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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *MORINGA CONCANENSIS* NIMMO LEAVES AND EVALUATION OF THEIR ANTIMICROBIAL AND CYTOTOXIC ACTIVITY

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Abstract

Background and Objectives: Silver nanoparticle (AgNP) synthesis and characterization is an area of vast interest due to their broader application in the fields of science and technology and medicine. Plants are an attractive source for AgNP synthesis because of its ability to produce a wide range of secondary metabolites with strong reducing potentials. The main objective of the present study was to the synthesis of AgNPs from aqueous leaves extract of *Moringa concanensis* Nimmo and evaluation of its antimicrobial and cytotoxic activity.

Materials and Methods: The silver nanoparticles were synthesized by green route method. The synthesized AgNPs were characterized by UV–Visible Spectroscopy and Scanning electron microscopy (SEM). The antimicrobial activity of synthesized AgNPs was evaluated against the clinical isolates and cytotoxic activity was tested by using breast cancer cell lines (MCF-7).

Results: The synthesized silver nanoparticles possessed a good antimicrobial and cytotoxic activity. It was found that microbial growth was significantly inhibited in a dose dependent manner and showed a notable cytotoxic activity against MCF-7 cell lines.

Conclusion: Based on the above results, it was concluded that the synthesized AgNPs from an aqueous extract of *Moringa concanensis* Nimmo leaves could be used as a potential antimicrobial and cytotoxic agent for the commercial application.

Key words: *M.concanensis* Nimmo, silver nanoparticles, SEM, antimicrobial and cytotoxic activity

1. Introduction

The synthesis and characterization of noble metal nanoparticles such as silver, gold and platinum is an emerging field of research due to their important applications in the fields of biotechnology, bioengineering, textile engineering, water treatment, metal-based consumer products and other areas, electronic, magnetic, optoelectronics, and information storage [1]. The silver nanoparticles (SNPs) have been widely utilized in biology and medicine due to its attractive physicochemical properties [2]. Nanoparticle synthesis is currently a significant area of research, searching for an eco-friendly approach and green materials for current scenario.

This is due to the use of certain toxic chemicals in the synthesis protocol or formation of harmful by-products. Synthesis of metallic nanoparticles using plant extracts is inexpensive, easily scaled up, environmentally benign and especially suited for making nanoparticles that are free of toxic contaminants as required in therapeutic applications [3]. Silver nanoparticles (AgNPs), as antibacterial agents, are now used extensively in the fields of medicine and drug delivery [4]. Silver nanoparticles is a nontoxic, safe inorganic antibacterial agent used for centuries and are capable of killing more than 500 types of diseases causing microorganisms. It has a significant potential for a wide range of biological applications such as preventing infections, healing wounds, anti-inflammatory and use as an antibacterial agent for antibiotic resistant bacteria [5]. Synthesizing silver nanoparticles by medicinal plants is eco-friendly and possesses effective biological activities [6]. The plant *Moringa concanensis* Nimmo abundantly seen in around Perambalur district of Tamilnadu, commonly known as Kattu murungai or Peyi murungai [7] and in Warangal district of Andhra Pradesh, commonly known as Adavi Mulaga, Konda Mulga [8 & 9]. All part of the tree *Moringa concanensis* Nimmo is reported to be used in the treatment of as cites, rheumatism, venomous bites, painful swellings, gastrointestinal motility disorders as a cardiac and circulatory stimulant. Along with other therapeutic applications, 'The Ayurvedic Pharmacopoeia of India' indicated the uses of dried leaves in goiter, glycosuria, and lipid disorders [10]. The present study was designed to synthesize and characterization of silver nanoparticles from aqueous leaves extracts of *Moringa concanensis* Nimmo and evaluation of their antimicrobial and cytotoxic activity. The aqueous extract of *Moringa concanensis* Nimmo leaves was used to synthesize the silver nanoparticles on the basic of cost effectiveness, ecofriendly sources, easily available and its medicinal values.

2. Materials and methods

2.1 Collection and identification of plant

The healthy, matured and insect bites free leaves of *Moringa concanensis* Nimmo plant (Family - *Moringaceae*) was collected from Esanai village, Perambalur district, Tamilnadu, India (Latitude – 11.2982° N, Longitude – 78.8298° E) from the month of December 2016. The plant sample was identified and authenticated by Dr. C. Murugan, Scientist, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The identification number BSI/SRC/5/23/2016/Tech-152.

2.2 Synthesis of silver nanoparticles

2.2.1 Preparation of plant extract

The 10 gms of the dried *M.concanensis* Nimmo leaves powder were kept in a beaker containing 100 ml double distilled water and boiled at 80˚C for 10 minutes to obtained bioactive compounds from *Moringa concanensis* Nimmo leaves. The extract was cooled and filtered through normal filter paper followed by Whatmann filter paper No.1. The final extract was used to synthesize silver nanoparticles.

2.2.2 Synthesis of silver nanoparticles

The aqueous solution of silver nitrate at a concentration of 0.001M was prepared to synthesize silver nanoparticles from filtered aqueous leaves extract of *Moringa concanensis* Nimmo. Five ml *Moringa concanensis* Nimmo leaves aqueous extract was slowly added to 95 ml of aqueous solution of $AgNO_3$ while stirring for reduction into Ag ions. The formation reddish brown color was observed after 3hrs incubation at room temperature AgNPs solution was purified by repeated centrifugation at 10,000 rpm for 20 minutes to isolate AgNPs free from other bioorganic compounds present in the reaction medium. After centrifugation, the obtained particles were washed with distilled water for 10 to 20 minutes and kept in hot air oven for drying at 100˚C for 1 hour.

2.2.3 Characterization of silver nanoparticles

2.2.3.1 UV-Visible spectroscopy analysis

The optical measurement is the prime technique for characterizing the biological synthesis of nanoparticles. The formation and stability of silver nanoparticles in aqueous solution was confirmed by UV-Visible spectrophotometer analysis. The reduction of pure Ag^+ ions was monitored by measuring the UV-Visible spectrum

of the reaction medium at 540 nm for 12 hours. UV-Visible spectral analysis was done by UV-Visible spectrophotometer (UV- 2450, Shimadzu, Kyoto, Japan).

2.2.3.2 Scanning electron microscope (SEM)

Scanning electron microscope (SEM) analysis was done using Hitachi S – 4500 SEM machine. The silver nanoparticles were centrifuged at 10,000 rpm for 30 minutes and the pellet was redispersed in 10 ml of ethanol and washed 3 times with sterile distilled water to obtain the pellet.

The pellet was dried in an oven and thin films of dried samples (10 mg/ml) were prepared on carbon coated copper grid and analyzed for size determination. The particle size and texture of nanoparticles can be analyzed by using image magnification software compatible with SEM and helps in determining the presence and formation of silver nanoparticles.

2.3 Antimicrobial activity

2.3.1 Test microorganisms

The test organisms used were clinical isolates *viz., Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Klebsiella nemoniae.* The human fungal pathogens like *Candida albicans and Trichoderma viride,* which were obtained from Department of Microbiology, Hindusthan college of arts and science Coimbatore. The bacteria and the fungal cultures were maintained on nutrient agar medium and potato dextrose agar (PDA) medium respectively.

2.3.2 Preparation of Inoculum

The gram positive bacteria *Streptococcus pyogenes, Staphylococcus aureus* and gram negative bacteria *E.coli, Klebsiella pneumoniae* were pre-cultured in nutrient broth over night in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, the pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm).

The fungal inoculums *Candida albicans, Trichophyton rubrum,* were prepared from 5 to 10-day old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using a sterile spatula. The spore density of each fungus was adjusted with a spectrophotometer (A_{595}nm) to obtain a final concentration of approximately 10^5 spores/ml.

2.3.3 Antibacterial activity

The samples were tested by the well diffusion method [11]. Different concentration of the extracts (20, 40, 60 μg/ml) was prepared by reconstituting with methanol. The test microorganisms were seeded into the respective medium by spread plate method 10 μl (10 cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After solidification, the filter paper wells (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Chloramphenicol (10 μg) was used as a standard for the antibacterial test. The antibacterial assay plates were incubated at 37°C for 24 hrs. The diameters of the inhibition zones were measured in mm.

2.3.4 Antifungal Activity

The antifungal activity was tested by well diffusion method. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper wells (5 mm in diameter) impregnated with 100 μg concentrations of the synthesized silver nanoparticles were placed on test organism-seeded plates. Streptocycline (10 μg) used as positive control. The activity was determined after 72 hrs of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

2.4 Cytotoxic activity

2.4.1 Cell line

The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

2.4.2 Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give a final density of $1x10^5$ cells/ml. One hundred microliters per well of cell suspension were seeded into 96-well plates at a plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity.

After 24 hrs the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethyl sulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of the medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

2.4.3 MTT assay

3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 hrs of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows

$$\% \text{ Cell viability} = [A] \text{ Test} / [A]\text{control} \times 100$$

$$\% \text{ Cell inhibition} = 100 - [A] \text{ Test} / [A]\text{control} \times 100$$

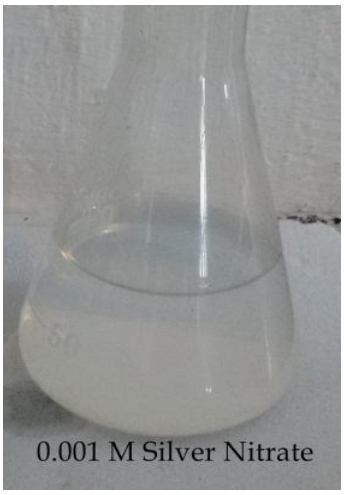
3. Results

3.1 Results of green synthesis of silver nanoparticles from *Moringa concanensis* Nimmo leaves

3.1.1 Visual observation

The silver nanoparticles were synthesized from an aqueous extract of *Moringa concanensis* Nimmo leaves. The formation of silver nanoparticles from reaction medium was confirmed by color change. The reaction mixture contains the aqueous extract of *M.concanensis* Nimmo leaves and aqueous silver nitrate solution. After the 24 hrs of dark incubation, the color of the reaction medium was changed from light brown to black (Figure 1). This result indicates the formation of silver nanoparticles by the reduction of Ag ions.

Figure 1: Synthesis of silver nanoparticles from *M.concanensis* Nimmo leaves



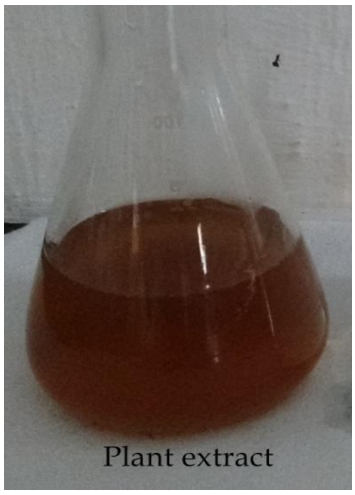


Figure – A **Figure – B** **Figure – C**

Figure A – Aqueous silver nitrate solution (0.001M)

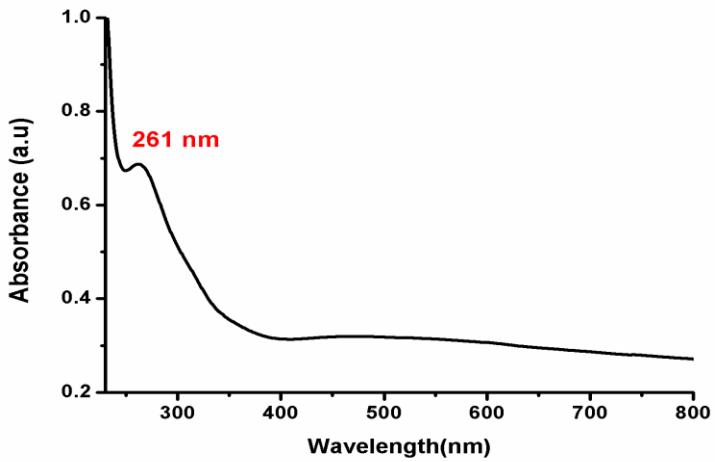
Figure B – Aqueous extract of *Moringa concanensis* Nimmo leaves

Figure C – Reaction medium after 24hrs dark incubation

3.1.2 UV-Visible Spectroscopy analysis of synthesized silver nanoparticles from aqueous extract of *M.concanensis* Nimmo leaves

The UV spectrum showed (Figure 2) the surface plasma AgNPs at increasing concentration was taken and the color changes were observed for nanoparticles. For silver color changes from colorless to dark brown color. Metal nanoparticles can be synthesized by reducing metal ions using some chemical molecules. In green synthesis, this is observed that natural material extract act as reducing agent for generation of metal nanoparticles**.**

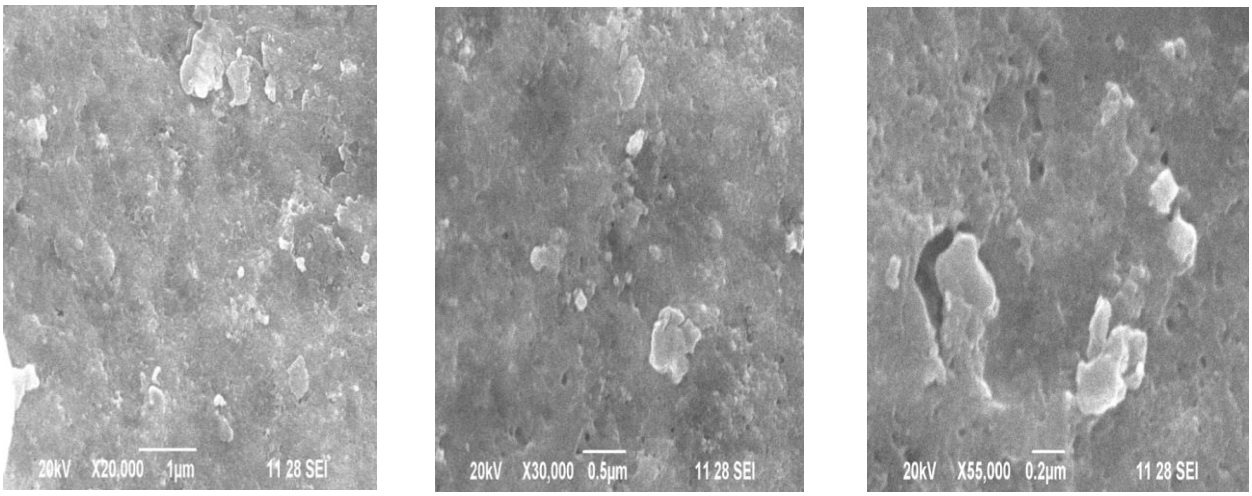
Figure 2: UV visible spectral analysis of silver nanoparticles



3.1.3 Scanning electron microscopic analysis of synthesized silver nanoparticles from aqueous extract of *M.concanensis* Nimmo leaves

The SEM image showed individual silver nanoparticles as well as a number of aggregates, SEM images of silver nanoparticles derived from the leaf extracts of *M.concanensis* Nimmo showed particles to be in spherical shape with size ranging from 0.2 to 1 µm (Figure 3).

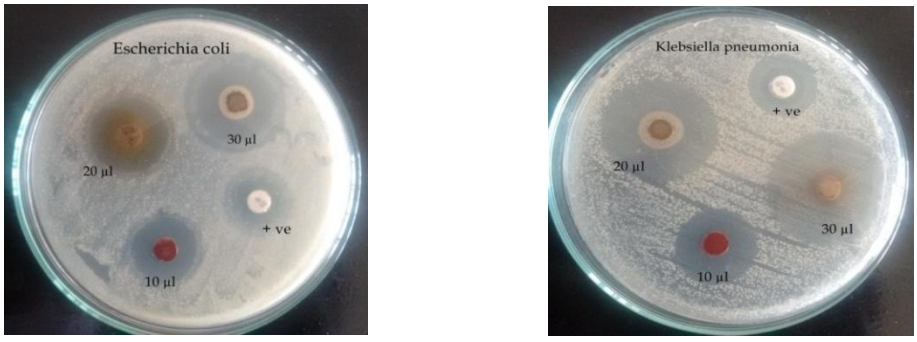
Figure 3: Scanning electron microscopic analysis silver nanoparticles



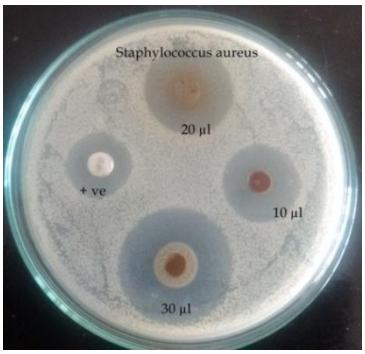
3.2 Results of antibacterial activity of silver nanoparticles of *Moringa concanensis* Nimmo

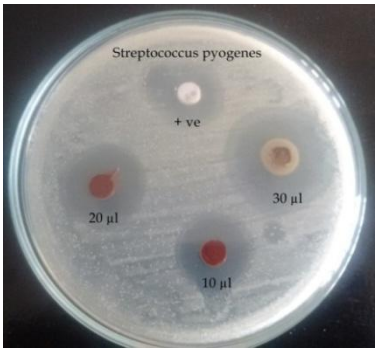
The antibacterial activity of synthesized silver nanoparticles of *Moringa concanensis* Nimmo leaves was depicted in (Table 2). All the concentrations of silver nanoparticles showed a markable activity. Especially *Staphylococcus aureus* and *Klebsiella pnemoniae* showed more sensitivity in 30 µl concentration. The inhibition zone length of *S.aureus* is 13 mm in 30 µl concentration, and the *K.pneumoniae* were 12 mm on 30 µl concentration.

Plate 1: Plates of antibacterial activity of synthesized silver nanoparticles of *Moringa concanensis* Nimmo.



Escherichia coli *Klebsiella pneumonia*



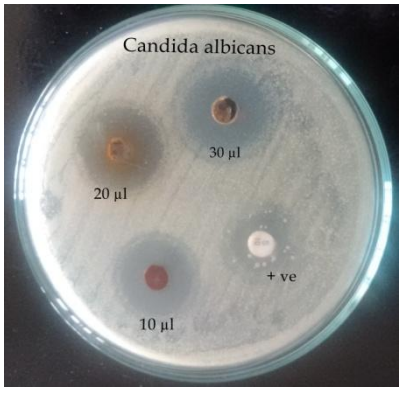


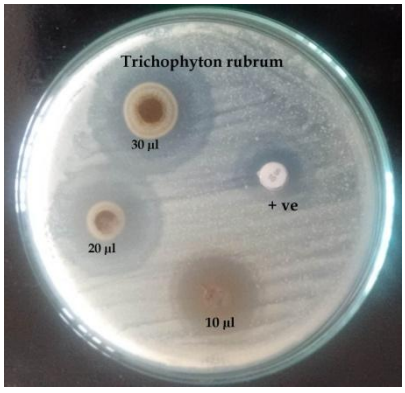
Staphylococcus aureus *Streptococcus pyogenes*

3.3 Results of antifungal activity of silver nanoparticles of *Moringa concanensis* Nimmo

In the antifungal activity of synthesized silver nanoparticles of *Moringa concanensis* Nimmo leaves, the *Trichophyton rubrum* showed maximum sensitivity against 30 µl concentration (Table 3). *T.rubrum* showed a maximum inhibition zone of 09 mm 30 µl concentration and *C.albicans* showed 08 mm inhibition zone in 30 µl concentrations.

Plate 2: Plates of antibacterial activity of synthesized silver nanoparticles from of *Moringa concanensis* Nimmo.





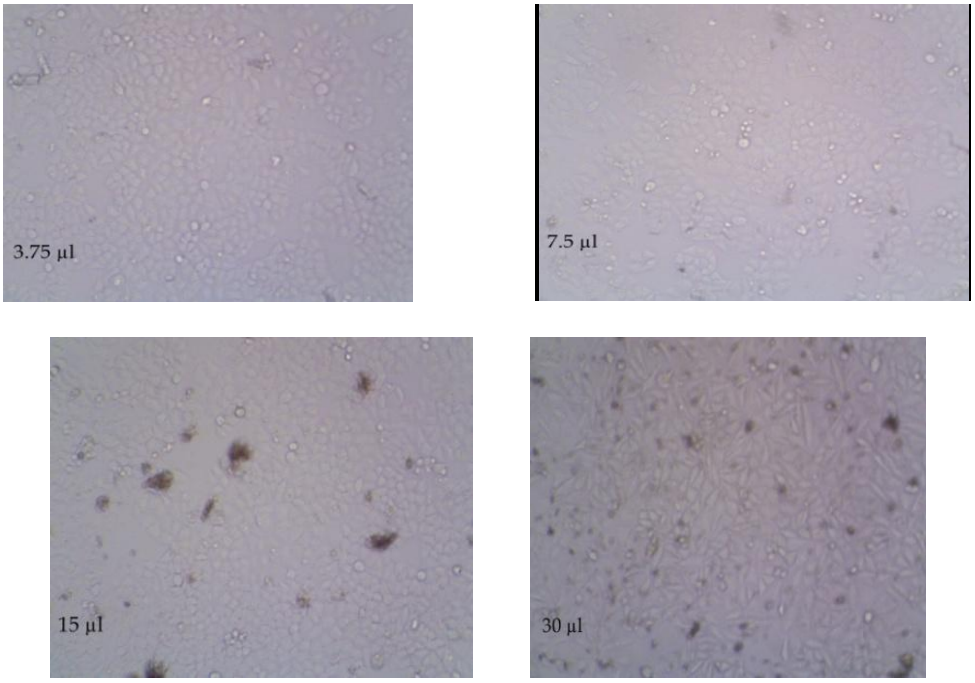
Candida albicans *Trichoderma rubrum*

4.3 Result of cytotoxic activity

The cytotoxic activity of synthesized silver nanoparticles of *Moringa concanensis* leaves is shown in table 4 and plate 3. The silver nanoparticles were tested against MCF-7 cell lines. The nanoparticles showed potent activity against the human breast cancer cell lines. The viability of the MCF-7 cells was decreased when the nanoparticle concentration was increased. The 1.88 µg/ml of nanoparticles was inhibited 7.88% of cell viability. The

synthesized silver nanoparticles from *Moringa concanensis* Nimmo aqueous leaves extract inhibited the cell viability of MCF-7 cell lines.

Plate 3: Plates of cytotoxic activity of synthesized silver nanoparticles from of *Moringa concanensis* Nimmo.



4. Discussion

In the present study, the silver nanoparticles were synthesized from *M.concanensis* aqueous leaves extract showed particle size ranging in between 0.2 and 10 µm, which may confer the ability to penetrate the cells/microbes and execute the bactericidal property. The mechanism of action of silver nanoparticles is ambiguous in a microorganism [12]. The silver nanoparticles synthesized from an aqueous extract of *Moringa concanensis* Nimmo leaves has shown remarkable antimicrobial and cytotoxic activity. Nanomaterials had proven to be the efficient mode of drug delivery in modern science [13]. The characterization of nanoparticles was done using various techniques such as UV–visible spectroscopy and scanning electron microscopy.

In the present study, the synthesized silver nanoparticles from an aqueous extract of *Moringa concanensis* Nimmo leaves was tested against various human pathogenic bacteria species like *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae.* And the fungal strains were *C.albicans* and *T.rubrum.* The antibacterial activity of ethanolic extract of *Moringa concanensis* Nimmo leaves was showed in (Table 2). All the concentrations of synthesized silver nanoparticle showed a remarkable activity. Particularly *S.aureus* and *K.pnemoniae* showed more sensitivity in 30 µl concentration. The inhibition zone length is 13 mm in *S.aureus* on

30 µl concentration and 12 mm in *K.pneumoniae* on 30 µl concentration. In the antifungal activity of silver nanoparticles of *Moringa concanensis* Nimmo leaves, the *C.albicans* showed maximum sensitivity against 30 µl concentration (Table 3). *T.rubrum* showed a maximum inhibition zone (09 mm) and *C.albicans* showed 08 mm inhibition zone in 30 µl concentration.

The cytotoxic effect of synthesized silver nanoparticles of *Moringa concanensis* Nimmo leaves against MCF-7 cell lines was assayed by MTT assay. MTT assay is suitable in vitro method for cytotoxicity against cancer cell lines and noncancer cell lines [14]. A large and increasing number of patients in the world use medicinal plants and herbs for health purposes. Therefore, scientific scrutiny of their therapeutic potential, biological properties and safety will be useful in making wise decisions about their use. The synthesized silver nanoparticles were tested against MCF-7 cells lines. The nanoparticles showed significant activity against the human breast cancer cells. When the drug concentration was increased, the viability of the MCF-7 cells was decreased. The *Moringa concanensis* leaves extract inhibited the cell viability of MCF-7 cells.

Silver nanoparticles have the ability to anchor to the bacterial cell wall and subsequently penetrate to the cell, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. There was a formation of pits on the cell surface, and there is an accumulation of the nanoparticles on the cell surface [15].

Silver nanoparticles, due to their antimicrobial properties have been used most widely in the health industry, medicine, textile coatings, food storage, dye reduction, wound dressing, antiseptic creams and a number of environmental applications [16]. Since ancient times, elemental silver and its compounds have been used as antimicrobial agents; and was used to preserve water in form of silver coins/silver vessels [17].

5. Conclusion

The biosynthesized silver nanoparticles using aqueous leaves extract of *Moringa concanensis* Nimmo leaves were characterized, they are crystalline, spherical and monodispersed nanoparticles. Synthesized silver nanoparticles revealed good antimicrobial activity against the selected pathogenic microorganisms and cytotoxic activity against human breast cancer cell lines (MCF-7). This green synthesis approach appears to be a cost-effective, non-toxic, eco-friendly alternative to the conventional microbiological, physical and chemical methods and would be suitable

for developing a biological process for large scale production. These silver nanoparticles may be used for reducing the microbial load.

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