry, promoting sustainable practices in nanotechnology[17].

In recent times, *Desmostachya bipinnata* (Db), one of the predominant native plants of Tropical regions,

familiarly known as Dharba grass, Halfa grass, big-cord grass, or salt-reed grass, considered as a sacred grass in the Vedic era, has been identified as a potent chemoprotective agent in gastrointestinal diseases. The long, acute, and linear leaves of this plant are used in traditional medicine as diuretics, anti-diarrheal, analgesic and anti-inflammatory agents[18]. Researchers have demonstrated hepato-protective activity in treating dysentery, jaundice, and menorrhagia and also have shown antioxidant effect *in vivo* by protecting yeast cells against oxidative stress, suggesting scavenging activity in a dose-dependent manner. They have also proven effective anti-cancer activity with minimal or no adverse effects[18-20].

Hence, the present study was undertaken to evaluate the anti-tumorigenic potential of *Desmostachya bipinnata* (Db) grass in the therapeutic management of oral lesions. To achieve this, the synthesis of silver nanoparticles (AgNPs) using Db extract was systematically optimised to ensure reproducibility, efficiency, biocompatibility, and enhanced therapeutic performance. Given the complex phytochemical profile of plant extracts—comprising flavonoids, phenolics, tannins, and alkaloids—whose solubility and functional activity are influenced by solvent polarity, it is crucial to determine the most suitable extraction medium. Optimisation allows for the identification of the ideal solvent system that yields nanoparticles with optimal size, stability, and bioactivity. Without this step, the synthesis process may result in variability, poor efficacy, or increased cytotoxicity, particularly detrimental in applications involving delicate tissues like the oral mucosa. By comparing solvents of varying polarity, this study aimed to develop a safe, scalable, and reproducible nanoparticle formulation tailored for biomedical applications, especially those requiring localised, site-specific delivery with minimal adverse effects.

2. Materials and Methods

2.1 Selection and Collection of Plant Material

Fresh leaves of *Desmostachya bipinnata* (Db) were collected from the banks of the Cauvery River in Tiruchirapalli, India. The botanical identification of the plant was carried out and authenticated by Dr. Lakshmi Narasimhan, based on the descriptions provided in the *Flora of the Presidency of Madras.*

2.2 Preparation of *Desmostachya bipinnata* (Db) Extract and Synthesis of Silver Nanoparticles

Fresh leaves of Db were thoroughly washed under running tap water to remove surface impurities such as dirt, mud, and other contaminants. The cleaned leaves were then air-dried at room temperature for 7 to 10 days. Once adequately dried, the leaves were cut into small pieces. After one week, 25.0 grams of the dried leaf material was accurately weighed using an electronic balance and set aside for extraction and subsequent analyses. For solvent extraction, 5.0 grams of the dried leaf powder was added separately to 100 mL each of different solvents—distilled water, ethanol, methanol, chloroform, and acetone. The mixtures were heated to a temperature range of 60–70 °C until they reached the boiling point. Each extract was then filtered through Whatman No.1 filter paper to obtain the clear DB solvent infiltrate.

To prepare the silver nanoparticle synthesis medium, 0.08 grams of silver nitrate (Sigma-Aldrich, Merck, USA) was dissolved in 50 mL of distilled water. Subsequently, 5 mL of each Db solvent extract was added individually to the silver nitrate solution. A visible colour change was observed, indicating the onset of nanoparticle formation. The reaction mixtures were incubated overnight and evaluated after 24 hours. A characteristic brown colouration developed in each sample, confirming the biosynthesis of silver nanoparticles through the reduction of Ag^{+} ions to elemental silver (Ag^{0}) by phytochemicals present in the Db extract.

The pH of the reaction mixtures during the initial stages of synthesis ranged between 6.1 and 6.5. Upon completion of the biosynthesis process, the pH increased slightly, stabilising between 7.2 and 7.5, which is conducive to nanoparticle formation.

2.3 Physico-Chemical Characterisation of Synthesised AgNPs

2.3.1 Ultraviolet–Visible Spectroscopy

The formation of silver nanoparticles was confirmed by UV-Visible spectroscopy. UV-Vis estimates the elimination of light passing through a sample.

Nanoparticles have distinctive optical properties that are sensitive to size, form, absorption, agglomeration state and refractive index near the nanoparticle surface, which makes UV –Vis a valued tool for identifying, illustrating and studying nanomaterials. The DB-based silver Nanoparticles of various solvents (Water, ethanol, methanol, acetone and chloroform) absorption under visible light was measured. The process of Ag NP's synthesis is a colored reaction and shows strong and sharp absorption bands under the visible light region in the range of 400-500nm.

2.3.2 Fourier Transmission Infrared Spectroscopy (FTIR)

FTIR is used to measure the AgNPs plant extract particle formation, also showing that the width and intensity of peaks in the spectrum will depend on the particle size. FTIR shows that when there is an increase in particle size, the width of the peak decreases, and there is also an increase in the intensity. The DB-based silver nanoparticles of various solvents (Water, ethanol, methanol, acetone and chloroform) were analysed for the functional group identification present in the extract and the possible interaction between the AgNP and biomolecule. Here, the DB-based AgNP of various solvents were dried at 80°C and crushed with anhydrous potassium bromide to form a pellet and characterised in FTIR at absorption frequency from 4000cm^{-1} to 400 cm^{-1} using Perkin Elmer Spectrum 2 model (PES-2).

2.3.3 SEM-EDS, Or Scanning Electron Microscopy Combined with Energy Dispersive X-Ray Spectroscopy

A concentrated beam of electrons with high energy is utilised by the SEM (Scanning Electron Microscope) to generate a variety of signals at the surface of a solid object. Information about the sample, such as its exterior morphology (texture), chemical makeup, and the crystalline structure and orientation of its constituent materials, can be gleaned from the signals produced by electron-plant extract solvent sample interactions. The systematic method known as X-ray spectroscopy with energy dispersive (EDS, EDX, or XEDS) enables the elemental analysis and chemical characterisation of constituents. When an energy source excites a model, a core-shell electron is released, which releases some of the absorbed energy. This allows for the compositional analysis of a given plant extract solvent sample volume that has been excited by the energy source. The SEM-EDS of various solvents (water, ethanol, methanol, chloroform and acetone), DB AgNP of solvents was carried out using Thermo-Fischer Scientific Helios Dual Beam.

2.3.4 Zeta potential of Db AgNP

The stability and charge of the nanoparticles were measured using Horiba SZ-100-Z. Zeta potential of DbAgNP suspension using dynamic light scattering techniques.

2.4 Biological Characterisation of Synthesised *Db* AgNPs

2.4.1 Minimal Inhibition Concentration Antibiotic Assay (MIC)

The superficial layer of a nutrient agar medium with a predetermined concentration gradient of the antibiotic tetracycline was utilised for a qualitative assessment of DbAgNPs. On a different agar plate, *Db* silver nanoparticles were evenly distributed in 100 µl of suspensions of water, ethanol, methanol, acetone, and chloroform. To maintain the cell density (CFU/mL), the bacterial inocula *Escherichia coli* (*E. coli*), *Enterococcus faecalis* (*E. faecalis*), *Streptococcus mutans* (*S. mutans*), and *Staphylococcus aureus* (*S. aureus*) were added separately to the solid nutritional agar media with the antibiotic within 30 minutes of this preparation. Each inoculum had a concentration of 108 CFU/ml. The petri plates are incubated at 35 ± 1 °C in a thermostat for 24 hours and observed respectively with all the suspensions containing nutrient medium covering silver NPs stabilised with oleic acid in different concentrations (0.5, 1.0, 1.5, 2.0, 4.0, 6.0 and 8.0 µg/ml). Similarly, the nutrient medium without nano-silver applied with bacterial suspension was kept as a control (0 µg/ml of silver). Gallic acid was kept as a control for Db.

2.4.2 Biofilm Growth and Inhibition Analysis

Ethanolic extracts of *Db* were evaluated for their effect on biofilm formation. The extracts were sterilised and individually introduced into sterile test tubes containing 3000 µL of Luria-Bertani (LB) broth. The assay was conducted in triplicate. To each tube, 5 µL of *E. coli* culture, adjusted to a concentration of 1×10^5

CFU/mL, was added to initiate biofilm development. The DB extract-inoculated cultures were incubated under standard conditions for 24 hours.

Post-incubation, the cultures were washed twice with 1 mL of sterile Phosphate-Buffered Saline (PBS) to eliminate non-adherent bacteria. Coverslips were then carefully retrieved for Scanning Electron Microscopy (SEM) analysis. These were fixed with 4% glutaraldehyde at 37 °C for 15 minutes, rinsed with PBS to remove residual fixative, and air-dried for an additional 15–20 minutes. The dried coverslips were then sputter-coated with platinum and visualised under SEM to assess biofilm morphology and structural integrity. The extent of biofilm inhibition was evaluated based on morphological damage to bacterial cells and disruption of biofilm architecture observed under SEM.

2.4.3 Nitric Oxide Scavenging Assay

The antioxidant potential of DbAgNPs synthesised using various solvents—water, ethanol, methanol, acetone, and chloroform—was assessed via *in vitro* Nitric Oxide (NO) radical inhibition. Gallic acid served as the standard antioxidant reference. The study aimed to evaluate the dose-dependent nitric oxide scavenging activity of freshly prepared Db-AgNP suspensions.

A 5 mmol/L solution of sodium nitroprusside was prepared in Phosphate-Buffered Saline (PBS, pH 7.4) and incubated with varying concentrations of Db-AgNP suspensions (derived from a 10 mg/mL stock solution prepared in each solvent). Reaction mixtures were incubated at 25 °C for 30 minutes. A control sample containing only distilled water (no extract) was maintained under identical conditions.

Following incubation, 1.5 mL of each reaction mixture was mixed with 1.5 mL of freshly prepared Griess reagent, consisting of: 1% sulphanilamide, 2% phosphoric acid, 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride. The formation of a chromophore due to the diazotisation reaction was measured spectrophotometrically at 546 nm. The nitric oxide scavenging activity was calculated as a percentage of inhibition compared to the control using the following formula:

$$Nitric\ oxide\ scavenging\ activity(\%) = \left(\frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}}\right) \times 100$$

This assay provided insights into the solvent-dependent antioxidant efficacy of the Db-AgNP derivatives, with results demonstrating a dose-dependent increase in nitric oxide scavenging potential.

2.4.4 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Inhibition Assay

The antioxidant potential of *Desmostachya bipinnata* (Db) extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Extracts prepared in various solvents—water, ethanol, methanol, acetone, and chloroform—were assessed for their free radical scavenging activity. In addition, silver nanoparticle (AgNP) derivatives of Db extracts were also analysed. To prepare the DPPH working solution, 24 mg of DPPH powder was dissolved in methanol and filtered to obtain a stable solution with an absorbance of 0.955 at 525 nm. The working solution was used immediately to maintain radical stability.

Each test consisted of 100 µL of the respective Db extract suspension (from a stock prepared in 100 mL of each solvent), which was mixed with 3 mL of the DPPH working solution and made up to a final volume of 100 mL. A standard control was prepared using 3 mL of DPPH solution with 100 µL of methanol, and gallic acid served as the positive reference standard.

The reaction mixtures were incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 525 nm using a UV–Visible spectrophotometer. The percentage of DPPH radical scavenging activity (RSA%) was calculated using the following formula:

$$RSA(\%) = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right) \times 100$$

Where:

- **Abs_{control}** is the absorbance of the DPPH solution without extract
- **Abs_{sample}** is the absorbance of the DPPH solution with extract

This assay provided comparative insight into the solvent-specific antioxidant efficacy of the Db extracts and their nanoparticle-conjugated forms.

2.4.5 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) Cytotoxic Assay

The effectiveness of treatment was assessed by measuring cell viability across different solvent-based extracts (ethanol, methanol, water, acetone, and chloroform) using the Cell Survival Determination (CSD) method. HeLa cells were seeded in 96-well plates at a density of 1×10^5 cells/well and incubated at 37°C in a humidified atmosphere with 5% CO_2 until reaching confluence. Once confluent, the cells were treated with UV-irradiated extract samples and incubated for periods ranging from 1 to 3 days.

Following the incubation, the treatment medium was carefully removed, and the wells were rinsed with phosphate-buffered saline (PBS, pH 7.4) or serum-free DMEM to eliminate residual substances. A volume of 10 µL per well of MTT solution (0.5%, 5 mg/mL) was then added, and the plates were incubated for an additional 4 hours to allow for the formation of formazan crystals by metabolically active cells.

Subsequently, 100 µL of dimethyl sulfoxide (DMSO) was added to each well to solubilise the formed formazan crystals. The solution was gently mixed by pipetting to ensure complete dissolution. Absorbance was then recorded at 570 nm using a UV spectrophotometer, with DMSO serving as the blank control. The IC_{50} value, representing the concentration of the extract required to inhibit 50% of cell viability, was derived graphically.

Cell viability was calculated using the formula: % Cell Viability = (Absorbance of Treated Cells / Absorbance of Control Cells) × 100

Results were plotted with the percentage of cell viability on the Y-axis and the concentration of the test sample on the X-axis. Appropriate controls, including untreated cells and solvent-only samples, were included in each assay to ensure accurate assessment of cytotoxic effects.

2.4.6 Hemolysis Assay

The hemolysis assay offers a valuable initial screening tool for evaluating the endosomolytic activity of candidate cytosolic delivery agents using naturally occurring biomembranes. However, as noted by B.C. Evans *et al.,* it should be regarded as one component of a broader analytical framework for assessing intracellular drug delivery mechanisms.

In this study, 5 mL of peripheral blood was collected from healthy adult volunteers under aseptic conditions. To prevent coagulation, Ethylenediaminetetraacetic Acid (EDTA) was added at a final concentration of 1.6 g/L of whole blood. The samples were then centrifuged at 2000 rpm for 10 minutes at 4 °C to isolate erythrocytes (red blood cells, RBCs). The separated RBCs were washed thoroughly with phosphate-buffered saline (PBS, pH 7.4) to remove plasma and other cellular debris. After multiple washes, the RBCs were resuspended in PBS to achieve the required concentration for the hemolysis assay.

For assay setup, 950 µL of distilled water was mixed with 50 µL of the RBC suspension to serve as the positive control, representing 100% hemolysis. The negative control consisted of 950 µL of PBS mixed with 50 µL of RBCs, indicating baseline or minimal hemolysis. The experimental groups were prepared by adding predetermined concentrations of the test sample to 1 mL aliquots of the RBC suspension in PBS. All tubes were incubated at 37 °C for 1 hour with gentle agitation to ensure uniform exposure.

Post incubation, samples were centrifuged again at 2000 rpm for 10 minutes. The supernatants were carefully collected, and the extent of hemolysis was quantified by measuring the absorbance at 540 nm using a UV–Visible spectrophotometer. The degree of haemoglobin release, indicative of membrane disruption, was calculated relative to the positive control.

The percentage of hemolysis was calculated using the formula:

Hemolysis (%) = [(Absorbance of Sample – Absorbance of Negative Control) / (Absorbance of Positive Control – Absorbance of Negative Control)] × 100

2.4.7 In Vitro Cytotoxicity Assay

Zebrafish (*Danio rerio*) are widely used in toxicological and biomedical research due to their high genetic homology with humans—approximately 70% of human genes have orthologs in the zebrafish genome. This genetic conservation, combined with transparent embryonic development and rapid organogenesis, makes zebrafish a valuable and cost-effective model for evaluating cytotoxic effects and exploring molecular mechanisms of toxicity.

The cytotoxic potential of the ethanolic extract of Db-AgNPs was evaluated using zebrafish embryos following the OECD Test Guideline 203. Six fertilised zebrafish eggs per group were exposed to different concentrations of Db-AgNPs (10, 20, 30, 50, 75, and 100 µg/mL). Each group of embryos was carefully transferred into 100 mL of Hank's balanced salt solution containing the respective concentration of the test compound. The embryos were then individually placed in multi-well plates for developmental monitoring.

Embryonic development was assessed at 24-hour intervals under a compound microscope (40× magnification). Morphological parameters, including the development of the tail, head, liver, and eyes, were recorded. The incubation temperature was maintained at 24 °C throughout the study to ensure optimal embryonic development.

The primary endpoints included: Embryo mortality rate; Time to hatching; Morphological abnormalities; Survival rate post-hatching. Care was taken to prevent contamination, ensuring that dead embryos were promptly removed to avoid cross-interference with viable specimens. This assay provided insight into the dose-dependent embryotoxicity of the Db-AgNPs, contributing to the safety evaluation of the synthesised nanoparticles for biomedical applications.

3. Results and Discussion

3.1 Physico-Chemical Characterisation of Synthesised AgNPs

3.1.1 UV VIS Spectrophotometry

The synthesis of AgNPs using *Db* leaf extract in various solvents—water, ethanol, methanol, acetone, and chloroform—was confirmed through UV–Visible spectroscopy. AgNP formation is typically associated with a colour change due to Surface Plasmon Resonance (SPR), which results in strong and distinct absorption bands in the visible light region, particularly between 400–500 nm. In the present study, all solvent-based Db-AgNPs exhibited a prominent SPR peak around 420 nm, indicative of successful nanoparticle formation. The broadening of this peak further suggested a polydisperse nature of the synthesised nanoparticles (Figure 1).

Maximum reduction of Ag^+ ions was observed approximately 3 hours after the addition of the Db extract to the silver nitrate solution, with ethanol-based Db-AgNPs showing the most pronounced absorbance at 420 nm. This enhanced peak intensity in the ethanolic extract reflects its superior efficiency in nanoparticle synthesis, attributed to its better solubility profile and phytochemical interaction, which facilitate particle formation, stabilisation, and reactivity of the resulting biopolymer.

These findings agree with Kumar *et al.,* .[18], who reported a UV–vis SPR peak at 433 nm for AgNPs, confirming their formation. Similarly, studies by Ramadan MA and Safwat NA[19], Awaad AS *et al.,* .[20], and Mohanapriya P *et al..*[21] demonstrated SPR peaks ranging between 413–560 nm for plant-derived nanoparticle systems. The SPR profile observed in the present study aligns with previously reported plant-based extracts such as *Eclipta alba*, *Taraxacum officinale*, *Musa paradisiaca*, *Madhuca longifolia*, *Punica granatum*, and *Vitex negundo*, which have been explored for anticancer applications in liver, colon, and breast cancers.

The stability assay was done on the 1st, 3rd, 5th and 7th day, which showed not much significant changes over time, which indicates that the sample shows good stability without degradation or aggregation (all the samples showed their reference peak around 420nm) (Figure 2).

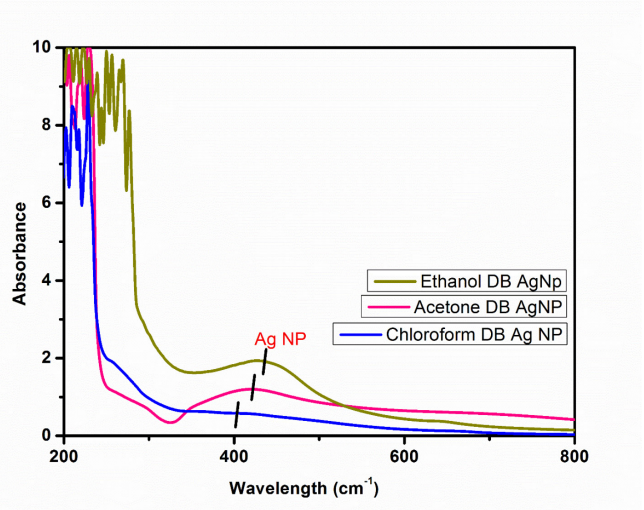


Figure 1. Ultraviolet spectra representing the formation of Silver Nanoparticles in various solvents of *Desmostachya bipinnata*.

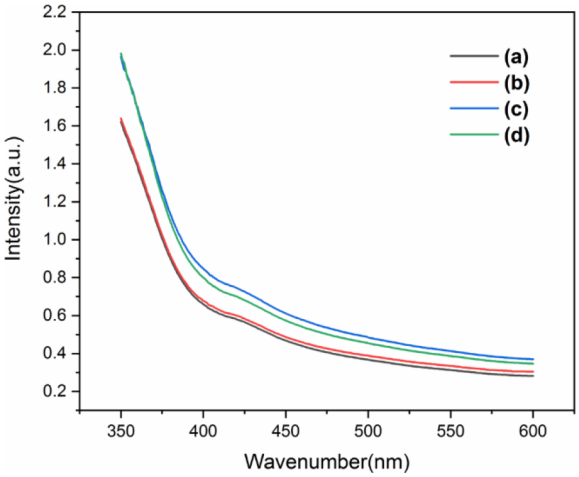


Figure 2. Stability assay of *Db* AgNPs on various time intervals (a-Day-1; b-Day-2; c-Day-3; d-Day 4).

3.1.2 FTIR (Fourier Transform Infrared Spectroscopy)

The *DB* plant extract of various solvents (Water, ethanol, methanol, acetone and chloroform) was analysed for the particle size, width and intensity of peaks that determine the possible interaction and stabilisation of the Ag nanoparticles. The FTIR measurements of the Db solvent extract showed absorption peaks at 3435.97 cm $^{-1}$ and 3467.7 cm $^{-1}$ characteristic to hydroxyl functional group in alcohol and phenols, peaks at 2354.4 cm $^{-1}$ and 1635.12 cm $^{-1}$ exhibited to carbonyl functional group in unsaturated aromatic carboxylic acids (C=O) and peaks at 3467.7 cm $^{-1}$ and 3435.92 cm $^{-1}$ exhibited to Alkanes/Alkyl groups (CH) respectively in agreement to Guntur SR *et al.*., indicating the availability of represented functional groups (Figure 3).

The FTIR spectra of synthesized AgNPs at different solvents by Db extract leaves showed the alcohol and phenols AgNPs group absorption peaks at 1046.27 cm $^{-1}$ and 1045.40 cm $^{-1}$ exhibited to O-H group from sugar alcohols, peaks at 1638.4 cm $^{-1}$ and 1638.20 cm $^{-1}$ exhibited to carbonyl functional group in unsaturated aromatic carboxylic acids (C=O) and peaks at 3467.7 cm $^{-1}$ and 3435.92 cm $^{-1}$ exhibited to Alkanes/Alkyl groups (CH) respectively. In the ketonic group (Acetone) and Chloroform AgNPs group absorption peaks at 1092.12 cm $^{-1}$ and 1015.03 cm $^{-1}$ exhibited to O-H group from sugar alcohols, peak at 1635.12 cm $^{-1}$ exhibited to carbonyl functional group in unsaturated aromatic carboxylic acids (C=O) and peaks at 3455.61 cm $^{-1}$ and 3435.97 cm $^{-1}$ exhibited to Alkanes/Alkyl groups (CH) respectively. Guntur SR *et al.*,[12] showed FTIR peaks at

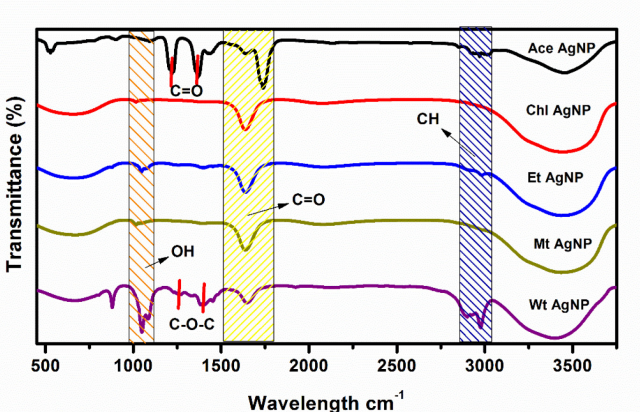


Figure 3. Fourier Transmission Infrared Spectroscopy (FTIR) spectra of various solvents of *Db*-based silver nanoparticles

1587 cm^{-1} for C-C, 1055 cm^{-1} represented the C–O and C–H stretching bonds corresponding to the presence of functional amines, alcohols, phenols and alkanes, like the present study.

3.1.3 Scanning Electron Microscopy- Energy Dispersive X-Ray Spectroscopy (SEM-EDS)

Scanning Electron Microscopy (SEM) was employed to characterise the surface morphology, particle size, and distribution of silver nanoparticles (AgNPs) synthesised using *Desmostachya bipinnata* (Db) leaf extract. SEM utilises a high-energy electron beam that is scattered over the surface of a solid sample, producing high-resolution images. The acceleration of electrons by an applied electric potential shortens their wavelength relative to photons, thereby enhancing image resolution and depth of field.

SEM micrographs (Figure 4A–E) revealed that the biosynthesised Db-AgNPs were predominantly spherical, with particle sizes ranging from 20 to 30 nm and an average diameter of approximately 28 nm. These morphological features confirm the successful formation of well-dispersed nanosized particles. Comparable findings were reported by Guntur SR *et al.*, where physicochemical analysis indicated uniformly round-shaped AgNPs with an average diameter of 53 nm, within a range of 30–60 nm. The SEM spectrum in the current study also affirmed the crystalline nature and nanoscale size of the synthesised particles, consistent with prior studies involving other plant leaf-mediated AgNPs. Such characteristics further support the applicability of Db-derived AgNPs in biomedical and therapeutic applications.

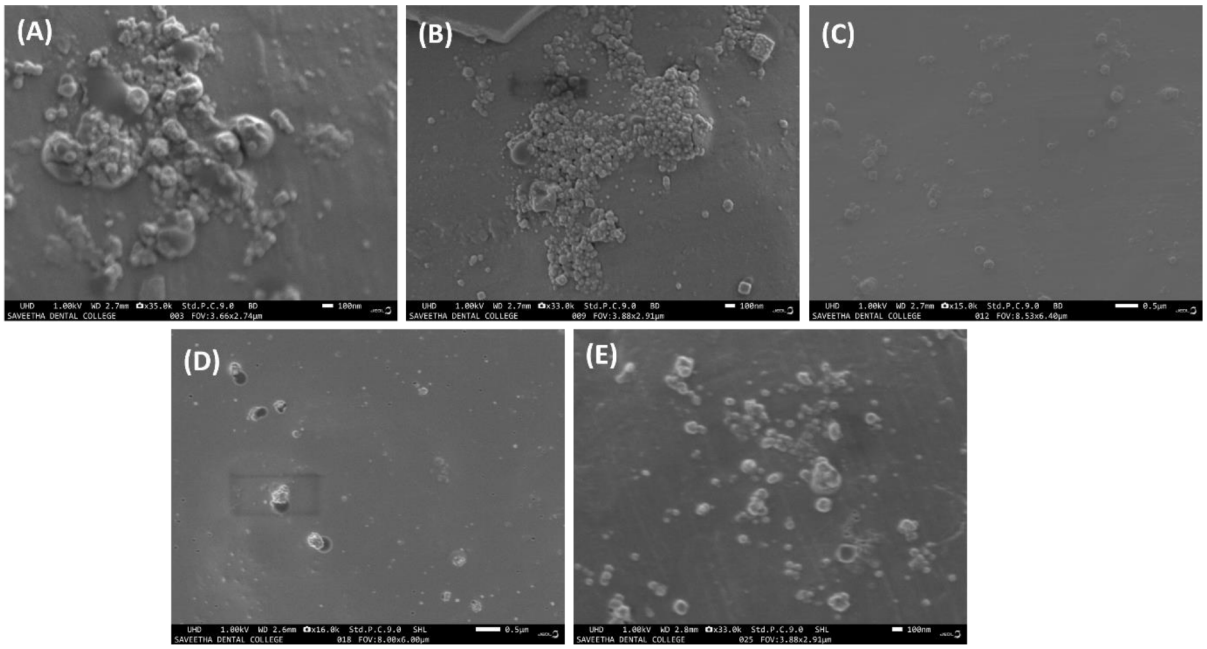


Figure 4. Scanning Electron Microscopy of *Desmostachya bipinnata*-based Silver Nanoparticles of various solvents. A) Water; B) Ethanol; C) Methanol; D) Chloroform; E) Acetone.

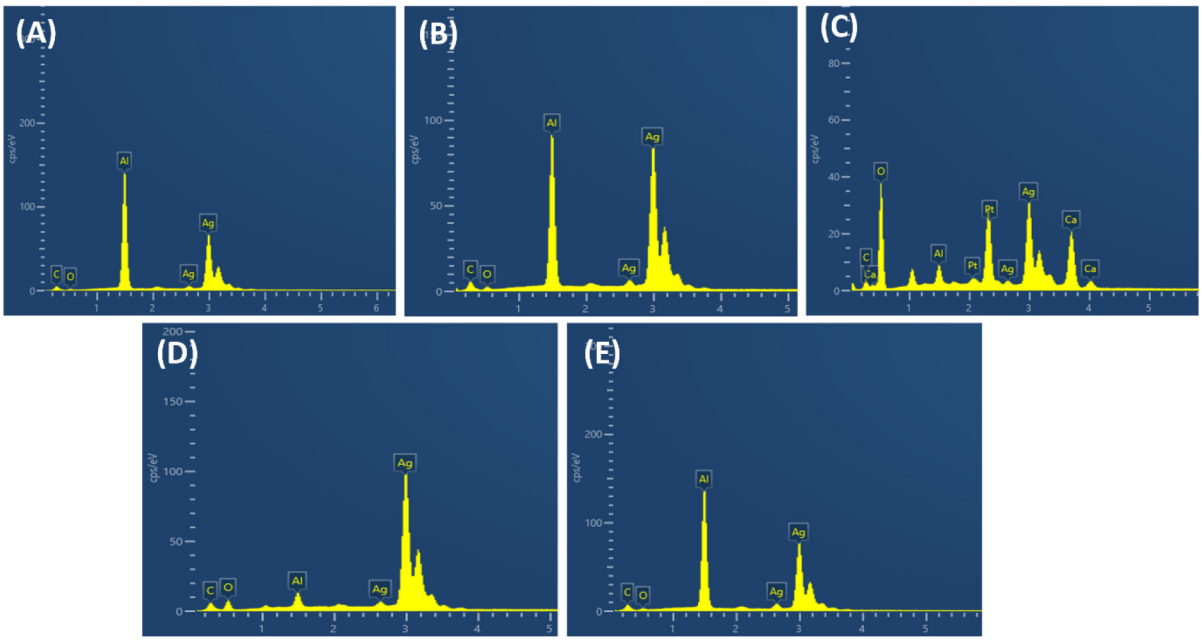


Figure 5. Energy Dispersive X-Ray Analysis of various solvents of *Desmostachya bipinnata* based Silver Nanoparticles (Db AgNPs). A) Water; B) Ethanol; C) Methanol; D) Chloroform; E) Acetone.

Elemental composition of the synthesised silver nanoparticles (AgNPs) was determined using Energy-Dispersive X-ray Spectroscopy (EDS/EDX) integrated with Scanning Electron Microscopy (SEM). The SEM-EDS/EDX analysis was performed on AgNPs synthesised using Db leaf extract as a bioreducing and stabilising agent in different solvents—water, ethanol, methanol, acetone, and chloroform.

The EDS/EDX spectra confirmed the presence of elemental silver as the major constituent in all samples, validating the successful formation of AgNPs (Figure 5A–E). Characteristic strong signals corresponding

to silver were observed in the spectra of spherical nanoparticles, indicating their metallic nature. Minor peaks corresponding to carbon and oxygen were also detected, which could be attributed to the presence of phytochemicals from the Db extract capping the nanoparticles. These findings confirm the elemental purity and the role of the plant extract in the biosynthesis and stabilisation of silver nanoparticles across different solvent systems.

The synthesised silver nanoparticles (AgNPs) using *Desmostachya bipinnata* (Db) extract were observed to be predominantly spherical, with particle sizes ranging from 20 to 30 nm and an average diameter of approximately 28 nm, as confirmed by SEM analysis. Energy-dispersive X-ray spectroscopy (EDS/EDX) revealed strong elemental signals corresponding to silver (Ag) and aluminium (Al), along with weaker signals for carbon (C), oxygen (O), calcium (Ca), and platinum (Pt).

The presence of Ag confirmed successful nanoparticle synthesis, while the detection of carbon and oxygen is likely attributable to organic phytoconstituents from the Db extract, which act as natural reducing and capping agents. This is consistent with the findings of Guntur SR *et al.*, .[12], who similarly reported the presence of carbon and oxygen alongside silver in green-synthesised AgNPs, reflecting the diverse phytochemical composition and associated physicochemical properties of plant-derived nanoparticles.

Interestingly, the detection of aluminium (Al) and trace amounts of platinum (Pt) suggests the involvement of unrefined bioactive fractions within the Db extract, potentially functioning as stabilising and reducing mediators during nanoparticle formation. Previous studies have indicated that platinum traces in plant-based nanoparticle systems can enhance thermal conductivity, catalytic activity, and structural stability of the resulting nanoparticles. These findings collectively reinforce the multifunctional role of *Desmostachya bipinnata* in the green synthesis of bio-functionalized, stable AgNPs with potential biomedical and catalytic applications[12,16-18].

3.1.4 Zeta potential of Db Ag NP

The zeta potential of the ethanolic extract of DbAgNP was determined as -13mV, which shows that it may be susceptible to aggregation and precipitation.

Over time, particularly in solutions with electrolytes or other destabilising factors (Figure 6). This result is almost similar to that of Edison *et al.* (2012), stating that its specific surface chemistry of the AgNPs, including any capping agents or surface modifiers derived from plants, can influence the zeta potential. Functional groups present on the surface may contribute to the observed negative charge.

3.2 Biological Characterisation of Synthesised *Db* AgNPs

3.2.1 Minimal Inhibition Concentration Antibiotic Assay (MIC)

For a qualitative estimation of *Db* silver NPs, on the surface of a nutrient agar medium containing a predefined concentration gradient of tetracycline antibiotic, the bacterial inoculum *E.coli, E. fecalis (Entero spp) and S. mutans, S. aureus* with 108 CFU/ml concentration was added individually to the solid nutrient agar media with the antibiotic to maintain the cell density (CFU/mL). The petri plates are incubated at 35 ± 1 °C in a thermostat for 24 hours and observed respectively with all the suspensions containing nutrient medium covering silver NPs stabilised with oleic acid in different concentrations (0.5, 1.0, 1.5, 2.0, 4.0, 6.0 and 8.0 µg/ml). It was observed that in the control sample (0 µg/ml of silver), bacterial growth was quite intensive, and as the concentration of the *Db*AgNPs increased, the efficiency of suppressing the microorganism growth increased. After 24 hours at increasing concentration

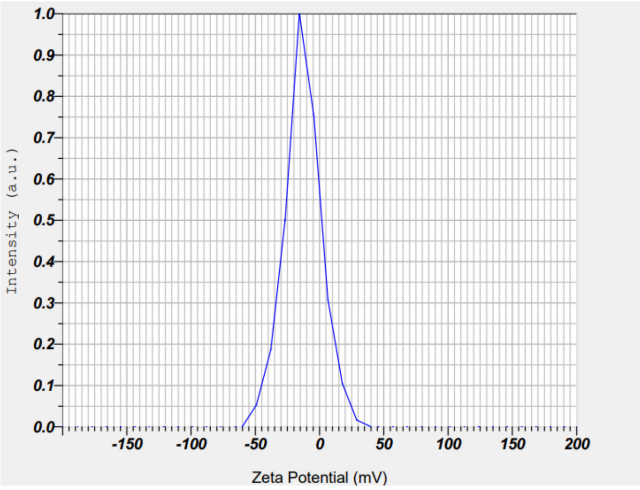


Figure 6. Zeta potential of *Desmostachya bipinnata* Silver Nanoparticles.

(0.5, 1.0, 1.5, 2.0), observation of the % (percentage) of inhibition at *E. coli* reached maximum of 79% in ethanol suspension followed by water (76%) with least being Methanol (71%) at 0.5 µg/ml of *Db* AgNPs. When the silver concentration reached 1 µg/ml, almost a total suppression of *E. coli* growth was observed in ethanol cell suspension (100%). In case of *E. fecalis* observation, the % (percentage of inhibition at *E. fecalis* reached a maximum of 72% in ethanol suspension, followed by water (68%), with the least being Methanol (61%) at 0.5 µg/ml of *Db*AgNPs. When the silver concentration reached 1.5 µg/ml, almost a total suppression of *E. fecalis* growth was observed in ethanol cell suspension (100%) with a mean zone of inhibition ranging between 6mm to 12mm (Figure 7).

In the case of S. aureus, ethanol suspension reached maximum inhibition of 81% followed by water (77%) and Chloroform (71%) at 1 µg/ml and the minimum inhibition concentration of *Db* silver NPs for the stabilization of nanosilver was 2 µg/ml where as in *S. mutan*s ethanol suspension reached maximum inhibition of 85% followed by water (81%) and Chloroform (77%) at 1 µg/ml and the minimum inhibition concentration of *Db* silver NPs for the stabilization of nanosilver was same as that of *S.aureus* at 2 µg/ml suggesting MIC susceptible or resistant at 24hours in 2 µg/ml as clinical breakpoints which is comparatively lower than those of Acetone and chloroform suspension at 4 µg/ml and 6 µg/ml respectively. When using oleic acid for the stabilisation of NPs, their MIC considerably increased and reached 8 µg/ml for both test *Entero spp* and *Strepto spp* strains. Guntur SR *et al.* (2018)[12], showed the highest zone of inhibition against both gram-positive and negative microorganisms at an AgNPs concentration of 20 mg/mL. Ramadan MA and Safawat NA[19] observed a MIC of 40 µg/ml for potential anti-helicobacter activity against *Helicobacter pylori* (*H. pylori)*. Similarly, Kumar KA *et al.* revealed that a strong antibacterial activity was observed against *S.epidermidis, S. aureus, E. coli* and *P. aeruginosa* at all dilutions, with MIC at > 4 µg/ml. Gram-negative organisms showed more resistant activity attributed to their complex cell membrane compared to gram-positive microorganisms[18]. Subramaniam S *et al.*.[23] found β-Sitosterol-d-glucopyranoside, a Db leaf extract bioactive compound, effective in inhibiting microorganisms at MIC 6–50 µg/ml. It was also observed that this bioactive compound acts synergistically with ciprofloxacin and kills pathogens within a 5 to 10 hours half-life activity (Graph 1; Figure 8 (a-c).

The observed increase in the Minimum Inhibitory Concentration (MIC) of ethanol-stabilised *DbAgNps* dispersions may be attributed to the relatively long aliphatic chains (–CH) present in the Db extract. These structural features contribute to a higher

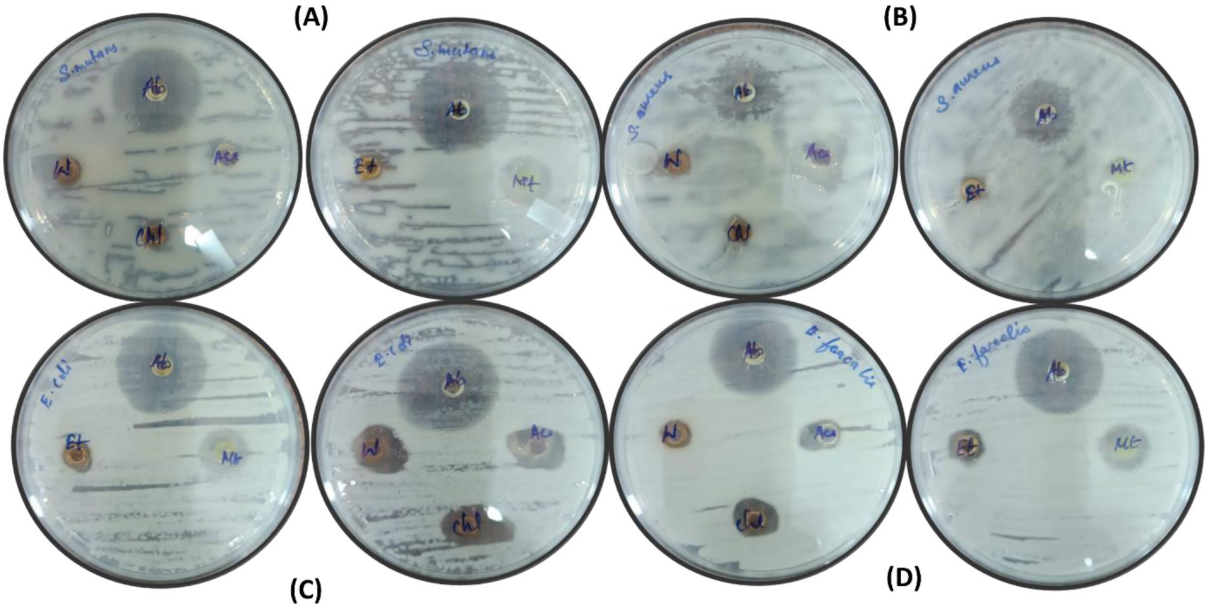


Figure 7. Zone of Inhibition of DbAgNPs on *S. mutans (A), S. aureus (B), E. coli (C), E. fecalis (D)* using various solvents.

hydrophobic character, which enhances membrane permeability. As a result, the Db-AgNPs exhibit improved ability to traverse lipopolysaccharide-rich bacterial membranes and effectively infiltrate the cytoplasmic space.

Additionally, the cytotoxicity of these nanoparticles may be amplified by the presence of silver ions and hydrocarbon residues, particularly those containing peroxide functional groups. The enhanced antibacterial efficacy of ethanol-stabilised Db-AgNPs can also be linked to the absence of significant aggregation, improved colloidal stability of the silver hydrosol, and a synergistic interaction between the silver nanoparticles and the bioactive stabilising agents present in the extract. These combined effects contribute to greater nanoparticle dispersion and sustain antimicrobial activity.

Desmostachya bipinnata has synergistic effects in medicinal, environmental, agricultural, and phytoremediation domains. The interactions between bioactive compounds in the plant and its microbiome led to improved outcomes and ecosystem services. DB possesses inherent anti-microbial properties attributed to various bioactive compounds such as alkaloids, flavonoids, phenolics and terpenoids. The synergistic interactions between these bioactive compounds enhance the overall antibacterial efficacy of Db extracts. Different compounds may target bacterial cells through multiple mechanisms, including disruption of cell membranes, inhibition of cell wall synthesis and interference with bacterial enzymes or metabolic pathways[18,23].

3.2.2 Nitric Oxide Scavenging Assay

In vitro inhibition of nitric oxide radical as a measure of antioxidant activity of the *Db* Silver NPs compound in each of the suspension (water, ethanol, methanol, acetone and chloroform) was performed using Gallic acid as standard and the scavenging potential of the *Db* Silver NPs fresh derivative using standard preparation exhibited a dose dependent activity as depicted. The percentage inhibition of the standard Gallic acid increased with increasing concentration at the given solvent for *Db* Silver NPs and showed maximum inhibition of 97.43% at 2.0µg/mL concentration and a minimum of 24.1% at 0.5 µg/mL concentration. Similarly, for the solvents ethanol, methanol, acetone, chloroform and water, the percentage scavenging increases linearly with increasing concentration.

The half percentage of the maximal inhibitory effect, IC 50, of *Db* silver NPs for the stabilisation of nanosilver was observed to be 1.02 µg/ml, with total inhibition effect at 2.2 µg/ml. At this concentration, ethanol solvent suspension provided 98.47% inhibition, followed by methanol (83.95%) and least being acetone (70.69%). Golla U and Bhimathati S observed IC50 of 264.18±3.47 µg/mL while Khyade VB *et al.,*[15,24] showed IC50 of 127.07±6.44 µg/mL using hydrogen peroxide radical assay, suggesting scavenging activity in a dose-dependent manner. It was evident that the toxicity of NO increases on reaction with superoxide radicals, forming a highly reactive peroxynitrite anion (ONOO-). The nitrous oxide reacts with oxygen to form nitrite at a concentration higher than the standard, thus contributing to the scavenging activity. However, the

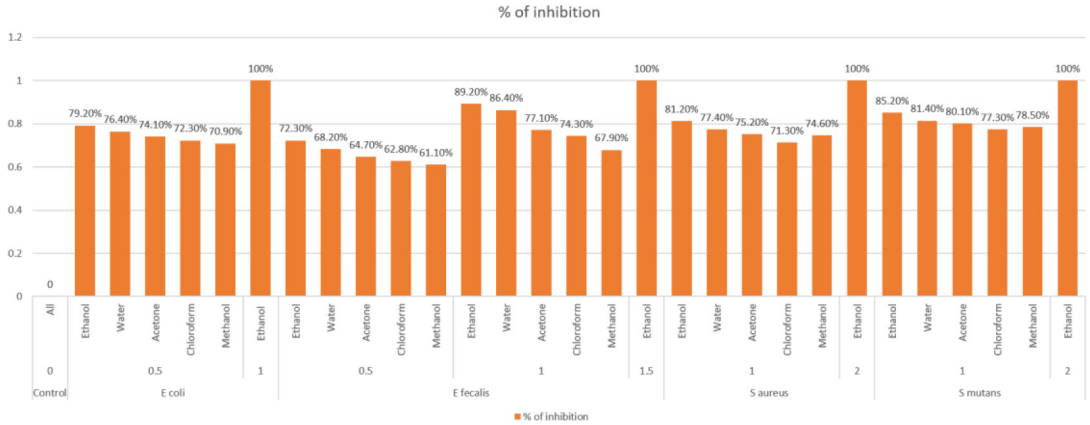


Figure 8. Minimal Inhibition Concentration Antibiotic Assay (MIC) of various solvents against *E. coli, E. fecalis, S. aureus* and *S. mutans*.

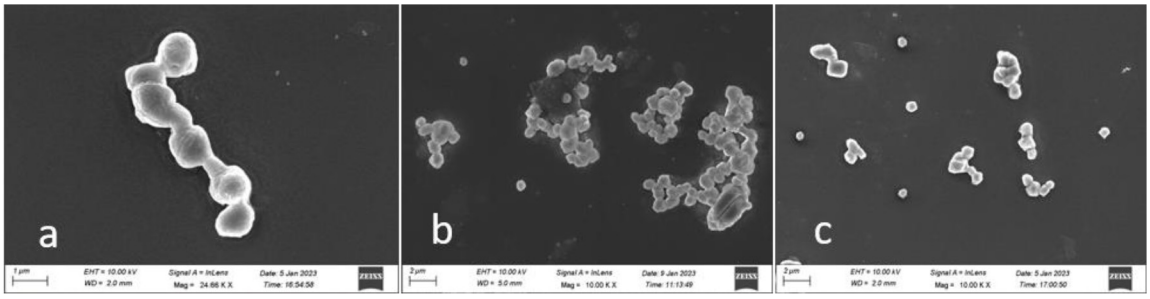


Figure 9 (a-c). Scanning electron microscopic view of biofilm assay of Ethanolic extract of DbAgNP against *E. coli.*

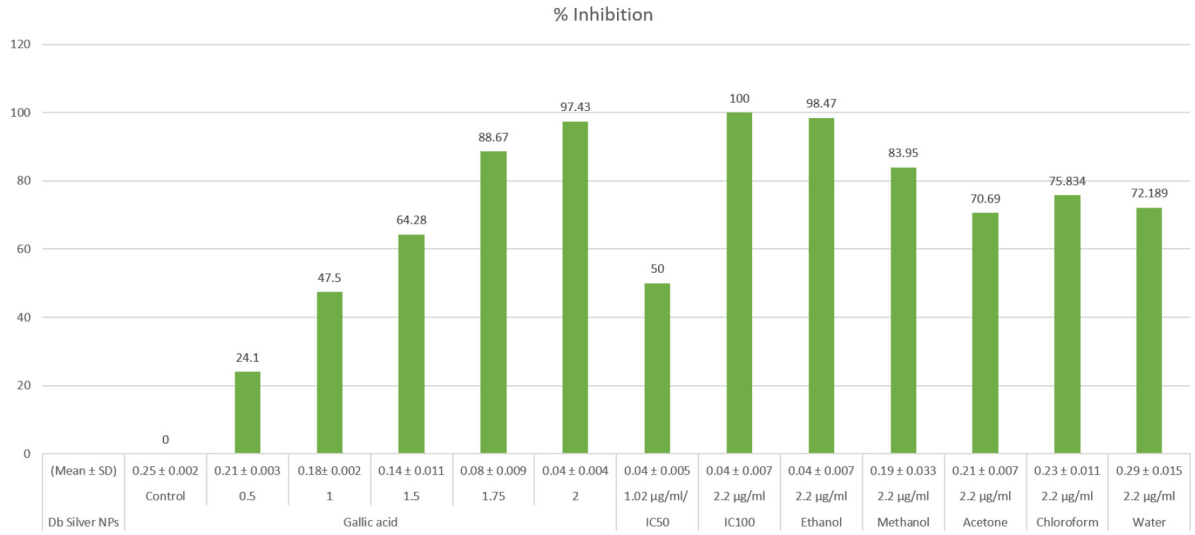


Figure 9. Nitric oxide scavenging assay of DbAgNPs of various solvents.

lowest concentration of the Db extract AgNPs (2.2 µg/mL) exhibited the highest percentage of inhibition, which is similar to that of AgNPs' maximum inhibition of 97.43% at 2.0µg/mL concentration and minimum 24.1% at 0.5 µg/mL concentration.

3.2.3 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Inhibition Assay

Db possesses potent antioxidant properties attributed to its high phenolic contents, flavonoids and other antioxidants. The synergistic interactions among these antioxidants enhance the overall scavenging capacity of Db extracts against Reactive Oxygen Species (ROS) and free radicals. Antioxidants present in Db protect cells and tissues from oxidative damage by neutralizing ROS, inhibiting lipid peroxidation, and enhancing the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)[24].

2, 2-diphenyl-2-picryl hydrazyl (DPPH) technique is based on the reaction of DPPH with antioxidants such as superoxide radicals in aqueous or organic solvents such as ethanol, methanol, acetone, chloroform and water, producing a bleaching effect by interacting with particles at 525nm. This interaction results in quenching of the solvents present at the given concentration to produce RSA (radical scavenging activity%). It was observed that *Db* Silver NPs possess an antioxidant property of 0.805 at 525nm, with a proportion of antioxidant at 84.36% in ethanol solvent, with the least being methanol solvent dissolved *Db* Silver NPs with an antioxidant property of 0.678 at 525nm, with a proportion of antioxidant at 71.025%. The IC50 value of the standard, which is the concentration of the standard required to inhibit 50% of the DPPH free radical of *Db* silver NPs, was calculated using the log dose inhibition curve. The half of the maximal inhibitory effect, IC 50 of *Db* silver NPs for the stabilisation of nanosilver, was

Table 1. DPPH assay of DbAgNPs of various solvents

Solvents	Water	Ethanol	Methanol	Acetone	Chloroform
24-hour sample absorbance at 525nm	**0.771**	**0.805**	**0.678**	**0.730**	**0.701**
% inhibition	80.76923	84.366	71.025	76.472	73.40468

observed to be 1.05 µg/ml with total inhibition effect at 2.2 µg/ml (Table 1).

Guntur SR *et al.,* demonstrated the highest percentage of inhibition of 44.41% ± 2.3% at the lowest concentration of 1 µg/mL, while Joshi KB *et al.* using a DPPH assay observed the highest activity of inhibition concentration at 50% (IC50) using acetone extract (at 17.42 *µ*g/mL) followed by methanol (at 25.83 *µ*g/mL) and least being ascorbic acid (at 7.5 *µ*g/mL). The possible reasons could be attributed to the fact that the acetone extract retains antioxidant properties and could assist as free radical inhibitors or scavengers, thus aiding as a potential natural antioxidant substitute. Lower absorbance of the reaction mixture indicated higher free radical activity and suggests that scavenging ability increases with increasing concentrations of the extract[25,26].

3.2.4 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) cytotoxic assay

The treatment efficacy was determined by using cell survival determination (MTT), cytotoxicity of cells to control, ethanol, methanol, water, acetone, and chloroform. The MTT assay has confirmed the best responses with the Ethanol extract. Ethanol solvent has shown 97% cytotoxicity at 48 hours compared to Water (87%), chloroform (71%), methanol (69%) and acetone (70%). Water extract of *Db*AgNPs at 24 hours showed 81% toxicity and 69% at 72 hours, while ethanol extracted *Db*AgNPs showed 79% at 24 hours and 88% at 72 hours. It was also observed that Methanol extracted *Db*AgNPs produced lower cytotoxicity of 71% at 24hrs and 62% at 72hrs respectively (Figure 9). Acetone-extracted *Db* Silver NPs possessed comparatively higher cytotoxicity at higher concentrations of 72% at 24 hours and 63% at 72 hrs. Chloroform extracted *Db* Silver NPs showed 73% at 24 hours and 64% at 72 hours. In the ethanol extracted *Db*AgNPs at 15.6 and 23.4 J/cm^2 dose, cytotoxicity was perceived compared with other solvents. Guntur SR *et al.,* .[12], observed significant cell death of HepG2 cells, where reduction

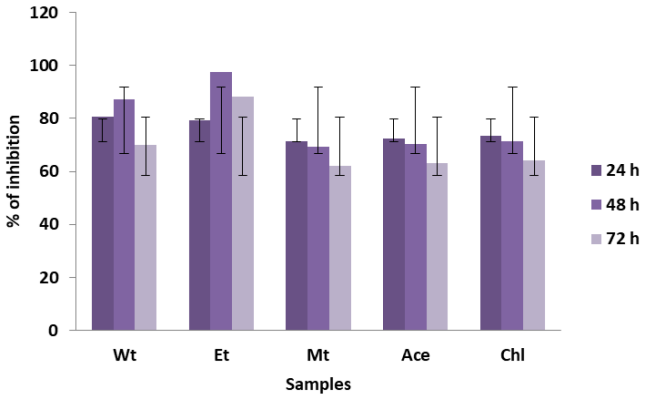


Figure 10. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) cytotoxic assay of DbAgNPs of various solvents at different time intervals.

in viability is caused by AgNPs rather than the extract of Db itself. Rahate K *et al.,* observed methanolic extract of Db at a concentration of 400 µg/ml with anti-tumour activity and showed HeLa cell line inhibition between 25 – 400 µg/ml. In contrast, the present study witnessed that cytotoxicity of various solvents in *Db* Silver NPs at 48 hours has shown high cytotoxicity as clinical breakpoints at a lower concentration between 20 to 40 µg/ml using HeLa cell lines[22].

3.2.5 Hemolysis Assay

The hemocompatibility of nanoparticle formulations is a critical parameter when considering their biomedical applications, particularly in drug delivery and systemic circulation. In this study, the hemolytic potential of *Desmostachya bipinnata*-synthesised silver nanoparticles (Db-AgNPs), extracted using different solvents (water, ethanol, methanol, acetone, and chloroform), was evaluated to assess their biocompatibility with Red Blood Cells (RBCs).

The control sample exhibited a complete absence of hemolysis, confirming the validity of the assay and the stability of RBCs under normal conditions. Among the test samples, methanol-extracted Db-AgNPs demonstrated the lowest hemolytic activity at 2.0%, followed closely by ethanol (2.3%) and water-based

extracts (2.5%). Acetone-extracted AgNPs exhibited slightly higher hemolysis at 2.9%, while the chloroform-extracted formulation showed the highest value of 3.2% (Figure 10). However, all observed hemolysis levels remained well below the internationally accepted threshold of 5% hemolysis, indicating excellent hemocompatibility of the formulations.

These findings suggest that the Db-AgNPs, regardless of the solvent used for extraction, pose minimal risk to erythrocyte membrane integrity. The relatively higher hemolysis observed with chloroform and acetone extracts may be attributed to residual organic compounds or solvent-derived phytoconstituents that can mildly disrupt membrane permeability. In contrast, ethanol and methanol-based extracts demonstrated superior RBC compatibility, possibly due to a cleaner reduction and capping process with minimal solvent toxicity—a finding supported by the stability of their absorbance and uniformity in size distribution.

Edison, Nandini J, and Shatviths PC propose that the mild variations in hemolytic index may be influenced by the solubility and polarity of phytochemicals present in each solvent system, which in turn affect the interaction of nanoparticles with the phospholipid bilayer of RBC membranes. Furthermore, the bioactive compounds from *Desmostachya bipinnata* likely play a stabilising role, forming a natural capping layer around the AgNPs, thus limiting direct cytotoxic interactions[33-35].

In agreement with existing literature, hemolysis below 5% is generally considered non-toxic and acceptable for nanomaterial use in biomedical applications, especially in systemic and intravenous formulations. These results reinforce the safety profile of *Desmostachya bipinnata*-derived AgNPs, particularly those synthesised using ethanol and methanol, for potential clinical applications such as antimicrobial agents, wound healing materials, or carriers for targeted drug delivery.

3.2.6 *In vitro* Cytotoxicity Assay of *Desmostachya bipinnata*-Silver Nanoparticles in Zebrafish Embryos

To evaluate the *in vitro* cytotoxicity of *Desmostachya bipinnata*-mediated silver nanoparticles (Db-AgNPs), varying concentrations (10, 20, 30, 50, 75, and 100 µg/mL) of the nanoparticles were prepared in 100 mL of Hank's balanced salt solution. Zebrafish (*Danio rerio*) embryos were selected as the model organism for toxicity assessment due to their high genetic homology to humans and their established utility in studying developmental toxicity and nanoparticle bio-interactions[26,27].

Previous studies have highlighted the sensitivity of zebrafish embryos to silver nanoparticles. Gao *et al.* reported cardiovascular developmental abnormalities at 1000 µg/L using AgNPs of 50 nm. Similarly, Wu and Zhou[28] observed toxicity across a concentration range

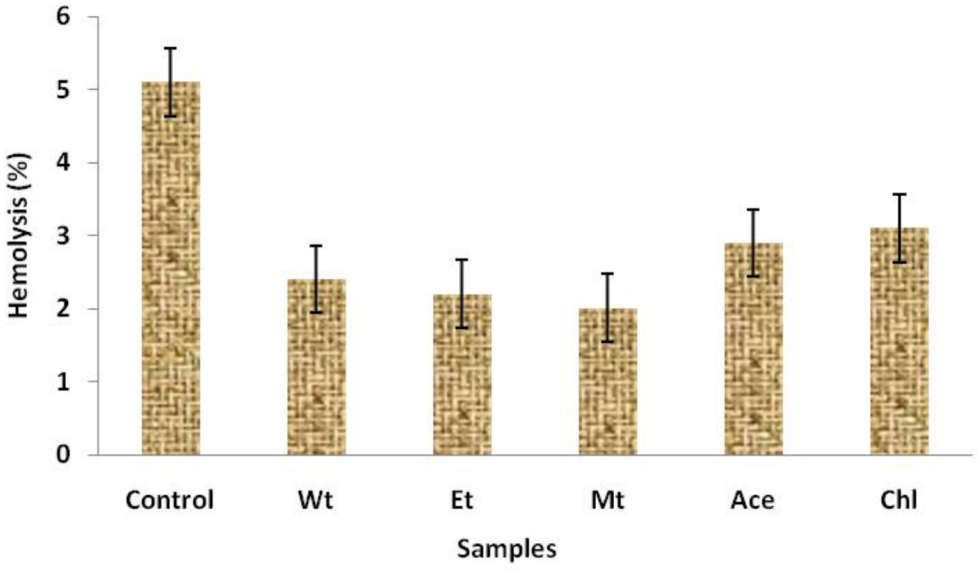


Figure 11. Hemolysis (%) of various solvents of *Desmostachya bipinnata* Silver Nanoparticles with control.

of 3 to 1000 µg/L for AgNPs sized 16–58 nm. Xin *et al.*.[29,30] reported nanoparticle accumulation in the head and trunk regions within 24 hours, while Osborne *et al.*.[30] noted deposition in the gills and intestines after four days of exposure to AgNPs sized 20–110 nm.

In the current study, Db-AgNPs with an average diameter of 28 nm showed visible accumulation in the head and trunk regions of zebrafish embryos at 30 µg/mL after 48 hours. This observation aligns with prior reports[31,32] and reinforces the understanding that nanoparticle size and surface charge significantly influence biodistribution, cellular uptake, and potential developmental toxicity.

Despite detectable nanoparticle accumulation, embryos exposed to lower concentrations (10–50 µg/mL) demonstrated normal development, with no evidence of acute toxicity. Observations made between 96 and 120 hours post-fertilisation revealed only mild morphological alterations—such as slight head-tail disarrangement and minor ocular deformities—which were non-lethal. These findings suggest that green-synthesised Db-AgNPs are relatively biocompatible at early developmental stages and exhibit a favourable safety margin up to 100 µg/mL[33,34].

This assay was performed to evaluate the biosafety of Db-AgNPs in a complex living system, simulating early organogenesis and membrane interactions, which is critical for validating their suitability for biomedical applications, particularly in oral mucosal drug delivery. The oral mucosa is a highly permeable, vascularized site often targeted for local and systemic delivery of antimicrobial and anticancer agents. Ensuring minimal cytotoxicity is essential for any formulation intended for use in this sensitive tissue environment[35,36].

By confirming the low embryotoxicity of Db-AgNPs, this study supports their potential incorporation into oral therapeutic formulations, such as mucoadhesive gels, patches, and mouthwashes for managing conditions like oral mucositis, candidiasis, leukoplakia, and Oral Squamous Cell Carcinoma (OSCC). The nanoparticle's ability to maintain efficacy at non-toxic concentrations highlights its promise as a biocompatible carrier system for targeted and sustained delivery across the oral epithelium, with minimal risk of systemic toxicity.

These results underscore the importance of *in vivo* biocompatibility screening in models like zebrafish to bridge the translational gap between nanoparticle synthesis and clinical application.

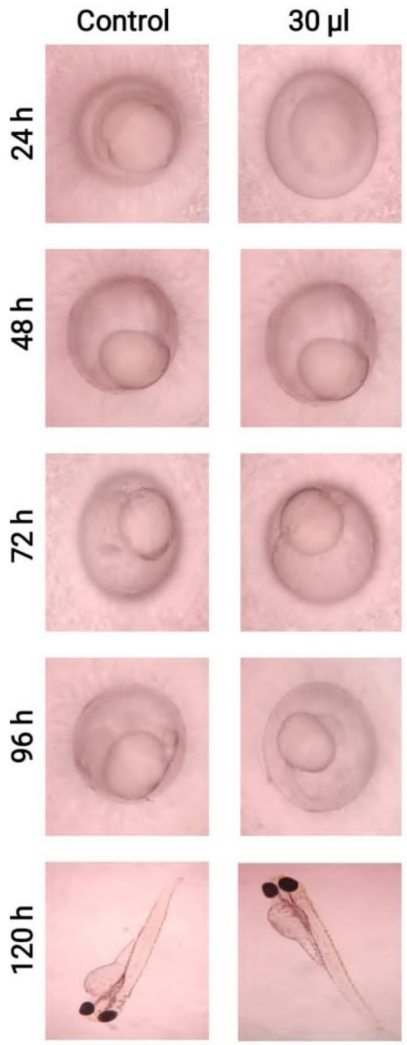


Figure 12. Embryotoxicity of *Desmostachya bipinnata* based silver nanoparticles (ethanolic extract) in the Zebra fish model.

4. Conclusion and Future Perspectives

In conclusion, the synthesis and characterisation of silver nanoparticles (Db-AgNPs) using *Desmostachya bipinnata* leaf extract represent a significant advancement in the field of nanomedicine, offering a natural, eco-friendly, and biocompatible platform with promising biomedical applications. Through comprehensive characterisation involving UV–Visible spectroscopy, Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray Spectroscopy (EDS), Fourier Transform Infrared Spectroscopy

(FTIR), and the physicochemical profile of the synthesised Db-AgNPs was thoroughly elucidated.

The biosynthesised Db-AgNPs demonstrated robust antimicrobial activity against a range of pathogenic bacteria, with ethanol-derived nanoparticles showing particularly potent efficacy against *Escherichia coli*. Furthermore, antioxidant assays such as nitric oxide and DPPH radical scavenging confirmed the strong free radical neutralising potential of Db-AgNPs in a dose-dependent manner. *In vitro* cytotoxicity studies using HeLa cell lines revealed significant anticancer potential, particularly for ethanol-based extracts. Importantly, hemolytic assays confirmed minimal erythrocyte damage, suggesting a high degree of biocompatibility and safety.

Embryotoxicity studies in zebrafish models further validated the biocompatibility of Db-AgNPs, with no significant developmental anomalies observed at therapeutic concentrations. These results highlight the potential translational relevance of Db-AgNPs in clinical settings.

In the context of oral mucosal applications, Db-AgNPs hold specific promise due to their anti-inflammatory, antimicrobial, and antioxidant properties. The oral mucosa—being a highly vascularized and accessible tissue—serves as an ideal site for localised drug delivery, particularly in conditions such as oral mucositis, leukoplakia, oral candidiasis, and Oral Squamous Cell Carcinoma (OSCC). Db-AgNPs can be incorporated into mucoadhesive gels, patches, mouthwashes, and nanofiber scaffolds for sustained and targeted drug delivery to the affected mucosal sites. Their ability to disrupt microbial biofilms, modulate oxidative stress, and induce selective cytotoxicity further enhances their therapeutic value in oral precancer and cancer management.

Nanoparticle-based drug delivery systems offer numerous advantages, including enhanced solubility, bioavailability, targeted delivery, reduced systemic toxicity, prolonged circulation time, and amplified therapeutic efficacy. Herbal-based nanoparticles—including liposomes, dendrimers, hydrogels, transdermal patches, nano-hydrogels, xanthan gum matrices, cellulose scaffolds, alginate carriers, polymeric micelles, metallic nanoparticles, quantum dots, and crystal nanoparticles—are widely investigated as advanced drug delivery vehicles.

Desmostachya bipinnata-based nanoparticles, when conjugated with such delivery platforms, can offer a tunable and multifunctional system for oral mucosal disease interventions. The nanoscale dimensions allow greater surface area for bioactive interactions, facilitating rapid drug dissolution, localised action, and reduced side effects—particularly advantageous for the dynamic environment of the oral cavity.

Future research should focus on validating the in vivo efficacy and toxicity of *Desmostachya bipinnata*-derived silver nanoparticles (Db-AgNPs) in relevant oral cancer models to establish their translational potential. Additionally, the development of Db-AgNP-based mucoadhesive drug delivery systems, such as gels, films, or patches, could provide sustained and targeted therapeutic action for oral mucosal lesions. Investigating combinatorial treatment **strategies** that incorporate Db-AgNPs alongside conventional chemotherapeutic agents may offer synergistic effects, improve treatment outcomes while minimising systemic toxicity.

Finally, well-designed clinical trials are essential to assess the safety and therapeutic efficacy of these nanoformulations in patients with Oral Potentially Malignant Disorders (OPMDs) and Oral Squamous Cell Carcinoma (OSCC), thereby advancing them toward clinical use. By leveraging the synergistic properties of herbal nanomedicine and targeted delivery systems, *Desmostachya bipinnata*-derived AgNPs can be effectively positioned as next-generation therapeutics for oral mucosal applications and beyond.

5. Acknowledgement

The authors greatly acknowledge Department of Health Research-Human Resource Development Scheme (Women Scientist 2022-1188) for the financial support.

6. Author Contribution

Nitya K: Conceptualization, methodology, original manuscript drafting Ramya Ramadoss: Review and editing of manuscript, validation Bargavi P: Characterization and analysis of synthesized nanoparticles Keerthi Narayan: Critical review of experimental design and manuscript Venkatesan

Muthukumar: Characterization support and manuscript review. All authors have read and agreed to the published version of the manuscript.

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