

Biosynthesis of nanoparticles and study of its applications

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Abstract— Development of an eco-friendly process for the synthesis of nanoparticles (NPs) has increased in the recent years, due to its low toxicity, ease in handling and inexpensiveness. In the present study synthesis of silver, copper and zinc oxide nanoparticles, by the bioreduction of 1mM silver nitrate, 3mM copper sulfate and 1 mM zinc acetate, respectively, using vegetable and fruit peel extract was done. Structural, morphological and optical properties of the nanoparticles was studied using UV–Vis spectrophotometer, FTIR and SEM analysis. SEM analysis showed that the synthesized AgNPs, CuNPs and ZnONPs had the average size of 50nm, 60nm and 90nm respectively, with predominantly spherical shape. The antibacterial action of the nanoparticles was evaluated using the agar well diffusion method. It was observed that zinc oxide nanoparticles exhibited the highest anti-bacterial property, against both Gram negative *Escherichia coli*, *Salmonella typhi* and Gram Positive *Staphylococcus aureus*, *Streptococcus pneumoniae*. It was found that zinc and silver nanoparticles showed a greater inhibition of both pure culture biofilms as well as on biofilms from water treatment plants (65% inhibition with AgNPs and 47% inhibition with ZnONPs). The malachite green dye removal using Luffa sponge (LS) and modified Luffa sponge with nanoparticles respectively (NPs-LS) were employed as adsorbents. The adsorption studies showed that LS coated with zinc oxide nanoparticles showed the highest adsorption capacity of 19.2mg/g. Zinc oxide nanoparticles were also tested for its cytotoxic effects against human leukemia- 60 cell lines.

Key words— nanoparticles, bioreduction, biofilms, cytotoxic effects, malachite green, nanoparticles, SEM.

1 INTRODUCTION

Nanotechnology is a field of modern research and scientific innovation that deals with the synthesis and manipulation of particles within the nanometer scale range (1-100nm)¹. This technology is capable of providing miscellaneous novel applications that range from innovative fabric compounds, food processing and agricultural production to sophisticated medicines. At the nanometer level, the properties and the functions of living and anthropogenic activities are defined. Nanoparticles present a higher surface to volume ratio with the decrease in its size². Metallic nanoparticles have a high specific surface area and a high fraction of surface atoms have been studied extensively due to their unique physicochemical characteristics such as catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties. Biosynthesis of NPs is a type of bottom-up approach where the main reaction occurring is reduction/oxidation.

Various micro-organisms such as bacteria, fungi, and yeasts have been suggested as nanofactories for intra- and extra-cellular synthesis of metal. Synthesis of metal NPs using plant extracts is very cost effective, so can be used as an economic and valid alternative for the large-scale production of metal nanoparticles³.

The present study explores the synthesis, anti-bacterial and anti-biofilm action of three metallic nanoparticles, silver, copper and zinc oxide nanoparticles were synthesized by using vegetable and fruit peel extract as the reducing agent. The antibacterial mechanisms of silver ion action have been studied for a long time. Silver ions can interact with the bases in DNA, rather than with the phosphate groups, and affect the DNA ability to replicate. It was assumed that AgNP can release silver-ions and this mechanism plays a significant role in AgNP antimicrobial effects⁴. In case of copper nanoparticles, the toxicity is due to accumulation and dissolution of NPs in the bacterial membrane changing its permeability, generation of reactive oxygen species (ROSs) or/and their corresponding ions from NPs, followed by depletion of intracellular ATP production and disruption of DNA replication⁵. ZnO NPs are also able to interact with membrane lipids, hindering the structure of the membrane, reducing membrane integrity, causing malfunction and eventually bacterial cell death⁶.

Another important aspect of this study is the removal of dyes from waste water using nanoparticle treated luffa sponge. *L. cylindrica* is a natural material consisting of cellulose and lignin (1.4:2.9% of sponge dry weight), belongs to Cucurbitaceae family. Treatment of nanoparticles without

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being immobilized on a support material for removal of azo dyes from water comes with pollution and contrary effects (AgNPs due to antimicrobial activity eliminate of balance of microbial flora, the increase of nanoparticles in water, etc.). Consequently, use of immobilization materials in water remediation works to be done with use of nanoparticle is vital to eliminate these difficulties⁷. ZnO NPs have also received much attention for their implications in cancer therapy. Studies have shown that ZnO NPs induce cytotoxicity in a cell-specific and proliferation-dependent manner, with rapidly dividing cancer cells being the most susceptible, and quiescent cells being the least sensitive. Clearly, the type of cell in question is important when considering the cytotoxicity of ZnO NPs toward mammalian cells. To date, however, the anticancer activity of ZnO NPs, especially the underlying mechanisms of apoptosis in cancer cells due to ZnO NP treatment, is largely lacking⁸. Understanding the effect of zinc oxide nanoparticles on human leukemia – 60 cell lines, is also a major focus of the present study.

2 MATERIALS AND METHODS

2.1 Collection of kitchen waste

The wastes from kitchen, containing peels of fruits such as *Citrus reticulata* (orange), *Punicagranatum* (pomegranate), *Musa* (banana) and vegetables such as *Momordicacharantia* (bitter gourd), *Moringaoleifera* (drumstick) and *Solanumtuberosum* (potato) were collected. 100g of peels were washed thoroughly and crushed in 200ml of distilled water. This extract was filtered with a muslin cloth and was used for nanoparticle synthesis.

2.2 Synthesis of silver, copper and zinc oxide nanoparticles

For the reduction of Ag^+ ions, 10 ml of the peel extract was added to 90ml of 1mM aqueous $AgNO_3$ and heated at 60° C to 80° C for 20minutes⁹. In case of copper nanoparticle synthesis, 50 ml peel extract was added to 50 ml of 3mM $CuSO_4$. This reaction mixture was kept at 80°C-90°C for 20 minutes in a boiling water bath and then incubated at 35°C-37°C in an incubator for 24 hours¹⁰. The 1mM zinc acetate [$Zn(O_2CCH_3)_2(H_2O)_2$] salt was dissolved in 50 ml distilled water and kept in stirrer for 1 h for the synthesis of zinc oxide nanoparticles. 20 ml of 1N NaOH solution was then added slowly into the zinc acetate solution and 25 ml of plant extract was added to the same. This solution was stirred continuously for 3 hours, using a magnetic stirrer. Each of these mixtures was then centrifuged at 3000 rpm for 10 minutes. The pellet obtained was washed thoroughly with distilled water and dried at 80°C for 2 hours¹¹.

2.3 Characterization of nanoparticles

Morphological Examination: The change in color of the incubated mixture was observed and recorded which indicated the synthesis of nanoparticles.

UV-Vis Spectrometry Analysis: The nanoparticles synthesized after were subjected to optical measurements, which were carried out by using a UV-Vis spectrophotometer (INKARP SCAN 2301) and scanning the spectra between 300-500 nm.

SEM analysis: To determine shape and size of synthesized nanoparticles, SEM (ZEISS ULTRA FIELD EMISSION SEM) was done. SEM micrograph images of the synthesized nanoparticles were used for morphological characterization.

FTIR Analysis: FTIR analysis was carried out in order to determine the role of functional groups involved in the stabilization (capping) and synthesis of silver nanoparticles present in the vegetable peel extract. 1 mg of the powdered sample of each of the nanoparticles was subjected to FTIR (JASCO FT/IR-4100 type A model of FTIR) analysis, at a resolution of $4cm^{-1}$.

2.4 Evaluation of antibacterial effect of nanoparticles

Agar well diffusion method was used to study the antibacterial effect of the nanoparticles against Gram positive organisms, *Staphylococcus aureus* and *Streptococcus pneumoniae*; and Gram negative organisms, *Escherichia coli* and *Salmonella typhi*. Pour plate method was used for seeding the organisms on nutrient agar medium. Wells were made on each of the plates using a sterile cork borer of diameter 7mm. The antibacterial analysis was carried out in triplicates, with each plate containing three wells filled with each of the nanoparticle sample, along with the positive control and negative control, being silver nitrate ($AgNO_3$) and distilled water, respectively. The plates were incubated at 37°C and were checked for the zones of inhibition after 24 hours. The diameter of the zone indicated the effectiveness of each of the nanoparticles on the test organisms.

2.5 Evaluation of effect of nanoparticles on biofilms

Biofilm inhibition was carried out in a 96 microtitre well plate, adopting modified method of biofilm spectrophotometric assay. Four pure cultures, *Salmonella typhi*, *Escherichia coli*, *Streptococcus pneumonia* and *Staphylococcus aureus*; a consortium of different pure cultures; and samples scrapped from the walls of bucket, toilet seat and water treatment plant were used, to grow biofilms invitro on the microtitre plates, for studying the effect of nanoparticles on each of the samples. The organisms were grown on the wells of the microtitre plate. The biofilms formed were treated with each of the synthesized nanoparticles. 0.1% crystal violet was used to stain the biofilms and 30% acetic acid, for solubilising the cells, for a quantitative assay¹². The percentage of inhibition was calculated using the formula,

$$\% \text{ inhibition} = \frac{[O.D. (\text{CONTROL}) - O.D. (\text{TEST})] \times 100}{O.D. (\text{CONTROL})}$$

3 Results and Discussion

3.1 Characterization of nanoparticles

3.1.1 Visual Observation

A noticeable colour change from light to dark brown was observed in each of the reaction mixtures, after the incubation period. This provided a primary observational confirmation of the formation of nanoparticles. This change is due the reduction of the metallic ions. The colour may vary from pale green to dark brown.

3.1.2 U.V. Visible Spectroscopy

UV-visible spectroscopy is one of the simplest and most used technique for the preliminary characterization of nanoparticle. The optical signature of metal nanoparticles is given by the surface plasma resonance (SPR) and it is crucial to understand the number, position, and width of the SPRs as a function of the NP shape, size, and environment. SPR is affected by properties like surface charge distributions, dielectric medium, and surface-absorbed species, which are directly associated with nanoparticle size¹³. The surface plasmon resonance of the synthesized silver nanoparticles produced a peak at 380 nm, whereas in case of zinc oxide and copper nanoparticles, the absorption maxima were seen at 340 nm and 342 nm, respectively.

3.1.3 FTIR spectroscopy analysis

The FTIR analysis of the biosynthesized nanoparticles were carried out to study the interaction between the biomolecules. In case of silver and copper nanoparticles, the results of the analysis showed sharp absorption bands, located at about 1612.2 cm⁻¹ and 3400.85 cm⁻¹. Absorption band at 1612.2 cm⁻¹ may be assigned to the amide I bond of proteins arising due to carbonyl stretch in proteins, and peaks at 3400.85 cm⁻¹ are assigned to OH stretching in alcohols and phenolic compounds¹⁴. The absorbance band at 1612.2 cm⁻¹ are known to be associated with the stretching vibrations for -C=C- (aromatic), whereas, the band at 2924.52 cm⁻¹ are generally assigned to the alkyl C-H stretching (Jain *et al.*, 2009). For ZnO nanoparticles, the absorption bands at 1598.7 cm⁻¹, 1407.78 cm⁻¹, and 1112.73 cm⁻¹ can be ascribed to organic impurities originating from reaction intermediates, which can be identified as Zn hydroxy acetate complex, while the one at 3352.64 cm⁻¹ may be assigned to the OH groups on the surface of ZnO¹⁵. These IR spectroscopic studies confirmed that

carbonyl group of amino acid residues have strong binding ability with metal suggesting the formation of layer covering metal nanoparticles and acting as capping agent to prevent agglomeration and providing stability to the medium. These results confirm the presence of possible proteins acting as reducing and stabilizing agents, for the nanoparticles¹⁶.

3.1.4 Field Emission Scanning Electron Microscopy Analysis (FE-SEM)

Field emission scanning electron microscopy (FESEM) has narrower probing beams at low and high electron energy, so it provides improved spatial resolution while minimizing sample damage. The FESEM micrographs clearly showed a versatile and spherical shape distribution of each of the nanoparticles. Fig. 1 shows the images of the synthesized nanoparticles.

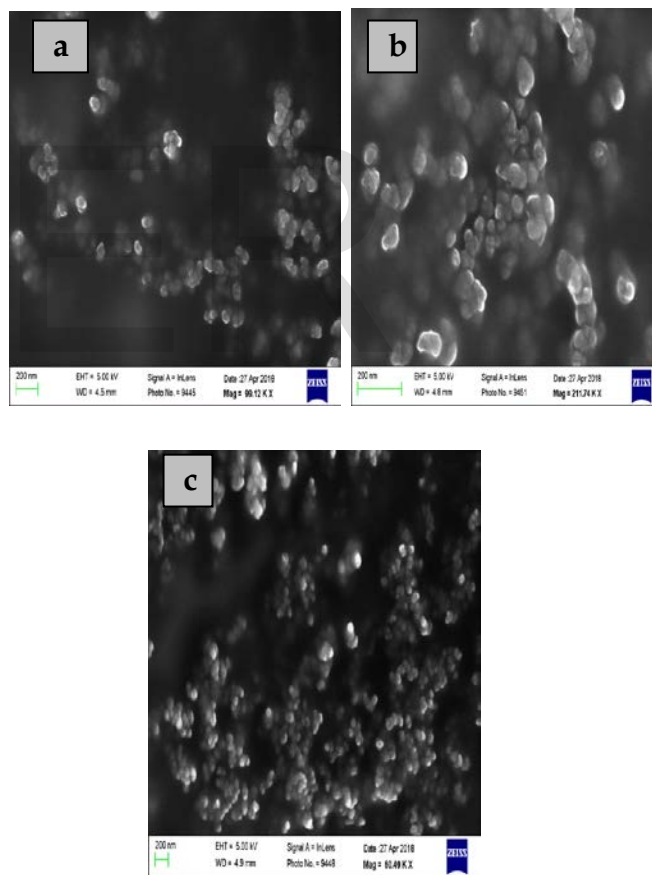


Fig. 1: SEM images of a) AgNPs; b) CuNPs; c) ZnONPs, at a scale of 200nm.

The images were observed at a scale of 200nm¹⁷. All the three nanoparticles were found to be spherical in shape. The size of silver and copper nanoparticles were found to be within a range of 50-60nm, where the particle size of ZnONPs were found to be in the range of 90-100nm. Determining the size and the

shape of the nanoparticles is important in understanding the impact of size and shape on the toxicity of the particles against bacterial or mammalian cells. NPs with different shapes can cause varying degrees of bacterial cell damage through interactions with periplasmic enzymes¹⁸.

3.1.5 Antibacterial Effect of Nanoparticles

The antibacterial properties of AgNPs, ZnONPs and CuNPs were evaluated using agar well diffusion method, against Gram positive *Staphylococcus aureus* and *Streptococcus pneumoniae*; and Gram negative *Escherichia coli* and *Salmonella typhi*. The three nanoparticles showed significant activity against all the four test organisms. Due to the presence of a thick peptidoglycan cell wall, Gram positive organisms are generally more resistant to antibacterial agents. However, all the three nanoparticles were seen to be effective against both gram positive and gram negative organisms; with zinc oxide nanoparticles showing the highest efficacy. Fig.2. shows the comparative effect of all the three nanoparticles on the test organisms, with AgNO₃ as the positive control.

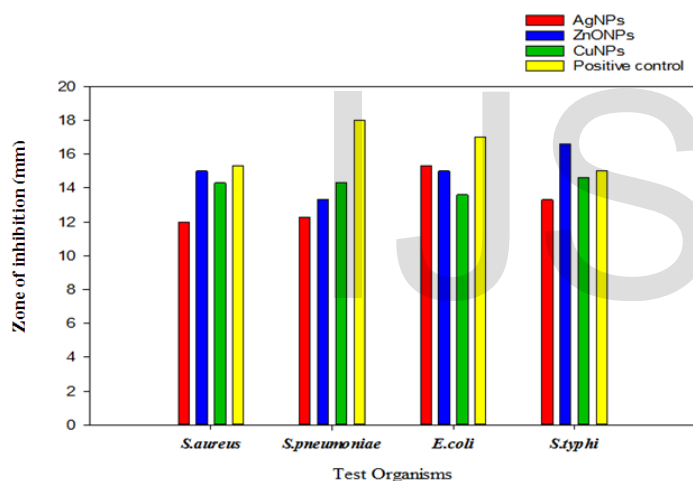


Fig.2. Comparison of the antibacterial effect of AgNPs, ZnONPs and CuNPs, against Gram positive *S. aureus*, *S. pneumoniae*; and Gram negative *E. coli* and *S. typhi*.

It was observed that AgNPs and CuNPs also showed effectiveness against the organisms, however zinc oxide exhibited greater efficacy. Hence, it can be effectively used as an alternative to AgNPs, as they are comparatively cheaper. The mode of action of all the three nanoparticles may vary. The penetration of silver nanoparticles into the bacterial cell may lead to DNA damage, or even cell death, by altering the normal functioning of bacterial DNA; and the interaction of Ag⁺ ions with the proteins containing sulfur present in the bacterial cell wall irreversibly causing the disruption of the bacterial cell wall are some of the proposed mechanism, deduced as the main antibacterial mechanism in evaluating the antimicrobial activity³. ZnO NPs are believed to destruct lipids and proteins of the bacterial cell membrane, resulting in

a leakage of intracellular contents and eventually the death of bacterial cells. In addition, generation of hydrogen peroxide and Zn²⁺ ions were suggested to be key antibacterial mechanisms of ZnO NPs¹⁹. In case of CuNPs, the interaction with cell wall may have initiated a large rate of oxidation, contributing to the release of Cu ions, than oxygen available in the solution. Furthermore, electrostatic attraction between Cu²⁺ and plasma membrane and also membrane-based reductases can lead to its reduction to cuprous ions²⁰.

3.1.6 Effect of Nanoparticles against biofilms.

The efficacy of the nanoparticles on biofilms was studied using the standard crystal violet assay. Pure cultures as well as biofilm samples from their habitat were collected and tested for nanoparticle susceptibility. The results of the study showed that the pure culture biofilms exhibited a greater susceptibility as compared to the biofilms from the natural habitat (Table 1).

Table1. Biofilm inhibition (%) by AgNPs, ZnONPs and CuNPs .

Biofilm Sample	Biofilm Inhibition (%)		
	AgNPs	ZnONPs	CuNPs
<i>Staphylococcus aureus</i>	46	94	8
<i>Streptococcus pneumoniae</i>	73.3	66	40
<i>Escherichia coli</i>	85.7	87.75	79.5*
<i>Salmonella typhi</i>	87.9*	91.37	63.7
Bucket surface	30	49	9
Toilet seat surface	49	59*	11
Consortium	40	37.5	5
Water treatment plant	65*	47	26*

The interactions between NPs and the biofilm can be viewed as a three-step process: (1) transport of NPs to the vicinity of the biofilm; (2) attachment to the biofilm surface; and (3) migration within the biofilm. At each of these steps, the interactions are a complex interplay of a myriad of factors including: (i) physical protections in the biofilms; (ii) interactions among biofilm microorganisms; (iii) the slow growth rate of certain biofilm microorganisms. More than one mechanism may occur simultaneously^{21,22}. It was observed that the zinc oxide nanoparticles showed a higher efficacy as compared to the other two counterparts. The particle size could have an effect on the antimicrobial efficacy of NPs as demonstrated by Yamamoto *et al*²³, whereby increasing particles sizes of ZnO powders from 0.01 to 0.08 μm increased its antimicrobial efficacy against *S. aureus* and *Escherichia coli*.

This could possibly explain the increased effectiveness of ZnONPs, as it was seen to have a particle size almost twice than that of CuNPs and AgNPs.). This study provides us with a basic understanding of the extent of effectiveness of nanoparticles for combating the major problem of biofilms.

3.1.7 Effect of nanoparticles on dye removal from waste water.

Recently, the surface modification of natural polymers has received great consideration with new developments in science and technology. Lignocellulosic materials developed with substantial alteration in physicochemical properties have been mainly employed as electrical insulators, thermal insulators, vacuum sealants, adsorbents and matrix materials for composites, etc²⁴. In this study, the dried fruit of *Luffa cylindrica* (Lc) has been used as source lignocellulosic fiber, which has been modified by using the synthesized nanoparticles. The absorbance of the samples was read at an interval of 10 minutes at 618nm using UV-Visible spectrometer.

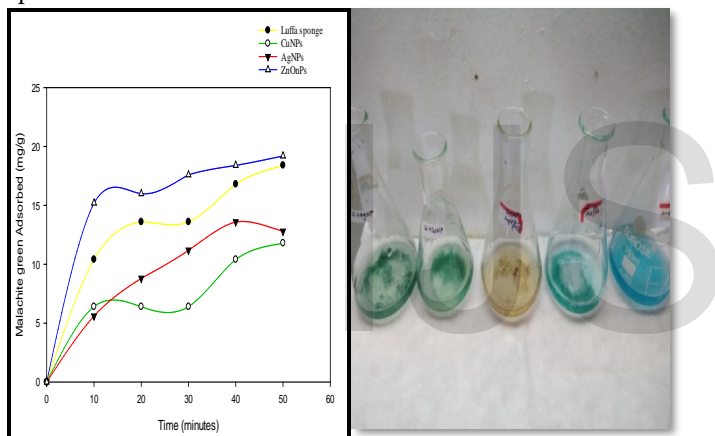


Fig.3: Graph of effect of contact time on adsorption of malachite green on luffa sponge and removal of malachite green dye from waste.

It was observed that the absorbance of the samples decreased with the increasing time, indicating the increase in the adsorption with time and finally stabilizing. The amount of the dye adsorbed on the surface of the luffa sponge was calculated. Metal or metal oxide nanomaterials have been widely studied as advanced adsorption materials in the environmental remediation. Some metal (Ag) or metal oxide (Fe, Co and Ni oxides) nanomaterials can be prepared by a simple process. As another common metal oxide, zinc oxide (ZnO) nanoparticles can be readily produced in large-scale with low cost and can be applied in many fields. However, up to date, few reports have been published on using of ZnO nanoparticles as adsorbents for dye removal²⁴. ZnO nanoparticles with a capacity of 27.6 mg/g, Farrokhiet *al.*²⁵ immobilized zinc oxide on magnetite nanoparticles and obtained the adsorption capacity of 22.1 mg/g for reactive

black. Jeonget *al.*²⁶, fabricated a ZnO shell on mesoporous SiO₂ particles for the adsorption and photocatalysis of methylene blue. In this study, the uncoated LS, had an adsorption capacity of 18.4 mg/g, which had increased to 19.2mg/g, after being coated with zinc oxide nanoparticle. Although, the difference between the capacities is low, the time taken for absorption by ZnONP coated LS was comparatively lesser than the uncoated LS. Adsorption of the dye varies with temperature and pH, hence, adjusting these parameters may lead to increased adsorption (Fig.3).

3.1.8 Effect of Zinc oxide nanoparticles on cancer cell line.

Metal oxide NPs are widely employed in creams, implants, drug carriers or as contrast agents, but for the potential use of these NPs in biomedicine strict toxicity rules should be followed. This study also examined the toxicity profile of Human Leukemia-60 cell line (HL-60) to zinc oxide nanoparticles because cellular response is dynamic and the ultimate phenotype is affected by a myriad of competing or overlapping signals present in the microenvironment (Fig. 4).

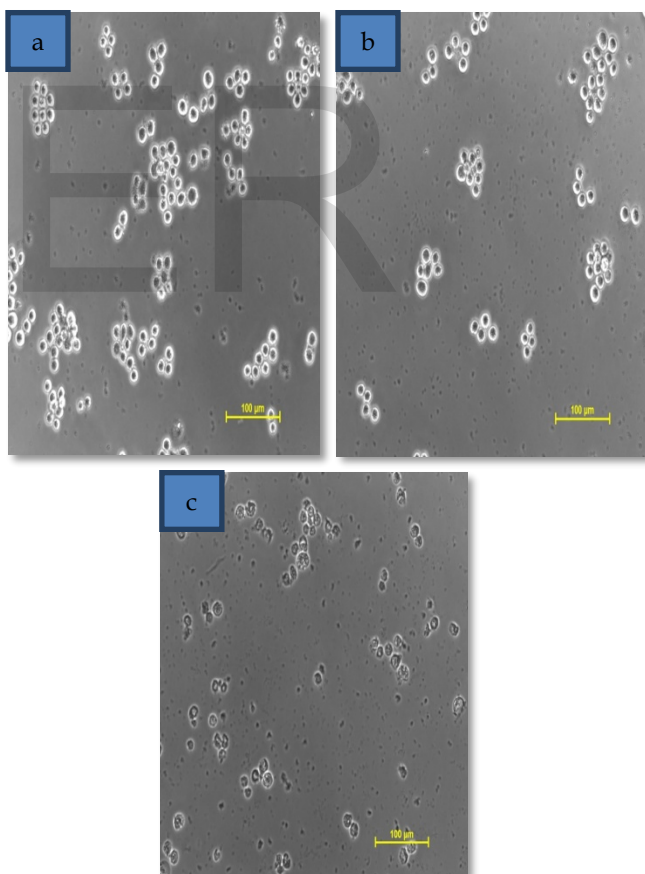


Fig.4: Human Leukemia – 60 cells a) Negative control b) Positive control-ADR c) ZnONP treated

It was observed (Fig.5) that zinc oxide nanoparticles that were synthesized did not show an expected impact on the HL-60 cell line. There could be a variety of reasons for this ineffectiveness. Nanomedicine aims to overcome the problems

related to human diseases at the nano scale level, where most of the biological molecules exist and operate. In nanoparticles, the major criterion that defines its property is the size and the shape of the nanoparticles. The smaller the size of the nanoparticle, the greater is the toxic effect on the cancer cells²⁷. It was observed through SEM analysis that the average size of the synthesized ZnONPs were about 90nm in diameter, which could be one of the major reasons for the decreased impact on the HL-60. The higher size of NPs in aqueous suspension might be due to the tendency of particles to agglomerate in an aqueous state. It was observed that the growth of the cells had not been affected even by increasing the concentration of the zinc oxide nanoparticles and the GI50 value was found to be non-evaluable. For zinc oxide nanoparticles, GI50 value $\leq 20\mu\text{g/ml}$ is considered to demonstrate activity. This non-evaluable data may be because of the low solubility of the nanoparticles or due to its large particle size. It has been studied that polygonal shaped nanoparticles showed a greater inhibition as compared to rod shaped and spherical nanoparticles⁸. ZnO nanoparticles induce toxicity in a cell-specific and proliferation dependent manner, with rapidly dividing cells being the most susceptible and quiescent cells being the least sensitive²⁹. The absence of rapidly dividing cells can possibly be a factor that may have resulted in low effectiveness of the synthesized ZnONPs on the HL-60 cells. Also, the concentration of the nanoparticles determines its effect on the cells. Although these ZnO nanoparticles can greatly inhibit cancer cell proliferation in vitro at higher concentrations, they have little effect on target cancer cells at lower concentrations³⁰. The presence of low concentration of the nanoparticles in the diluted solution, used to treat the HL-60 cells, may be another distinguishable factor for its ineffectiveness.

4 Conclusion

A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for synthesis of metallic nanoparticles. This study demonstrated the productive use of vegetable and fruit peels, which is generally a major part of the waste generated. The use of a natural, low cost biological reducing agent can produce metal nanostructures, through efficient green nanochemistry methodology, avoiding the presence of hazardous and toxic solvents and waste. The biosynthesized nanoparticles also proved to show an excellent antimicrobial activity. It was found that silver being a costly metal, can be replaced by zinc, which is relatively less expensive, since both silver and zinc oxide nanoparticles, exhibit the same extent of antibacterial activity. Zinc oxide nanoparticles, showed a greater activity against gram positive organisms also, as compared to the other two. Copper nanoparticles, on the other hand, were found to be effective against bacteria, however, it was seen

that most of the organisms had developed resistance and were able to grow on the zones of inhibition. Biofilms have been a major problem in a wide range of industries, and because of the resistance of biofilms to many disinfectants and the difficulty in combating it, has led to the search for new methods of its prevention and removal. Nanoparticles have been widely used for the studying the anti-bacterial action, however, not much work has been done to use in against biofilms. It was observed that nanoparticles exhibited a distinguishable effect on biofilms. The natural biofilms also showed inhibition to a considerable extent. In case of water treatment plants, silver nanoparticles had a greater effect followed by zinc oxide nanoparticles. Thus, coating the walls of the plants with nanoparticles, can possibly act as a preliminary step in preventing the growth of biofilms.

Waste water from the textiles and laboratories contain many types of hazardous waste which also include dyes. The resulting pollution has significantly affected the water sources, affecting the aquatic life. Luffa sponge is a cellulosic fibre that has been used for the adsorption of dyes and metals from waste water. Nanoparticles are widely known for their property of dye adsorption. Immobilizing nanoparticles on luffa sponge, helped in increasing the capacity of dye adsorption. Zinc oxide nanoparticles coated luffa sponge had an absorption capacity of 19.2 mg/g. This property can be utilized in bioremediation. Luffa sponge being an inexpensive and easily available source of fibre can be used in large quantities for the removal of the dye from polluted water. The results of the present investigation indicate that LS-ZnONPs, a low-cost adsorbent could be employed as an alternative to commercial adsorbents for removal of TB from aqueous solutions.

Nanoparticles have been use widely in cancer therapy. ZnONPs have shown to exhibit a cytotoxic effect on cancer cells. The present study demonstrated that the concentration of the nanoparticles used for treatment played a major role in determining its effect on the cell. Also, the size and the shape of the nanoparticles must be controlled, in order for it to show anti-cancer activity. Thus, further experiments can be conducted, by varying the size and the shape and the concentration of the nanoparticles, to have a clear understanding of the exact effect of zinc oxide nanoparticles against cancer cells.

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6. References

- [1] Bhosale R, Kulkarni A, Gilda S S, Aloorkar N H, Osmani R A & Harkare B R, Innovative Eco-friendly Green Synthesis Of Silver Nanoparticles, *International Journal of Pharmaceuticals and Nanotechnology*, 7(1)(2014).
- [2] Ahmed S, Ahmad M, Swami B L & Ikram S, A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise, *Journal of Advanced Research*, 7(1), (2016) 17-28.
- [3] Keat C L, Aziz A, Eid A M & Elmarzugi N A, Biosynthesis of nanoparticles and silver nanoparticles. *Bioresources and Bioprocessing*, 2(1) (2015) 253-58.
- [4] Morones J R, Elechiguerra J L, Camacho A, Holt K, Kouri J B *et al.*, The bactericidal effect of silver nanoparticles. *Nanotechnology*, 16(10) (2005), 2346-2353.
- [5] Chatterjee A K, Chakraborty R & Basu T, Mechanism of antibacterial activity of copper nanoparticles, *Nanotechnology*, 25(13) 2014, 1-7.
- [6] Xie Y, He Y, Irwin P L, Jin T & Shi X, Antibacterial Activity and Mechanism of Action of Zinc Oxide Nanoparticles against *Campylobacter jejuni*, *Applied and Environmental Microbiology*, 77(7) (2011), 2325-2331.
- [7] Nadaroglu H, Cicek S & Gungor A A, Removing Trypan blue dye using nano-Zn modified Luffa sponge, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 172 (2017) 2-8.
- [8] Akhtar J, Ahamed M, Kumar S, Khan M, Ahmad J & Alrokayan S, Zinc oxide nanoparticles selectively induce apoptosis in human cancer cells through reactive oxygen species, *International Journal of Nanomedicine*, (2012) 845.
- [9] Prasad T N, Elumalai E K & Khateeja S, Evaluation of the antimicrobial efficacy of phyto-genic silver nanoparticles. *Asian Pacific Journal of Tropical Biomedicine*, (2011), 82-85.
- [10] Paul G, Philip J, Raj B, Das P K & Manna I, Synthesis, characterization, and thermal property measurement of nano-Al₉₅Zn₀₅ dispersed nanofluid prepared by a two-step process, *International Journal of Heat Mass Transfer*, 54 (2011) 3783-3788.
- [11] Meena S K, Goldmann C, Nassoko D, Seydou M, Marchandier T *et al.*, Nanopase segregation of Self-Assembled Monolayers on Gold Nanoparticles, *ACS Nano*, 11 (7) (2017) 7371-7381.
- [12] O'Toole G A, Microtiter dish biofilm formation assay, *Journal of Visualized Experiments*, 47 (2011) 2437-2441.
- [13] Barapatre A, Aadil K R & Jha H, Synergistic antibacterial and antibiofilm activity of silver nanoparticles biosynthesized by lignin-degrading fungus, *Bioresources and Bioprocessing*, 3(1) (2016)
- [14] Kong J & Yu S, Fourier Transform Infrared Spectroscopic Analysis of Protein Secondary Structures, *Acta Biochimica et Biophysica Sinica*, 39(8) (2007), 549-559.
- [15] Anzlovar A, Crnjak O Z, Kogej K & Iigon M, Polyol-mediated synthesis of zinc oxide nanorods and nanocomposites with poly(methyl methacrylate), *Journal of Nanomaterials*, 55(2012), 1-9.
- [16] Jain D, Daima H K, Kachhwaha S & Kothari S L, Synthesis of plant mediated nanoparticles using papaya fruit extract and evaluation of their antimicrobial properties, *Digest Journal of Nanomaterials and Biostructures*, 4(3) (2009), 557-563.
- [17] Singh A K, Experimental Methodologies for the Characterization of Nanoparticles. *Engineered Nanoparticles*, 2(2016) 125-170.
- [18] Wang L, Hu C & Shao L, The antimicrobial activity of nanoparticles: present situation and prospects for the future, *International Journal of Nanomedicine*, 12, (2017) 1227-1249.
- [19] Mirhosseini M & Firouzabadi F B, Antibacterial activity of zinc oxide nanoparticle suspensions on food-borne pathogens. *International Journal of Dairy Technology*, 66(2) (2012), 291-295.
- [20] Bogdanovic U, Lazic V, Vodnik V, Budimir M, Markovic Z & Dimitrijevic S. Copper nanoparticles with high antimicrobial activity, *Materials Letters*, 128, (2014) 75-78.
- [21] Sheng Z & Liu Y, Effects of silver nanoparticles on wastewater biofilms, *Water Research*, 45(18) (2011), 6039-6050.
- [22] Ikuma K, Decho A W & Lau B L, When nanoparticles meet biofilms" interactions guiding the environmental fate and accumulation of nanoparticles, *Frontiers in Microbiology*, 6 (2015), 59-65.
- [23] Yamamoto O, Influence of particle size on the antibacterial activity of zinc oxide, *International Journal of Inorganic Materials*, 3(2001), 643-646.
- [24] Zhang F, Chen X, Wu F & Ji Y, High adsorption capability and selectivity of ZnO nanoparticles for dye removal, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 509(2016), 474-483.
- [25] Farrokhi M, Hosseini S, Yang J & Shirzad-Siboni M, Application of ZnO-Fe₃O₄ Nanocomposite on the Removal of Azo Dye from Aqueous Solutions: Kinetics and Equilibrium Studies, *Water, Air, & Soil Pollution*, 225(9)(2014). 3-8.
- [26] Jeong B, Kim D H, Park E J, Jeong M, Kim K, *et al.*, ZnO shell on mesoporous silica by atomic layer deposition: Removal of organic dye in water by an adsorbent and its photocatalytic regeneration, *Applied Surface Science*, 307 (2014), 468-474.
- [27] Gupta V K, Pathania D, Agarwal S & Sharma S, Amputation of congo red dye from waste water using microwave induced grafted *Luffa cylindrica* cellulosic fiber, *Carbohydrate Polymers*, 111(2014), 556-566.

- [28] Nair S, Sasidharan A, Divya Rani V V, Menon D, Nair Set al., Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. *Journal of Materials Science: Materials in Medicine*, 20(S1) (2008), 235-241.
- [29] Premanathan M, Karthikeyan K, Jeyasubramanian K&Manivannan G, Selective toxicity of ZnO nanoparticles toward Gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation, *Nanomedicine: Nanotechnology, Biology and Medicine*, 7(2) (2011), 184-192.
- [30] Li J, Guo D, Wang X, Wang H, Jiang H & Chen B, The Photodynamic Effect of Different Size ZnO Nanoparticles on Cancer Cell Proliferation In Vitro, *Nanoscale Research Letters*, 5(6) (2010), 1063-1071.

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