Green Synthesis of Silver Nanoparticles using the Mucuna pruriens plant extract into Biofilm Formulation and its Characterization

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ABSTRACT

In recent times, the green synthesis of nanoparticles via biological research has attracted enormous attention. In this study, the bioactive compounds are synthesized using silver nanoparticles from Mucuna pruriens to enhance the epithelialization period, granular tissue formation, reduced susceptibilities to antimicrobial agents, and host defense. The bioactive compounds are incorporated into the biofilm to make a physical barrier. The biofilm, designed using eco-friendly and biocompatible material, serves as a controlled delivery system for the bioactive component of Mucuna pruriens. The nanocomposites are characterized using Scanning Electron Microscopy (SEM), Fourier-Transform Infrared Spectroscopy (FT-IR), Antioxidant activity, Cell line -HEK, and the antimicrobial activity was done using Gram – Negative bacterium(E-Coli) has been recorded using the disc diffusion method. This research contributes to the development of sustainable strategies for biofilm management. As a result, the synthesis nanoparticles promise to be a great biomedical material with eco-friendly high antibacterial properties.

Keywords: *Mucuna pruriens, Green synthesis, Silver Nanoparticles (AgNPs), Bioactive components*

1. INTRODUCTION

1.1 NANOTECHNOLOGY

Nanotechnology is a branch of science that deals with designing, producing, and using structures, devices, and systems by manipulating matter at the atomic scale, otherwise known as the “nanoscale” [1]. Nanotechnology has extensive applications in the field of health and medicine as “nanomedicine”, which facilitates the early detection, prevention, precise diagnosis, effective treatment, and ongoing monitoring of diseases, thereby significantly advancing healthcare capabilities. In nanomedicine, the nanoparticles are used for site-specific drug delivery where precise drug dosages are administered, leading to a reduction in side effects, as the therapeutic agent is selectively delivered to the morbid region only,

which minimizes the impact on healthy tissues and in addition it reduces the drug toxicity and cost of treatment and also improves bioavailability [2]. Solid lipid nanoparticles (SLNs), liposomes, nanostructured lipid carriers (NLC), fullerenes, nanoshells, quantum dots (QD), superparamagnetic nanoparticles, and a diverse array of inorganic materials such as gold, silica, or iron oxide nanoparticles are among the frequently utilized nanoparticles in nanomedicines for drug delivery and therapeutic applications [3].

1.2 SILVER NANOPARTICLES

The utilization of silver nanoparticles (AgNPs) is experiencing a rapid surge across various sectors, including medical, pharmaceutical, healthcare, and many more. This escalation can be attributed to the antimicrobial properties of silver nanoparticles, coupled with their non-toxic nature to humans. Studies have shown that silver nanoparticles can actively release silver ions, contributing to their antimicrobial effectiveness by targeting microbes[4]. Furthermore, their large surface-to-volume ratio and specific crystallographic surface structure render silver nanoparticles promising candidates as antibacterial agents[5]. The antimicrobial effectiveness of silver nanoparticles was significantly greater against gram-negative bacteria when compared to gram-positive bacteria. Silver nanoparticles are also utilized in wound healing because they can expedite the differentiation of fibroblasts into myofibroblasts, consequently enhancing the healing rate[6]. In addition to the aforementioned uses, silver nanoparticles exhibit a diverse range of applications, including antiviral, antifungal, anti-inflammatory, anticancer, antioxidant activities, and numerous others[7].

1.3 GREEN SYNTHESIS

The preference for green synthesis of silver nanoparticles (AgNPs) over physical and chemical methods stems from several drawbacks associated with the latter, including the utilization of hazardous chemicals, low yield, solvent contamination, high energy consumption, and occasionally uneven distribution of nanoparticles. In the synthesis of silver nanoparticles using this approach, three primary components are employed: a solvent, a reducing agent, and non-toxic materials[8]. Various substances such as leaf extracts, bark, roots, stems, leaves, bacteria, algae, fungi, and more are harnessed for nanoparticle synthesis. Among these, plants and their extracts hold particular favor due to their robust capping and reducing properties, along with rapid proliferation capabilities. Plants and their extracts utilized for nanoparticle synthesis contain alkaloids, terpenoids, phenols, flavonoids, tannins, quinines, and various other biomolecules, all recognized for their role in mediating the synthesis of nanoparticles[9]. Increasingly, contemporary practices are witnessing a rise in the adoption of economical, eco-friendly synthesis techniques that utilize biological agents in the fabrication process of silver nanoparticles (AgNPs).

1.4 *MUCUNA PRURIENS*

Mucuna pruriens is a medicinal plant that belongs to the family of Leguminosae, commonly known as velvet bean or cowitch, and is present in the plains of India. *M. pruriens* is an annual vine capable of growing up to 15 meters in length Its leaves are arranged alternately and measure between 6.3 to 11.3 cm. The leaflets are membranous, with smaller terminal leaflets and very unequal lateral leaflets. Its flowers, numbering between 6 to 30, in color of dark

purple, resembling larger pea flowers with distinctively curved petals, arranged in drooping racemes. The fruits and elongated curved pods contain 4 to 6 seeds, reaching about 10 cm in length. Urinary tract, neurological, and menstruation disorders, constipation, edema, fever, tuberculosis, and ulcers have all been traditionally treated with the *Mucuna pruriens*. *M. pruriens* seeds are rich in LDOPA, an uncommon non-protein amino acid that serves as a direct precursor to the neurotransmitter dopamine. Also, *Mucuna pruriens* exhibit diverse pharmacological properties, encompassing antimicrobial, antioxidant, aphrodisiac, and anti-glycemic activities, suggesting its potential therapeutic benefits across various health domains[10].

1.5 BIOFILM-BIODEGRADABLE BAND-AID

Biodegradable bandages, made from sustainable and natural materials, offer a departure from traditional adhesive bandages, which frequently incorporate harsh chemicals. This environmentally conscious decision promotes the natural decomposition of the bandages upon disposal, resulting in a reduced environmental footprint. In contrast, plastics derived from non-renewable petroleum by-products pose substantial environmental risks, underscoring the significance of embracing biodegradable alternatives to alleviate ecological damage[11]. A significant drawback of plastic bandages is that many individuals experience skin allergies when using them, often due to the barrier they create that impedes normal skin respiration[12]. In this study, a novel, environmentally friendly material for Band-Aids has been developed, aimed at enhancing breathability and mitigating the adverse effects of skin allergies. Moreover, this material boasts biodegradability and safety, ensuring its suitability for widespread application. The innovative material underwent thorough examination and analysis of its toxicity, and various other characteristics to assess its performance and suitability for intended applications. Therefore, our study is centered around characterizing the synthesized silver nanoparticles through the utilization of UV-visible spectrophotometry, scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR) [13]. These techniques will provide valuable insights into the structural, optical, and chemical properties of the nanoparticles. Furthermore, the antimicrobial and antioxidant properties of synthesized silver nanoparticles were analyzed, and wound-healing properties of band-aids were studied using assays like Cytotoxicity in HEK cell line, and scratch wound assay.

2. MATERIALS AND METHODS

2.1 COLLECTION OF SEED EXTRACT

Mucuna pruriens seeds were collected from the local surroundings of Coimbatore, India. The seeds were rinsed with tap water to remove any dust particles and then washed with distilled water to improve the quality of the extract. After that, they were dried in a hot air oven for an hour to remove any remaining moisture. Finally, the seeds were crushed using a mixer to obtain a powdered seed extract.

2.2 PREPARATION OF SEED EXTRACT

To prepare the seed extract, 5 grams of powdered seeds are weighed and mixed with 100 ml of distilled water. The mixture is then heated in a water bath for 5 to 10 minutes at 80°C and allowed to cool down to room temperature. Finally, the mixture is filtered using What-man filter paper.[14]

2.3 GREEN SYNTHESIS OF SILVER NANOPARTICLES

A beaker was filled with 20 ml of freshly obtained seed extract and 100 ml of silver nitrate solution. The silver nitrate solution was prepared by mixing 0.02 M silver nitrate with 98 ml of distilled water. After that, 80 ml of the silver nitrate solution was added to 20 ml of seed extract. The mixture was then placed on a magnetic stirrer and heated to 70°C while stirring at 250 rpm**.** In a separate step, 0.05 M of trisodium citrate was added to 10 ml of distilled water. The resultant mixture was then added dropwise to the silver nitrate solution while vigorously mixing until the color of the solution changed to a dark brown. The colloidal dispersion was centrifuged at 3500 rpm for 20 minutes to separate the pellets from the supernatant. The pellets were then dried in a hot air oven at 100°C for 1 hour.[15]

2.4 PREPARATION OF BIOFILM

A beaker containing 50 ml of distilled water was placed on a magnetic stirrer and heated until it boiled. Then, 3.75g of polyvinyl alcohol was slowly added to the boiling water, little by little, instead of all at once. Once the polyvinyl alcohol was fully dissolved, an amagnetic bead was added to the solution. The mixture was then heated to 80°C for 2 hours to attain a clear solution. Next, 0.5 g of pellets were dispersed in 15 mL of Polyvinyl alcohol solution and thoroughly mixed to ensure uniform distribution. The resulting mixture was then allowed to air dry at room temperature for 3 days. This drying process aimed to solidify the Polyvinyl alcohol matrix containing the pellets, forming a stable composite material suitable for further characterization and testing.[16]

2.5 SCANNING ELECTRON MICROSCOPE

Surface morphology was investigated using scanning electron microscopy (SEM). Specifically, the Zeiss Sigma 300 SEM equipped with field emission was utilized, operating at an acceleration voltage ranging from 0.02 to 30 kV. Notably, this instrument exhibits exceptional performance at low kilovoltage (kV), achieving a resolution as fine as 1.5 nm at 1 kV.

2.6 FOURIER TRANSFORM INFRARED SPECTROSCOPY

The attenuated total reflection crystal was placed with the investigated dried samples. The plant extract is used as a reducing and capping agent during the production of silver nanoparticles. The reducing and capping agent, or the groups preventing the aggregation of silver nanoparticles, are shown by the FTIR examination. Buffer-subtracted transmission spectra with wave numbers between 1000 and 4000 cm^{-1} were captured. ABB Bomem MB3000 series of FTIR is used for analyzing the sample, which is the market's most trustworthy FTIR laboratory analyser. This series will make sample collection, processing, and analysis easier.[17]

2.7 EVALUATION OF CYTOTOXICITY

The inhibitory concentration (IC50) value was determined using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cells were cultured at a density of 1×10^4 cells per well in a 96-well plate and allowed to grow for 48 hours until reaching 80% confluence. The medium was then replaced with fresh medium containing serially diluted samples, and the cells were further incubated for 24 hours. Afterward, the culture medium was removed, and 100µL of MTT solution (Hi-Media) was added to each well, followed by incubation at 37°C for 4 hours. Subsequently, the supernatant was removed, and 50µL of dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals, followed by a 10-minute incubation period. The optical density was then measured at 620 nm using an ELISA multiwell plate reader (Thermo Multiskan EX, USA).[18]

$$\text{Formula: \% of viability} = \frac{\text{OD value of the experimental sample}}{\text{OD value of experimental control}} \times 100$$

2.8 SCRATCH ASSAY

For the wound healing assay, A549 cells were seeded at a density of 1×10^5 cells per 35×11 mm dish and incubated for 24 hours at 37°C. Subsequently, the cells were subjected to various treatments according to our treatment schedules, including control/untreated conditions and concentrations of 5, 10, and 25 µg/ml. Once the cells reached confluence, a scratch was made in the cellular layer of each dish using a plastic pipette tip. The migration of cells at the edge of the scratch was then analyzed at 0 and 24 hours, during which microscopic images of the cells were captured.[19]

2.9 ANTIBACTERIAL ACTIVITY TESTS

The compound's antibacterial effectiveness against Gram-positive bacterial strains, including E. coli, was assessed using the disc diffusion/Kirby-Bauer method. To summarize, nutrient agar plates were inoculated with a 100-microliter suspension of freshly cultured bacteria (at concentrations of 10-4 and 10-6 colony-forming units (CFU)/mL of E. coli, respectively). Small, uniformly sized sterile paper discs (10 mm in diameter) impregnated with samples of the compound were then placed onto the agar plates. A disc containing ciprofloxacin served as a positive control. Following an incubation period of 24 hours at 37°C, the diameters of the zones of inhibition surrounding the discs were measured to evaluate antibacterial activity.[20]

2.8 ANTIOXIDANT ASSAY

The Silver nanoparticle was tested using 1-diphenyl-2-picryl hydroxyl (DPPH) to ascertain the extracts' capacity to scavenge free radicals. 1 ml of newly made0.1 MM Methanoic DPPH and 1 ml of plant extract were ingested. After 15 minutes of incubation at room temperature, the mixture's absorbance was detected at a wavelength of about 517 nm. Ascorbic acid served as the standard in this test.[21]

3. RESULTS AND DISCUSSION

3.1 COLLECTION AND PREPARATION OF SAMPLES

Mucuna pruriens seeds were collected from the local surroundings of Coimbatore, India. The seeds were rinsed with tap water to remove any dust particles and then washed with distilled water to improve the quality of the extract. Afterward, they were dried in a hot air oven for an hour to remove any remaining moisture. Finally, the seeds were crushed using a mixer to obtain a powdered seed extract. To prepare the seed extract, 5 grams of powdered seeds are weighed and mixed with 100 ml of distilled water. The mixture is then heated in a water bath for 5 to 10 minutes at 80°C and allowed to cool down to room temperature. Finally, the mixture is filtered using What-man filter paper. The collection and processing of natural products are vital processes for extracting and identifying bioactive compounds. This is essential for reducing contaminant levels and increasing the concentration of bioactive compounds, as long as the processing is carried out correctly to preserve compound stability.

3.2 SILVER REDUCTION

Monitoring the reduction of silver ions to silver nanoparticles often involves observing a noticeable colour change, which can be easily detected visually. Additionally, UV-Vis spectroscopy is commonly employed to track this conversion process. The technique described above has demonstrated significant utility in the analysis of nanoparticles, offering a straightforward and effective means of monitoring their formation and evolution.

Figure 1. Silver Nanoparticles

3.3 BIOFILM

The biofilm was meticulously prepared utilizing a combination of polyvinyl alcohol (PVA) and silver nanoparticles derived from the green synthesis method. This involved a systematic procedure wherein the silver nanoparticles, synthesized through eco-friendly means, were carefully incorporated into the PVA matrix to create a composite material with enhanced properties. The green synthesis method employed for the extraction of silver nanoparticles ensured not only the efficient utilization of natural resources but also minimized environmental impact.

Figure 2. Biofilm

3.4 UV-VIS SPECTROSCOPY

The silver nanoparticles underwent characterization using UV-Vis spectroscopy, a prominent technique for structural analysis in nanoparticle research. The absorption spectrum of the silver nanoparticle solution prepared using the suggested method, exhibited a distinct surface plasmon absorption band reaching its peak at 425 nm. This absorption profile strongly suggests the presence of spherical silver nanoparticles. Further confirmation of this structural morphology was obtained through SEM (Scanning Electron Microscopy) imaging. These images provided visual evidence supporting the spherical shape of the silver nanoparticles, thus corroborating the findings derived from UV-Vis spectroscopy. Collectively, these characterization techniques underscore the reliability and consistency of the proposed method for synthesizing spherical silver nanoparticles.

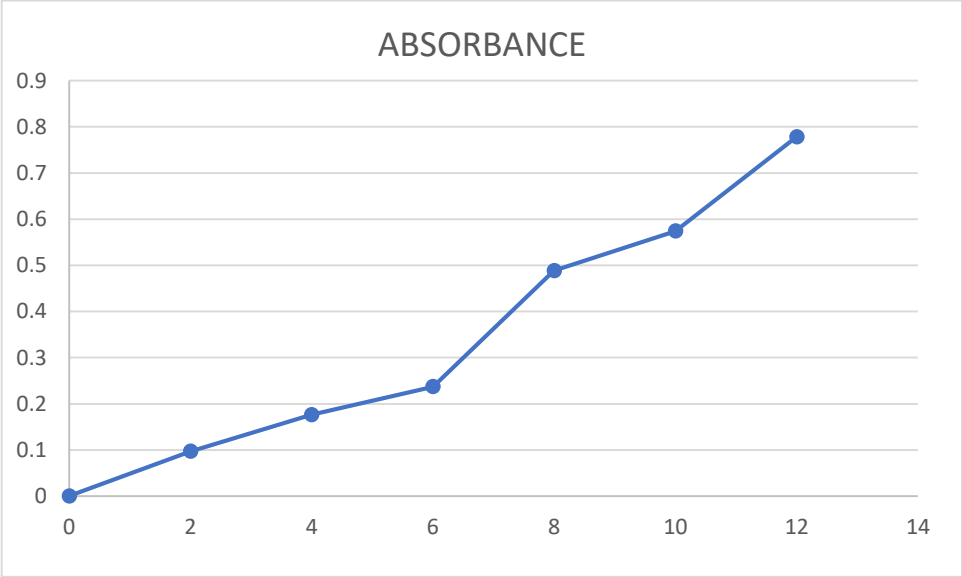

FIGURE 3. The graph represents the absorbance value of the sample

3.5 SEM ANALYSIS

Scanning electron microscopy (SEM) offered additional insights into the morphology and size characteristics of the silver nanoparticles. The figures depict scanning electron micrographs showcasing the silver nanoparticles produced through the suggested green synthesis method.

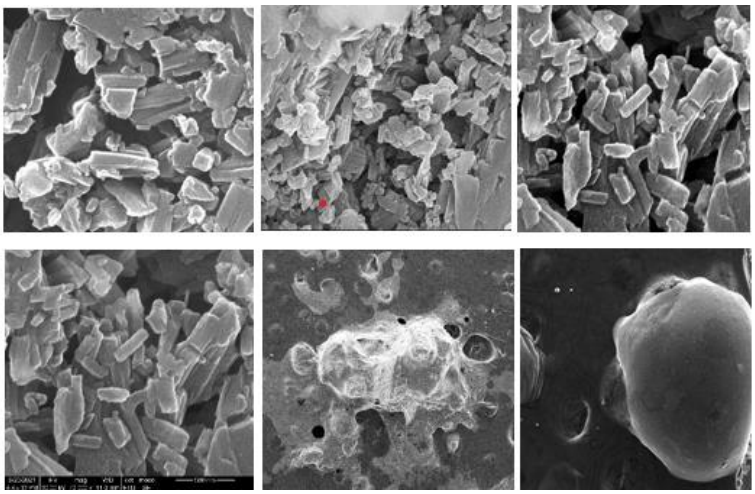

Figure 4. Morphology of Silver Nanoparticles

3.6 FTIR ANALYSIS

The FTIR analysis of silver nanoparticles confirmed the dual function of the plant extract, acting both as a reducing and capping agent. The presence of specific functional groups was also identified. A broadband appearing around 3648.66 cm^{-1} indicated the stretching vibration of groups NH2 and OH, with overlaps from water and *Mucuna pruriens* seed extract molecules. At 1428.03 cm^{-1}, an amide C=O stretching was observed, while a peak at 2361.41 cm^{-1} was attributed to the alkyne group present in the phytoconstituents of the extract. Peaks at 1115.62 cm^{-1} indicated the presence of C-O-C linkages or C-O- bonds, primarily associated with flavonoids and terpenoids abundantly present in the seed extract.

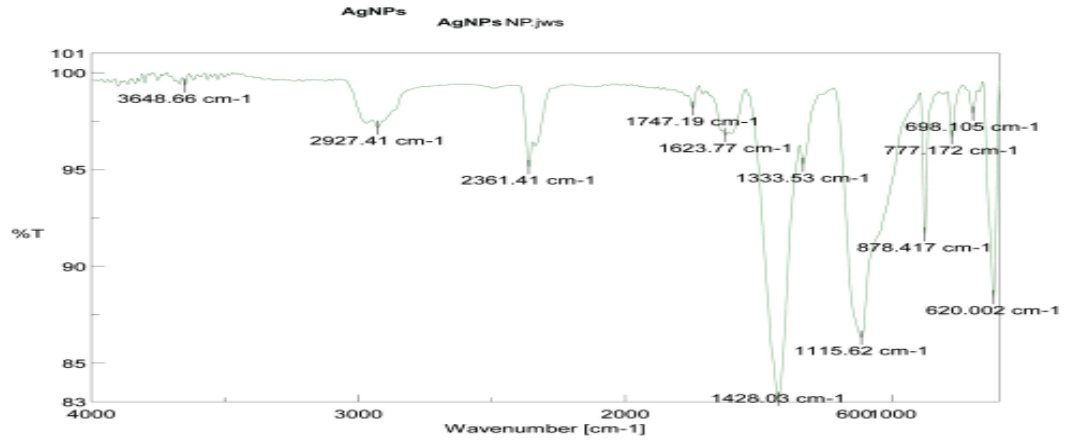

Figure 5. FTIR Spectral peak values

3.7 CYTOTOXIC SCREENING

A total of 3 samples which include silver nanoparticles, PVA, and PVA with silver nanoparticles were screened for cytotoxic activity against HEK cell lines. The cell viability of these three samples is compared in the given graph.

Cytotoxic activity of sample (µg/ml)

Sample	Sample (Inhibitory Concentration/ IC_{50}) HEK
Particle	Insignificant toxicity up to (100µg/ml) (Biocompatibility)
PVA	Insignificant toxicity up to (100µg/ml)
Particle +PVA	Insignificant toxicity up to (100µg/ml)

IC_{50} – Values of the respective sample (at 24 hrs)

Statistics:

All the *in vitro* experiments were done in triplicate, and the experiments were repeated at least thrice. The statistical software SPSS version 17.0 was used for the analysis. *P* value <0.05 was considered significant.

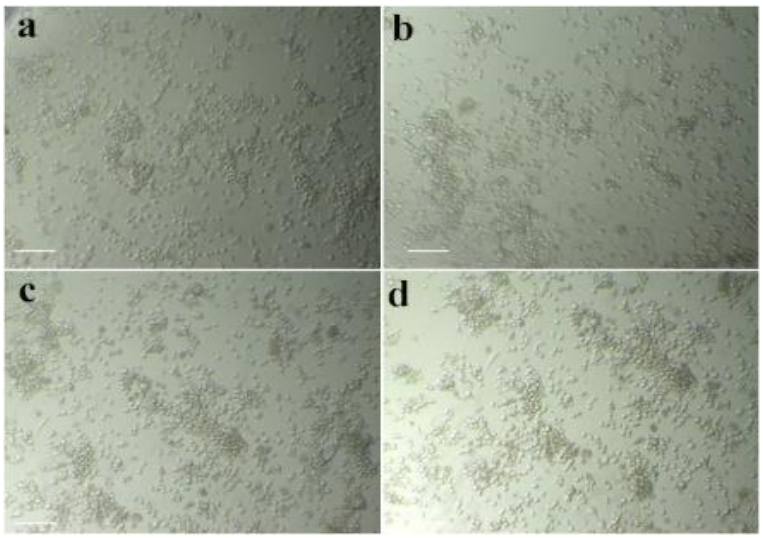

Figure 6. The cell cytotoxicity of the silver nanoparticles performed by MTT assay

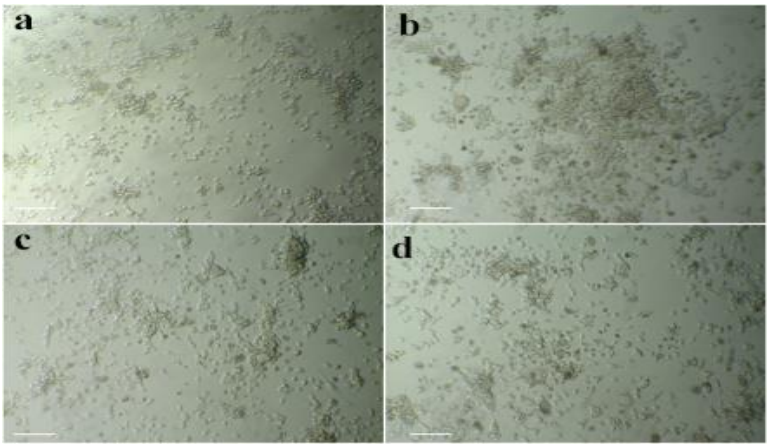

Figure 7. The cell cytotoxicity of the PVA performed by MTT assay

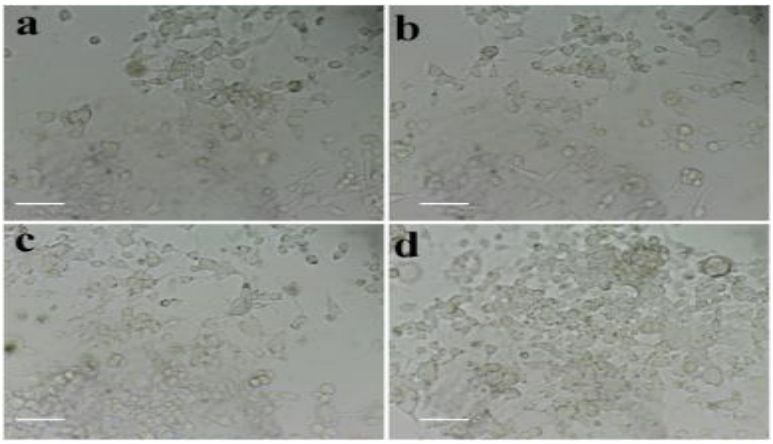

Figure 8. The cell cytotoxicity of the PVA and AgNp performed by MTT assay

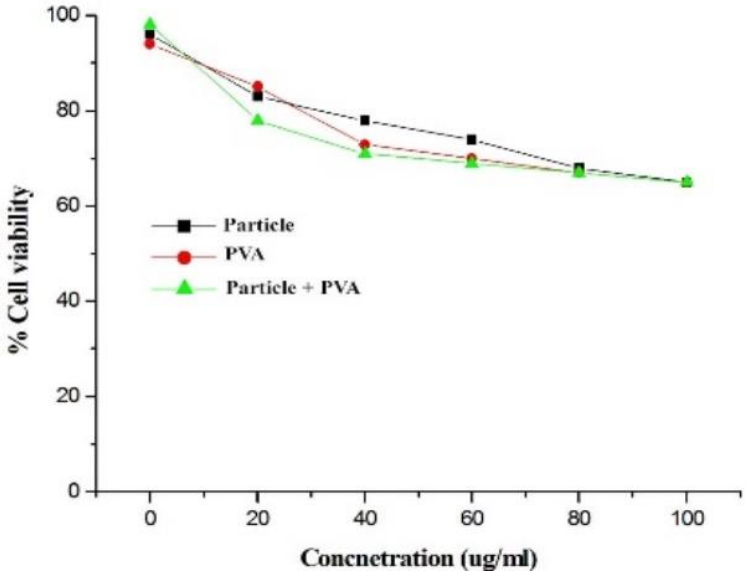

Figure 9. Graph showing the cell viability of the samples

3.8 SCRATCH ASSAY

The migration of cells was assessed via scratch assay at two-time points: 0 hours (control) and after 24 hours of incubation. The experiment involved the use of loaded hydrogels, at a concentration of 1 mg/ml. A549 cells were utilized in this study.

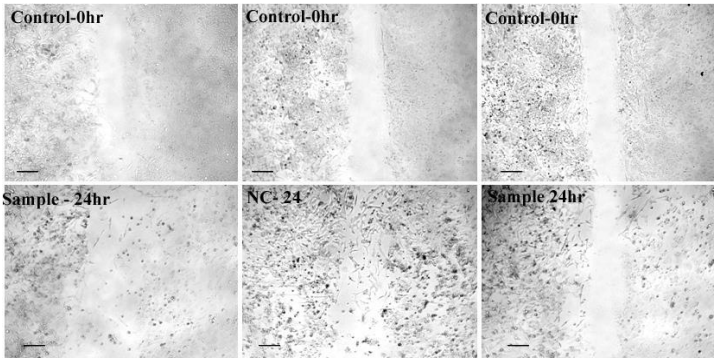

Figure 10. The migration of cells was observed by scratch assay

3.9 ANTIBACTERIAL ACTIVITY

We have explored the potential of utilizing silver nanoparticles mediated by *Mucucna pruri*ens extract as antibacterial agents. Both the plant extract and the resulting silver nanoparticles were promptly evaluated for their ability to inhibit the growth of gram-negative (*E. coli*) bacteria, as indicated by the zones of inhibition observed. The synthesized silver nanoparticles demonstrated significant antibacterial activity against *E. coli*, as evidenced by the notable zones of inhibition. However, neither the control nor the plant extract alone exhibited any discernible antibacterial activity. It is plausible to deduce that although the plant's leaf extract possesses inherent antibacterial properties, its effectiveness alone is compromised due to factors such as the extraction medium and lower concentration during experimentation. The results detailing the antibacterial efficacy of the prepared silver nanoparticles, determined through the disc diffusion method, are outlined in the subsequent table.

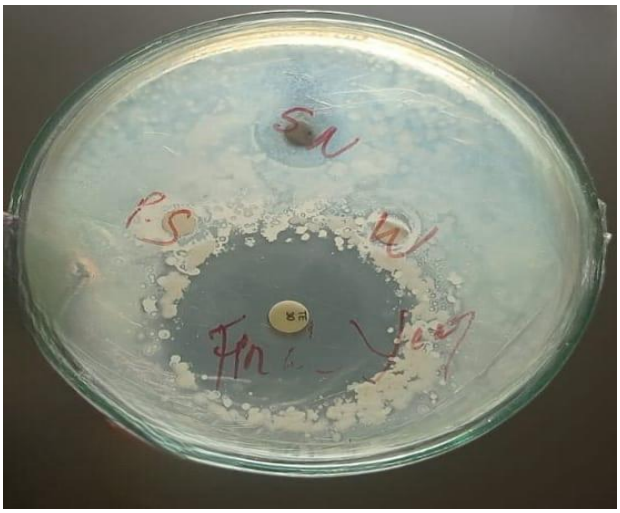

Figure 11.The antibacterial susceptibility of prepared compounds

3.10 ANTIOXIDANT ASSAY

The DPPH radical scavenging activities of the total extracts of the seed extract of *Mucuna pruriens* are shown in the following figure.

Figure 12. Antioxidant Assay

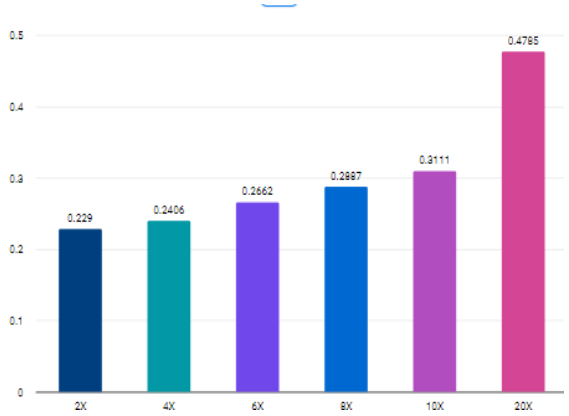

Figure 13. Standard graph for Antioxidant assay

4.0 CONCLUSION

Mucuna pruriens seed extracts were effectively employed as capping and reducing agents in the fabrication of silver nanoparticles. UV-visible characterization of these nanoparticles unveiled a moderate stability range. The investigation into antioxidants seeks to provide a defense against the potential harm inflicted by free radicals. SEM (Scanning Electron Microscopy) is additionally employed to examine the surface morphology of silver nanoparticles during characterization. Furthermore, FTIR analysis detected the existence of functional groups on the nanoparticles. When these compounds are integrated into biofilms, such as Band-Aids, they can serve as effective materials for wound dressings, offering enhanced therapeutic benefits and promoting healing.

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