

A Study on Silver Nanoparticles Antibacterial Efficacy against Multi-Drug Resistant Clinical Isolations

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Abstract – The present study reported an eco-friendly process for quick synthesis of silver nano particles (Ag-NPs) using an aqueous leaf extract of *Corchorus Capsularis*, with an aim to investigate the new and effective and cheap nano-therapeutic approach to *P. Aeruginosa*, *staphylococcus aureus* and coagulase negatives (CoNS, CRCP). At various intervals the formation of stable Ag-NPs usually provides range from 5 to 45 nm in diameter to spherical particles. The resultant Ag-NPs were analyzed by ultra-violet spectroscopy (UV-Vis), Fourier infra red transform (FT-IR), X-ray diffraction (XRD), Electron Microscopy (TEM) and Energy Dispersive Radiation Analytics (EDM) (EDX). XRD analysis indicates that the particles with face centered cubic shape are crystalline in nature. The production of Ag-NPs at 20.52 nm is shown by the TEM study. Multi Drug Resistant (MDR) *P. Aeruginosa*, *Staphylococcus Aureus* and CoNS isolates from post-surgical wound infections were examined for antibacterial activity from synthesized Ag-NP. This research demonstrates that Ag-NPs produced using CRCP aqueous leaf extract have considerable antibacterial activity in post-chirurgical wound isolates.

Keywords – Silver Nanoparticles, Multi Drug Resistant, Clinical Isolations;

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INTRODUCTION

Clinical isolates collection and conditions of growth

Swab samples were taken at the Ayder Referral and Teaching Hospital, Ethiopia, by the suspected individual who showed clinical symptoms from post-surface injury infection. Using regular saline and sterile gauze, the infected spot was cleansed. Two wound swabs have been taken using a patient's sterile cotton swab and transferred and then processed within 1 hour of collection at the College of Mekelle, Ethiopia, School of Clinical Microbiology. For first injury swab, a gram stain spray was employed, while Blood agar, Mac Conkey, and Mannitol-salt agar were infected on the other site and

kept 24 to 40 hours at a temperature of 37°C. In fact, gram defects, catalytic reactions, coagulase and homolytical activity of sheep's blood agar have been found to identify bacterial micro-organisms. Kilo micro-organisms based on colony Blood agar, Mac Conkey and chemical responses such as oxidase and triple sugar iron were later discovered Tsi. *P. Aeruginosa*, *Staphylococcus Aureus* and CoNS were the major bacterial isolates. The Muller Hinton agars

at the microbiology laboratory, which was kept at 4°C for Ag-NP antimicrobial effectiveness testing, were at times sub-growned to manage viability. The research has been authorized by the University of Mekelle Health Research College's Ethical Review Committee (#REC REF 2012-127).

Also at 37°C and shaking at 150 rpm, sub-cultural samples were identified in Müller Hinton (pH 7.3 ± 0.1; Oxiod, Oxshire, UK). For following antimicrobial efficacy experiments employing Ag-NPs, cells isolates were tested to McFarland's 0.5 TU for sterile solution dilute to achieve the ultimate concentration of 107 CFU/ml.

Disc Diffusion Assay

Disc diffusion tests were conducted to evaluate the effectiveness of antibiotic patients in laceration patients with CoNS and MRSA isolates. The inoculus for each isolation of the bacterium was aseptic and the cell density was adjusted to the 0.5 McFarland turbidity criterion to evaluate antimicrobial property. The 6 mm Whatman No-1, which could aseptically dry at room temperature for a 30 minute period, was coated with Ag-NPs of various temperatures, (20, 40 or 60 mg/mL). The

discs were coated with the Ag-NP and cultured 24 hours at 37°C on the contaminated MHA plate. As a control group Gentamycin (15 µg) was the standard antibiotic disc (Oxiod, Hampshire, UK).

Staphylococcus Aureus and adverse events against P. Aeruginosa Ag-NPs bactericidal conduct.

"106 CFU of P. Aeruginosa, Staphylococcus Aureus and CoNS postoperative wound infection isolates of P. Aeruginosa were treated individually with various levels of Ag-NPs (20, 40/60, 80/100µg/mL) and controls 100 µg/ml of lyophilized leaf extracts free from the Ag-NPs to determine the antibacterial effect of Ag-NPs. These formulae were incubated for 1 h at 37 °C and placed on plates with LB agar. The plates were incubated for 24 hours at 37 °C and the colonies were counted against the automated counter".

Determination of P. Aeruginosa, staphylococcus Aureus and CoNS kinetics of growth

The bacterial growth kinetics have been studied to determine how cells from each bacterial isolate in LB borde media produce the effects of Ag-NPs at various levels (20, 40, 60, 80, 100 µg/ml). Under the same circumstances in the presence of Ag-NPs a control (100µg/ml lyophilized plant extract) was maintained. It was incubated at 37°C and the optical density (OD) at an interval of 3 to 18 h was measured at 660 nms.

Ag-NPs test in vitro cytotoxicity

In the cell Vero of the African Green Monkeys cell, Cyto-toxicity testing was carried out for pure and lyophilized Ag-NPS. In DMEM, 10% of fetal bovine serum, 100 microns/ml IU/ml and 50.0 ml of Gentamycin, a cell was produced. Cell was grown using 25 cc DMEM flask. With the addition of DMEM, the cells were grown. Cells were cultivated as a

monolayer in cultivated flasks in CO₂ incubators, at 37°C in humidified air with 5% of CO₂. All tests have been conducted with 20 or fewer cells. Serum medium containing 200-1,5 µg/ml lyophilized Ag-NPs was changed in serum-free media and was dissolved in DMSO and kept at -20 degrees throughout the research period. DMSO had less than a 1.0 percent ultimate working level. In the Vero cultivated unit, a 96-wave (cell content adjusted to 1 10⁵ cell/well) suitable for 6 hours was planted and the cells treated were 6-12, 24, 48 and 72 h at a different concentration of lyophilized green Ag-NPs synthesized from the plant extract (concentration from 1,5 mg/mix/ml to 200 ml each serial well dilution). In fact, control groups with the same quantity of DMSO were maintained. For 2-3 hours, the cells were put in a moisturized 5% incubator. The cyto-toxicity of Ag-NPs to the development of the cells was evaluated after three weeks of fermentation

using a DMSO color and a 970 nm well filter syringe (BIO-RAD, Mod. 680, USA).

REVIEW OF LITERATURE

B. A. Iwalokun et. al. (2019) suggested the excess of mortality in hospitals and in the population continues to be accounted for by S. Aureus infections and constitutes an increasing worldwide health concern. However, there are no data on the effectiveness and operational mechanism in poor nations for alternate treatments such as silver nanoparticles. This research examined silver nano particles' (Ag-NP) anti-taphylococcal efficacy against indigenous strains in Nigeria. A total of 119 S. aureus clinical isolates from five MRSA (n = 52) and MSSA (n = 67) labs in Nigeria have been examined by PCR. Microbroth-based dilution technique was used to evaluate Ag-NPs MIC generated by a chemicals reduction process defined by surface plasmon resonance absorption and size equivalence. It has also studied its impact on the activity of protease and plasmids. MDR isolate phenotype MDR, conductivity of several plasmids (15-32 kb) inside the MSSA MDR and mean protease activity of 24.8-55.7U/mL have been identified as basic features of isolates. A high absorbance was obtained by the chemically produced Ag-NPs with a height of 4.58 nm. The mixtures of Ag-NP for isolates for MRSA and MSSA (P > 0.05) were 4.7 µg/mL and 4.9 µg/mL, respectively. The bactericidal effects on MSSA and MRSA isolates in 2.7–5.5 hours after in vitro exposure were found at Ag-NP at 2.5–5 µg/mL. Further research shows that the MDR MSSA isolates were treated to 5 µg/mL of Ag-NP and that extracellular protease activity was dose-dependently lowered by 84.6–93.1 percent.. Further analysis, the MIC range did not include Homolysis of Ag-NP human erythrocytes. conclusion: The safety and effectiveness in the use of plasmid expulsion and protease inhibition as modes of action against clinical MDR S. Aureus in Nigeria was shown by this research.

Faizan Abul Qais et. al. (2019) in this paper the development of pathogens multi-medicine resistance is becoming a world-wide issue with bacterial infection chemotherapy. The two main categories of troublesome MDR bacteria that have developed quickly in the recent past, including extended-spectrum β-Lactamase (ES βL-) producing enteric and methicillin-resistant Staphylococcus Aureus (MRSA). In this research, Murrayakoenigii extract was utilized to synthesize silver nanoparticles. The synthesised, UV-Vis spectroscopy, FTIR, XRD, SEM and TEM were used to characterize and their antibacterial potentials on various enteric bacteria generating ES βL- and Mresa were assessed. The nano particles were discovered mostly to have a spheroidal distribution of 5–20 nm in particle size. The percentage of MK Ag-NPs was 60.86% silver. Assessing antibacterial activities using disc-

diffusion analysis reveals that the development of test pathogens with different zones of inhibition is successfully suppressed by MK-Ag-NPs. MICs of both MRS and MMSSA strains were 32 µg/ml for MK-Ag-NP, while they varied from 32 to 64 µg/ml for ES βL producer *E. coli*. With a MIC of 16 µg/ml, *E. coli* (ECS) control strain was considerably more sensitive. The MBCs corresponded to the corresponding MICs. Growth kinetics analyzes showed that in the presence of 32 µg/mL of MK-Ag-NP, the growth of all tested strains of *S. Aureus* (to 90%), was suppressed. With a >81 percent inhibition of 16 µg/ml the sensitive strain of *E. coli* (ECS) was less resistant to MK-Ag-NPs. In this study, the in vitro effectiveness of green manufactured MK-Ag-NPs was encouraged and further in vivo evaluation of their therapeutic potential against MDR bacteria was required.

OBJECTIVE

The objective of the research is to explore the Silver nano particles antibacterial efficacy against Multi-Drug Resistant Clinical Isolations

METHODOLOGY

Experimental Section

The synthetic antibacterial activity of Ag-NP was investigated for multi-drug-resistant (MDR) *P. Aeruginosa*, *Staphylococcus aureus*, and CoNS insulation of postoperative wound infections. The study shows that MDR isolates from postoperative injury infections, produced by the watery CRCP leaf extraction, have high antibacterial activities.

Materials and Methods

Merck's silver nitrate was obtained (>99.9% pure) (India). Without further clearing, all reagents were used and analyzed for this research. The whole procedure was used with triple deionized water of 18.2 MΩcm⁻¹ resistivity. "The CRCP medicine leaf used to synthesize Ag-NPs was collected from Thiagarajar College, Madurai, Tamilnadu, India. For the analyzes of antimicrobial tests bacteria strains *P. Aeruginosa* resistant, *Staphylococcus Aureus* resistant, and Coagulase negative *Staphylococci* (CoNS) post-surgical infection isolates were utilized".

For the fundamental evaluation of Ag-NP size and shape TEM (JEOL JEM 2100) coupled to the EDX spectrometer were used. The Shimadzu FT-IR-8201 PC tool has been used to carry out FT-IR. For UV-spectral analysis, a double-beam Jasco V-560 spectrophotometer was employed. X-ray diffraction on pure Ag NPs using XPERTPRO data analysis (XRD, PW3050/60) was carried out on.

RESULTS AND DISCUSSION

In surgery, the increasing expenses of care, morbidity and mortality, post-operative wound infections are issues all around the globe, related to prolonged hospital stays. Most infections are received in hospitals and vary across facilities. Studies showed that 14, 8 - 60 percent of the infections produced by the most common post-chirurgical "*Staphylococcus Aureus*, CoNS and *P. Aeruginosa* bacteria". The lack of consistent criteria for diagnosis is a barrier to track the infectious epidemiology at the world wide location. In addition, the management of post-operative lesions is more complicated because of the development of high anti-microbial resistance of bacterial infections. Different biotechnology techniques using micro-organisms and vascular plants have been suggested. In lack of deleterious effects at very low bacterial concentrations Ag-NPs have been shown to be harmless and highly effective compounds, viruses and other eukaryotics. In order to make a practical application for the production of metal nanoparticles in various biofields, catalysis, detection and bacterium storage systems, many physical and chemical techniques were developed. The MNP study focuses on the investigation of benign Green Synthesis and applications in antibacterial, antioxidant, and anti-tumor properties. Plant extracts for synthetization without any chemical components were used in biosynthesis processes as a viable alternative for MNP creation. The active microbicidal activity of nano-silver on bacteria, fungi, and viruses is well known. The complex surface and the interaction between the microbial environment is a factor in the high antibacterial activity of Ag-NP. We report on the synthesis of CRCP sheet extract agents in this research as both a stabilizing and decreasing agent. CRCP leaves were widespread in use as an encouraging, laxative, pregnant, demulgent and stomach for the treatment of fiber, constipation, dysentery, liver illness and dyspeppia. A root or unripe fruit decoction was also used to cure diarrhea. "All biologically generated Ag-NPs were XRD, TEM, EDX, SAED and UV spectroscopy. The interaction between biomolecules in the extract and Ag-NPs were confirmed by FT-IR spectroscopy. Sufficient antibacterial activity study was conducted using different concentrations depending on the dissemination of disks and the kinetic growth. In addition, a test of kinetic growth, cytotoxicity testing and the bactericidal testing was performed with *P. Staphylococcus Aureus* and CoNS insulates, after post-operative wound infections".

A novel, efficient and cheap approach for "nanotherapy *P. Aeruginosa*, *staphylococci* and negative *staphylococci* coagulase" for the fast synthesis of Ag-NPs utilizing aqueous CRC leaf

extracts is used in this research (CoNS). Stable forms are usually roughly spherical between 5 and 45 nm in size. These Ag-NPs have been manufactured using UV, FTIR, XRD, TEM and EDX. The XRD test indicates that particles with face centered cubic geometry are of crystalline structure. Ag-NPs with a normal number of 20,52 nm formed in TEM investigations. The antibacterial effects generated by Ag-NPs were examined in *P. Aeruginosa*, *Staphylococcus Aureus* and CoNS isolates from post-operational wound infection. The study shows that MDR isolates from post-operative injury infections had significant antibacterial activity produced by a water leaf extract of CRCP.

Characterizations of Ag-NPs

Spectrophotometry is one of the most important techniques for structural characterization of MNPs. Fig. 1a UV-Vis shown showed the productivity and

stability of Ag-NPs by decreasing the Ag^+ ions. The spectra disclose the 435 nm belt which matches the spherical Ag-NPs resonance with the surface plasma indicating that Ag-NP is produced in the reaction mixture. During synthesis, the solution colour, which is absorbed after regular intervals, changed (“5 min, 10 min, 20 min, 30 min, 40 min, 50 min and 1 h”). The solution was maintained for approximately a month to assess the stability of generated nanoparticles. The stability was tested within 24 hours but absorption did not alter, showing (Fig. 1b) that the nanoparticles were stable, even in ambient settings ($28^{\circ}C$) for up to 24 hours.

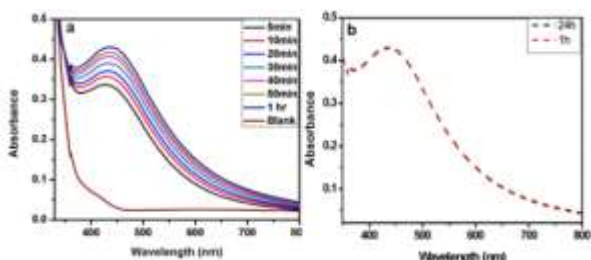


Fig. 1 (a) UV – The synthetic absorption of Ag-NP by means of leaf extract from *Corchorus Capsularis* (b) UV – Ag-NP spectrum absorption at different intervals. One hour 24 hours.

TEM has been investigated in particle size and shape of the Ag-NPs. The synthesized displayed in Figure 2 include the TEM, the histogram, SAED and EDX patterns. (a-d). The TEM picture shows nano, spherical and ellipsoidal silver particles. Images from the TEM microscope demonstrate an extreme part size range 5 nm to 45 nm. The particle size indicates that the dispersion size ranges from 5 to 60 nm. The size distribution in histogram was estimated to be 20.52 nm for produced nano particles.

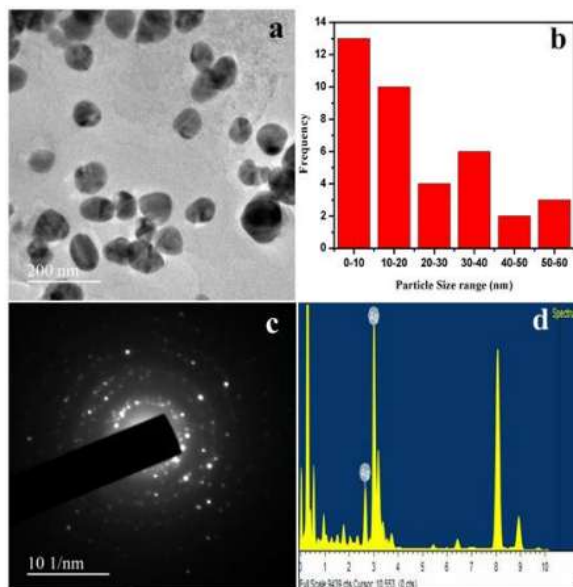


Fig. 2 Ag-NPs (a), SAED pattern (b) synthesized Ag-NPs (c) and EDX spectrum (d) history of frequent distribution

The SAED model of the Ag-NPs synthesized shows the brilliant circular rings (1 1), (2 0 0), (2 2 0), (3 1) corresponding to the planes, confirming further that the Ag-NPs have a great crystallinity. For one month at room temperature, these nanoparticles are stable in aqueous solutions. The locations are indexed by Ag’s fcc structure. The pristine crystalline character of the Ag-NPs is validated by XRD and SAED. The energy peak in the region of 3 KeV is to Ag may be inferred from the EDX spectra.

The XRD examination was conducted to investigate the crystalline nature of the Ag-NPs. Figure 2 indicates that there are four peaks at the grade of degree (2 θ), 38.17, 43.93, 64.42, and 77.32. The increase of the measured X-ray maximum is mainly because to the tiny size of the particle. On the Debye–Scherrer equation the mean Ag-NPs were estimated to be 21 nm.

$$D = K\lambda / \beta \cos\theta$$

“Where K is the constant of Scherrer (K = 0,94), X-ray is the wavelength, β is a full breadth of the diffraction line in radians at half a max (FWHM) and a half diffract angle – K is the maximum. A unique, shallow and strong XRD spike centered at 38.17 degrees indicates that a strong X-ray dispersion center in the crystalline phase is related to the nanoparticles capping and stabilization that can be referenced to the (1 1 1) metallic silver reaction with a fcc structure (JCPDS file no.65-2871). Weak diffraction peaks are well suited to (2 0 0), (2 2 0 and (3 1), respectively), 43.93, 64.42 and 77.32 of pattern”.

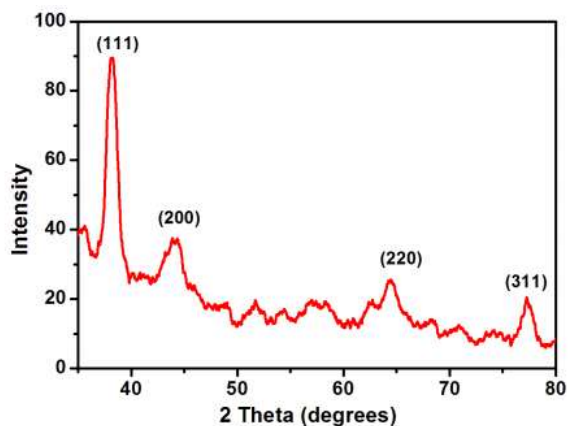


Fig. 3: XRD pattern of Ag-NPs using leaf extract of Corchorus Capsularis

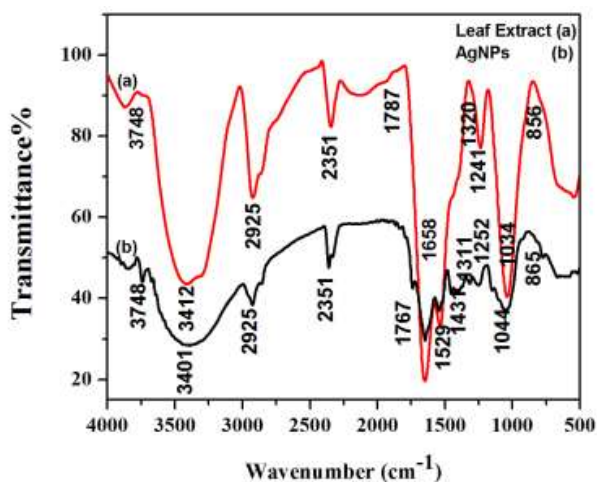


Fig. 4: FT-IR Ag-NP spektrum utilizing Corchorus Capsularis leaf extract before (a) and after (b) AgNO₃ addition.

The plant CRCP has really been examined for its potential to aid Ag-NPs synthesis and the production of Ag-NPs can be accomplished owing to its high phytochemical content. Further, the Spectroscopic FT-IR study indicates that phenolic – OH, carboxy and amino extracts from CRCP may serve as a nitrogen reduction and stability agent for aggregation formation and agglomeration prevention. The FT-IR study in Fig. 4 (a&b) indicates that the band's position in the CRCP may be described in Table 1. The main reduction of the Ag⁺ ion to AgO nanoparticles in CRCP extract leaves was observed as a result of changes in pinnacles and a decrease of the pinnacles of the FT-IR spectrum. The FT-IR spectroscopy also indicates that CRCP extracts are antiagglomeration and stabilizers of the phenolic OH group and protein. The amino acid carbonyl group has a strong binding metal capacity and thus proposes the formation of a layer covering Ag-NPs.

Table 1 Corchorus Capsularis leaf powder spectrum FT-IR data before (a) and after (b) Ag-NP encapsulation, according to different functional groupings

Band Positions (cm ⁻¹)		Assignment
(a)	(b)	
3748	3748	-COOH of carboxylic group
3412	3401	Hydroxyl groups
2925	2925	-C-H group in aromatic ring
1767	1787	Carbonyl group as in aldehydic or ketonic group
1658	1658	Hydroxyl groups in aromatic ring
1529	1529	C-C aromatic group
1431	1431	C-H group in aromatic ring
1252	1241	Carbonyl group as in aldehydic or ketonic group
1034	1044	C-O-C of phenolic

Disc Diffusion Assay

“A revised Kirby Bayer Disc Diffusion Assay for synthesized Ag-NPs for P. Aeruginosa, Staphylococcus Aureus and CoNS Isolates assessed the antibacterial effective effects of post-surgical wound infections”. The findings showed that the concentrations of Staphylococcus Aureus, CoNS and P. Aeruginosa in the presence of Ag-NPs in a mean inhibition area (ZOI) of 21, 20 and 15 mm correspondingly to 60 µg / ml.. The ZOI for MRSA and CoNS was 15 mm and 17 mm, with an Ag NP of 40 µg/ml. (Fig. 5.6 a-c).

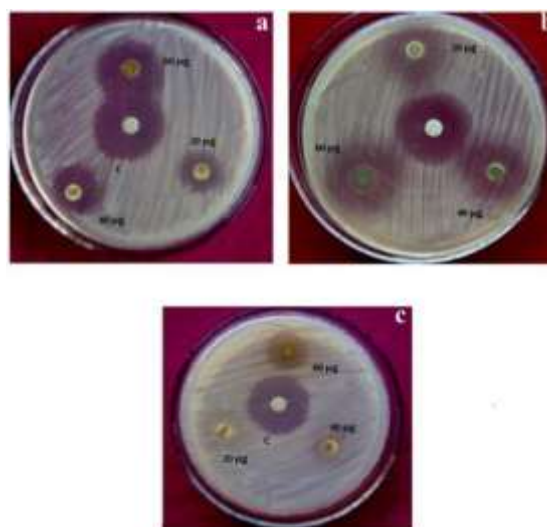


Fig. 5: Antibacterial action measurement of green generated Ag-NPs in post-surgical wound infections “(a), coagulase negative Staphylococci (CoNS) (b) and P isolate P.Aeruginosa (c) from post-surgical wound infections”.

From those findings, there is an apparent rise in the ZOI, consistent with many studies, in line with increased concentrations of Ag-NPs. The generated Ag-NPs exhibit a considerable antibacterial activity in contrast to an antibiotic called Gentamycin. Cell replication capability has been reduced by changing the permeability of the cellular filtration membrane when clinical bacterial pathogens have been exposed to Ag-NPs and cell growth has thus been suppressed. Ag-NPs may harm some macromolecules, including glycoproteins, leads to cell deterioration and mortality. Green Ag- NPs have been found to be biological-bacterial when they are size dependent and to be highly antibacterial in comparison to chemicals Ag-NPs and bio produced Ag-NPs. size-dependent, total area and a wide surface-to-volume ratio have been found to be antibacterial effectively.

Growth Kinetics Assay

In addition, “106 CFU were tested with various levels of Ag-NPs (20, 40 60, 80 and 100 µg/ml) at bacterial growth kinetics (Fig. 6.c.). At regular intervals of 3 to 24 h, growth kinetics was evaluated at 600 nm. The graph shows that with an increased concentration of Ag-NPs and exposure times the number of bacterial cells is decreasing. Moreover, bacterial culture alone does not stop growth without therapy for Ag-NPs and cells survive till they are in decline. It was discovered from the research that smaller Ag-NPs readily enter the cell wall and have increased antibacterial activity. The results of this research. The produced Ag-NPs first attach the bacterial cell wall and then pierce it producing structural alterations like piercing, leading to internal element release”.

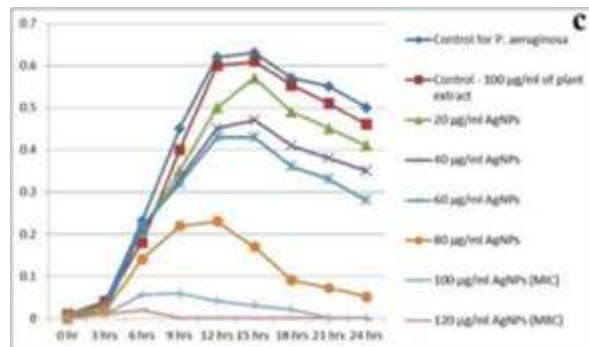
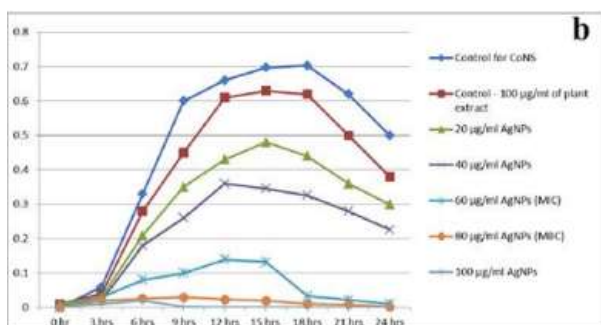
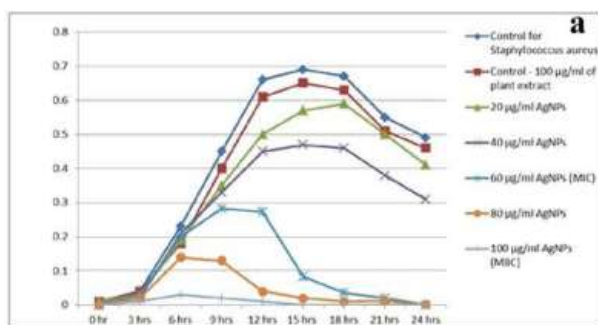


Fig. 6: Staphylococcus Aureus “(a), Coagulase-negative Staphylococci (CoNS) (b), and P. Aeruginosa (c) postoperative wound disease isolates Determination of growth kinetics”

The generated NPs discharge silver ions into the inside and form reactive oxygen (ROS) species that interact with protein molecules that change the electron transport chain. The ROS generated plays a significant role in antibacterial activity. A potential antibacterial mechanism was proposed for Ag-NPs, that may be produced by an electrostatic pull between the cell membrane negative charge and the weak positive charge of Ag-NPs. Ag-NPs may also serve as a means of transporting Ag^+ better to bacteria, the motive proton force of which will decrease the local pH and increase the release of Ag^+ . In addition, ag-np interacts with disulfide intracellular groups which contribute to metabolic inhibition. Ag-NPs gained significance as an antibacterial agent in several NPs in riding with a distinct mechanism of action from other NPs. The disulfide linkages of the intracellular enzymes interfere with membrane integrity and distort bacterial essential activities like as respiration, cell absorption and metabolism, while other nanoparticles cause anti-bird action by stimulating or preventing oxidative stress Mechanisms.

Cytotoxicity Assay

Cell viability was evaluated with the use of MTT tests for Green Ag-NPs utilizing the mouse embryo fibroblast cell lines. The cytotoxicity impact on the mouse embryo cell line of green produced Ag-NPs is shown in Table 2. It is evident from the MTT findings that Ag-NPs have little cytotoxicity to the cell line of the mouse embryo until the maximal incubation period. CRCP leaf extract produced green Ag-NP exhibits no toxicity until 48 hours of incubation in all doses. At 72 hours, $4,69 \pm 0,36\%$, $13,1 \pm 1,00\%$, $16,34 \pm 1,24\%$ toxicity is recorded at 50 µg/ml, 100 µg/ml and 200 µg/ml, correspondingly. No toxicity of up to 72 hours of incubation is shown for 3.125 µg/ml, 12.5 µg/ml or 25 µg/ml, and a very low toxicity of 50 µg/ml is seen. The Ag-IC50 NP's values in fibroblast cells of the mouse are not identifiable for different



incubation times up to the tested dose as given in table 2 below.

Table 2: Green NPs generated with Corchorus Capsularis leaf extract in vitro cytotoxicity effects on mouse embryo fibroblast cells

Ag-NPs (µg/mL)	Mouse embryo fibroblast cell line (10,000cells/well) (% of toxicity)					IC ₅₀ concentration of Ag-NPs
	6 h	12 h	24 h	48 h	72 h	
200	nt	nt	nt	nt	16.34 ± 1.24	-
100	nt	nt	nt	nt	13.1 ± 1.00	-
50	nt	nt	nt	nt	4.69 ± 0.36	-
25	nt	nt	nt	nt	nt	-
12.5	nt	nt	nt	nt	nt	-
6.25	nt	nt	nt	nt	nt	-
3.125	nt	nt	nt	nt	nt	-
1.5	nt	nt	nt	nt	nt	-

The notion that every medication effect includes tissue changes reflected in functional changes is a basic one in the drug examination. "A great deal of research has already been written on the use of cell cultivation methods in the assessment of test sheets on Ag-NP in vitro. The cytotoxicity of every natural and synthetic substance in a fixed cell line must be established as a fundamental step prior to in vivo testing". The studied lyophilized Ag-NPs indicate that mouse embryo fibroblast cells have no substantial toxicity. Microorganisms in clinical RDM create dangerous invasive infections, which put a wide range of drugs at risk to life and have shown to be resistant. The multidrug - resistant mechanism is complex. Based on the continuous increase of hospitalized bacterial MDR strains, alternative medications against MDR pathogens urgently need to be developed. The current study will be an extensive research effort into alternative (nanomedicine) therapy methods for "P. aeruginosa, Staphylococcus aureus and CoNS".

Bactericidal Activity Assay

For Staphylococcus aureus, Co NS and P. aeruginosa, the antibacterial activities of green ag-NPs were performed. About 10⁶ CFU bacterial isolates on MHA plates at various intensities (20, 40, 60, 80 and 100 µg/ml) have been treated. In MRSA and CONS, the percentage of Ag-NP colonies is reducing further following treatment, respectively, at 80 and 60 µg/ml. Please note that the colonial count is significantly decreased even with a small concentration of Ag-NP. When the cells are detected to be attached to different places and pierced in the membrane, increasing concentrations of Ag-NPs and

higher levels may result in complete suppression of cells and Ag-NPs that may lead to analyses.

CONCLUSION

Ag-NPs are produced using leaf extract CRCP in the current study. Tested for their antibacterial effectiveness against "P. Aeruginosa, Staphylococcus Aureus and Co NS", The narcotic isolates are characterized as Ag-NPs for postoperative injury infections. "The antibacterial efficacy of all Ag-NPs has proven itself to be highly hazardous to the bacterial strains as the aggressive levels grow. The same bactericide effect is claimed to have by CoNS and Staphylococcus Aureus (80µg/ml) but is different from P. Aeruginosa. Therefore the variation in its antibacterial activities may be linked with the concentration of Ag-NP suspension particles". The results of these studies indicate that green silver nanostructures produced via this method may be used to effectively treat wound infections in the area of nanomedicine. In future, green produced Ag-NPs may function to produce wound cure bandages as a biocompatible nanomotors material.

REFERENCES

1. B. A. Iwalokun, O. Akinloye, B. E. Udoh & K. O. Akinyemi (2019) Efficacy of silver nanoparticles against multi-drug resistant clinical Staphylococcus aureus isolates from Nigeria, Journal of Immunoassay and Immunochemistry, 40:2, pp. 214-236, DOI: 10.1080/15321819.2018.1555765
2. Faizan Abul Qais, Anam Shafiq, Haris M. Khan, Fohad M. Husain, Rais A. Khan, Bader Alenazi, Ali Alsalmeh, Iqbal Ahmad (2019), "Antibacterial Effect of Silver Nanoparticles Synthesized Using Murrayakoenigii (L.) against Multidrug-Resistant Pathogens", Bioinorganic Chemistry and Applications, vol. 2019, ArticleID 4649506, 11 pages. <https://doi.org/10.1155/2019/4649506>
3. K. Twum-Danso, M. J. Newman, N. Obeng-Nkrumah, and K. A. Krogfelt (2013), "High levels of extended-spectrum beta-lactamases in a major teaching hospital in Ghana: the need for regular monitoring and evaluation of antibiotic resistance," American Journal of Tropical Medicine and Hygiene, vol. 89, pp. 960–964. View at: Publisher Site | Google Scholar
4. M. N. Alekshun and S. B. Levy (2007), "Molecular mechanisms of antibacterial multidrug resistance," Cell, vol. 128, no. 6,

pp. 1037–1050, View at: [Publisher Site](#) | [Google Scholar](#)

5. D. Rawat and D. Nair (2010), “Extended-spectrum β -lactamases in gram negative bacteria,” *Journal of Global Infectious Diseases*, vol. 2, no. 3, p. 263, View at: [Publisher Site](#) | [Google Scholar](#)
6. S. Picozzi, C. Ricci, M. Gaeta et. al. (2013), “Do we really know the prevalence of multi-drug resistant *Escherichia coli* in the territorial and nosocomial population?” *Urology Annals*, vol. 5, no. 1, p. 25. View at: [Publisher Site](#) | [Google Scholar](#)
7. Li, Y.; Leung, P.; Yao, L.; Song, Q. W.; Newton, E. (2006). Antimicrobial Effect of Surgical Masks Coated with Nanoparticles. *J. Hosp. Infect.*, 62, pp. 58–63. DOI: 10.1016/j.jhin.2005.04.015.
8. Lara, H. H.; Ayala-Nuñez, N. V.; Ixtapan-Turrent, J.; Rodriguez-Padilla, C. (2010). Mode of Antiviral Action of Silver Nanoparticles against HIV-1. *J. Nanotechnol.*, 8, pp. 1.
9. Cottell, A.; Denyer, S. P.; Hanlon, G. W.; Ochs, D.; Maillard, J. Y. (2009). Triclosan-Tolerant Bacteria: Changes in Susceptibility to Antibiotics. *J. Hosp. Infect.*, 72(1), pp. 71–76. DOI: 10.1016/j.jhin.2009.01.014.
10. Arora, S.; Jain, J.; Rajwade, J. M.; Paknikar, K. M. (2008). Cellular Responses Induced by Silver Nanoparticles: In Vitro Studies. *Toxicol. Lett.*, 179, pp. 93–100. DOI: 10.1016/j.toxlet.2008.04.011.

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