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Terminalia Arjuna Bark Assisted Biosynthesis, Characterization and Bioactivity of Metal oxide Nanoparticles

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ABSTRACT

Aqueous solutions of Copper and Zinc nanoparticles with very good stability has been biosynthesized using Terminalia Arjuna bark extract as the inducer and stabiliser for the formation of nanoparticle. The formation of nanoparticles is confirmed and characterised by UV spectral and FTIR analysis. The SEM images revealed the surface morphology and size of the nanoparticles forms by bioreduction method. The copper nanoparticles are in spherical form with size ranging from 10-26nm whereas zinc nanoparticle had the particle size range between 15-25nm. XRD profile revealed the particle size of the biosynthesised nanoparticle. The antibacterial activity of the biosynthesised nanoparticles varied proportionally to the concentration. Copper nanoparticle exhibited maximum antibacterial efficacy than the zinc nanoparticle against the entire organism tested when compared to the positive control [Streptomycin]. Further, K.pneumoniae showed high resistivity to both the biosynthesised nanoparticle when compared to other tested organism. Both the biosynthesised nanoparticles exhibited antihemolytic activity against hypotonic and heat induced hemolysis of erythrocytes. CuNP exhibited maximum efficacy when compared to ZnNP.

Keywords: Green synthesis; Copper and Zinc nanoparticle; *Terminalia Arjuna*; Antibacterial activity

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INTRODUCTION

Due to the outbreak of infectious diseases caused by different pathogenic bacteria and the development of antibiotic resistance, the pharmaceutical companies and the researchers are now searching for new antibacterial agents. Recently, in this field nanotechnology represents a modern and innovative approach to develop new formulations based on metallic nanoparticles with antimicrobial properties. In the present scenario, nanoscale materials have emerged up as novel antimicrobial agents owing to their high surface area to volume ratio and the unique chemical and physical properties [1], [2]. In comparison to published reports on physical and chemical properties, very limited information is available on the antimicrobial properties of metal oxide nanoparticles. Green synthesis techniques make use of moderately pollutant free chemicals to synthesis nanomaterials and embrace the use of benign solvents such as water, natural extracts. Among the diverse biosynthetic approaches, the use of plant extracts has compensation such as easily available, safe to handle and possess a broad viability of metabolities.

The height of the Arjuna tree reaches upto 60 –85 feet. It is the evergreen tree with the yellow flowers and conical leaves. The Bark of the Arjuna tree contains calcium salts, magnesium salts, and glucosides has been used in traditional Ayurvedic herbalism. Its bark power possesses diuretic, prostaglandin enhancing and coronary risk factor modulating properties. It is also considered as beneficial in the treatment of Asthma. Its

bark is astringent and is used in fevers and in fractures and contusions. Arjuna is used for the treatment of coronary artery disease, heart failure, edema, angina and hypercholesterolemia.

The use of nanoparticles of silver and zinc oxide has been seen as a viable solution to stop infectious diseases due to the antimicrobial properties of these nanoparticles. The intrinsic properties of a metal nanoparticle are mainly determined by size, shape, composition, crystallinity and morphology [3]. The solution phase synthesis of metal oxide nanoparticles typically involves the reaction of a metal salt with hydroxide ions [4]. The antimicrobial activity of Cu Nps is linked with ions that are released from nanoparticles. The activity is further enhanced by its small size and high surface area to volume ratio that it allows them to interact closely with microbial membranes [5]. Antimicrobial activity is due to its tendency to alternate between its cuprous - Cu [I], and cupric - Cu [II], oxidation states. The antibacterial mechanism of ZnO NPs involves the direct interaction between ZnO nanoparticles and cell surfaces affecting cell membrane permeability; afterwards these nanoparticles enter and induce oxidative stress in bacterial cells, which results in the inhibition of cell growth and eventually cell death.

Realizing the potential antimicrobial applications of metal oxide nanoparticles, we designed experiments to synthesize ZnO and CuO nanoparticles using a green synthesis method utilising the bark extract of *Terminalia Arjuna* and subsequently tested their antibacterial activities against both Gram positive and Gram negative bacteria. The objective of this study was to compare the bactericidal effect of Copper and Zinc nanoparticles using various microbial strains. Such a comparative study would reveal strain specificities and would eventually lead to better utilization of nanoparticles for specific application.

EXPERIMENTAL SECTION

Biosynthesis of nanoparticles

The dried *Terminalia arjuna* bark were purchased from the local market at Chennai, Tamilnadu. Selection of plant was based on their availability and medicinal importance. The collected plant material was washed with sterile double distilled water, finely cut and air dried for a week. The dried plant materials were finely powdered and stored in airtight containers for analysis. The powdered bark was used for extract preparation. About 100 gram of powder was mixed with 1000 ml of double distilled water and this was allowed to stand for 24 hours and thus formation of plant extract. The obtained bark extract was filtered through whatman no 1 filter paper and further used for the synthesis of nanoparticle.

To synthesize copper nanoparticles, 180ml of 5mM $CuSO_4$ solution was taken in a sterile conical flask and 20 ml of aqueous plant bark extract was added to it. The solution was mixed well and kept in a rotator shaker for overnight. As a result, a blue to brown color solution was formed, indicating the formation of copper nanoparticles. It showed that aqueous copper ions could be reduced by aqueous extract of plants part to generate extremely stable copper nanoparticles in water [6].

To synthesise zinc nanoparticles, 180ml of 5mM zinc acetate solution was taken in a sterile conical flask and 20 ml of aqueous extract was added to it. The solution was mixed well and kept in a rotator shaker for overnight. As a result, a brown to dark black color solution was formed, indicating the formation of zinc nanoparticles.

Characterization of biosynthesised nanoparticles

The initial characterization of the biosynthesized CuNPs and ZnNPs were carried out after 24hrs between 300 nm to 800 nm using Perkin-Elmer lambda 25 UV-Vis spectrometer. The absorbance was taken after 5-fold diluting the sample with distilled water against distilled water as blank. FTIR was used to identify the possible functional groups responsible for the reduction of the metal ions and capping of the bioreduced Copper and Zinc nanoparticles synthesized. The samples were analyzed on a ABB Horizon MB 3000 spectrum instrument in the diffuse reflectance mode operation with the scanning range of 4000-400cm-1 at a resolution of 0.7 cm^{-1} utilising the DTGS [Deuterium Triglyane sulphate] detector. In order to obtain good signal/noise ratio 512scans were recorded. The peaks obtained were plotted as % transmittance in Y axis and wave number (cm^{-1}) in x axis. Combined with the intuitive Horizon MB^{TM} FTIR software, the MB3000 will facilitate easy acquisition, processing and analysis of samples. Scanning Electron Microscopy is done for revealing the surface morphology of particles. Structural studies of CuO and ZnO NPs were done by AURIGA- Cross beam FESEM (M/s Carl Zeiss, Germany). The characterization of the purified nanoparticles were conducted with an XRD 6000 X-ray diffractometry (shimadzu, Japan) operated at voltage of 40 kV and current of 30 mA with cu k radiation in θ 20 configurations. The crystallite domain size was calculated from the width of the XRD picks by assuming that they were free from non uniform strains and using the sherrer formula:

$$D = \frac{0.94}{\beta \, Cos \, \theta}$$

Where the D is the average crystalline domain size perpendicular to the reflecting planes, λ is the X-ray wave length, β is the full width at of maximum (FWHM) and θ is the diffraction angle. To eliminate the additional instrumental broadening, the FWHM was curetted using FWHM from a large grained Si sample. β corrected = $(FWHM^2 \text{ sample} - FWHM^2 \text{ n})^{1/2}$ This modified formula is valid only when the crystallite size is smaller than 100 nm.

Antibacterial activity of the biosynthesised copper and zinc nanoparticles

Preparation of standard bacterial suspensions:

Invitro antibacterial activity was examined for different concentrations of the biosynthesised Copper and Zinc nanoparticles. The microorganisms investigated were *Staphylococcus aureus, Pseudomonas aeruginosa, Klebseilla pneumonia* and *E.coli. Staphylococcus aureus* is a gram positive bacterium whereas all the other three organisms tested (*Pseudomonas aeruginosa, klebseilla pneumonia* and *E.coli*.) were gram negative organism. All the bacterial strains were maintained at 4°C on nutrient agar slants.

Antibacterial assay:

The bioassay used was the standard disk diffusion assay on Meuller Hilton Agar Media[7]. Test disks were prepared by dipping and saturating sterilized filter paper disks in the test suspensions. Same sized filter paper disks (6 mm diameter) absorbed the same volume of extract. Mueller Hilton media was poured in the petriplates and kept for 30 minutes for solidification. After 30 minutes the fresh overnight cultures of inoculum (100 µl) of four different culture were spread on to solidified nutrient agar plates using the sterile cotton swab.

Under aseptic conditions empty sterilized discs (Whatmann No.1 filter paper 5-6 mm diameter) were impregnated with 100µl of different concentrations (500 and 1000µg) of nanoparticle suspension in DMSO using micropipettes and the residual solvents were completely evaporated. A standard commercial streptomycin disc served as positive control and discs impregnated with DMSO alone served as the blank. All petridishes were sealed with the sterile laboratory parafilm to avoid eventual evaporation of the test samples. The plates were left for 30 minutes at room temperature to allow the diffusion of the extracts and then they were incubated at 37°C for 24 hours to allow the maximum growth of the microorganisms. The test materials showing antibacterial activity by inhibiting the growth of the thus shows a clear, distinct zone of inhibition surrounding the discs. After incubation, the diameters of the resulting growth inhibition zones were measured, averaged and the mean values were tabulated.

Determination of MIC and MBC:

The MIC and MBC were performed by a serial dilution technique using 96-well microtiter plates [8], [9]. Two different concenrations of ZnNP and CuNP were dissolved in methanol and added to luria broth with respective inoculum and the microplates were incubated for 72 hours at 37°C. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs. Methanol was used as a negative control, and commercial standard of streptomycin was used as positive control. All experiments were performed in triplicate and repeated three times.

The MBCs were determined by serial sub-cultivation of 2µl into microtitre plates containing 100µl of broth per well and further incubation for 72 hours. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate reader and compared with the standards streptomycin as the positive control.

Assay of Membrane Stabilization

Erythrocyte suspension:

The blood was washed three times using isotonic solution (0.9% saline). The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (pH 7.4) which contained in 1 L of distilled water: NaH_2PO_4. $2H_2O$,0.26 g; Na_2HPO_4, 1.15 g; NaCl, 9 g (10 mM sodium phosphate buffer). Thus the suspension finally collected was the stock erythrocyte (RBC) suspension.

Hypotonic solution induced hemolysis:

The membrane stabilizing activity of the extracts was evaluated by using hypotonic solution induced human erythrocyte hemolysis, designed by [10] with minor modification. To prepare the erythrocyte suspension, blood (7 ml) was obtained using syringes (containing anticoagulant EDTA) from male volunteers through puncture of the anti-cubital vein. The blood was centrifuged, using centrifugal machine, for 10 min at 3000 g and blood cells were washed three times with solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4). The test sample, consisted of stock erythrocyte (RBC) suspension (0.50 ml), was mixed with 5 ml of hypotonic solution

(5 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing either the biosynthesised Copper and Zinc nanoparticles (1.0 mg/ml) or acetylsalicylic acid (0.1 mg/ml) as positive control. The negative control sample, consisted of 0.5 ml of RBCs, was mixed with hypotonic-buffered saline alone. The mixture was incubated for 10 min at room temperature, centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm using UV spectrophotometer (Shimadzu, Japan). The percentage inhibition of either hemolysis or membrane stabilization was calculated using the following equation: % inhibition of hemolysis = 100 X (OD_1- OD_2/OD_1) Where, OD_1 = Optical density of hypotonic-buffered saline solution alone (control) and, OD_2 = Optical density of test sample in hypotonic solution.

Heat-induced hemolysis:

Aliquots (5 ml) of the isotonic buffer, containing 1.0 mg/ml concentration each of the biosynthesised copper and zinc nanoparticles from the bark extract of the plant were put into two duplicate sets of centrifuge tubes [11]. The vehicle, in the same amount without the addition of the nanoparticles, was added to another tube as control. Erythrocyte suspension (0.3mL) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54°C for 20 min in a water bath. The other pair was maintained at 0-5°C in an ice bath. The reaction mixture was centrifuged for 3 min at 1300 g and the absorbance of the supernatant was measured at 540 nm using UV spectrometer. The percentage inhibition or acceleration of hemolysis in tests and was calculated using the following equation: % inhibition of hemolysis = 100 × [1-(OD_2-OD_1/OD_3-OD_1)] Where, OD_1 = test sample unheated, OD_2 = test sample heated and, OD_3 = control sample heated.

RESULTS AND DISCUSSION

Preparation of aqueous extract and biosynthesis of copper and zinc nanoparticle

Aqueous extract of *Terminalia arjuna* bark was prepared and used for the synthesis of Copper and Zinc nanoparticle by biological method. As the plant extract was mixed in the aqueous solution to the copper sulphate and Zinc Acetate, the respective ions were reduced by the phytochemicals present in the bark extract.

Figure 1: Solutions of extract, copper sulphate and zinc acetate

Figure 2: Solution of extract, zinc and copper nanoparticle synthesised after 24 hrs

It started to change the color which may be the preliminary indication of formation of Copper and Zinc nanoparticles [Figure 1 and Figure 2]. *Terminalia arjuna (T. arjuna)* bark extract is used to reduce Cu(2+)→Cu(0) and also for the reduction of zinc ions (Zn2+) to zinc nanoparticles (Zn0). The intensities of the colors increased on standing the solutions at room temperature for several hours and then remained constant after 48 Hrs.

Characterization of biosynthesised nanoparticles

UV-VIS Spectral analysis:

Metal nanoparticles exhibit strong plasmon resonance extinction band in the visible spectrum in consequence of interaction between conduction electrons of metal nanoparticles and incident electromagnetic radiation [12].

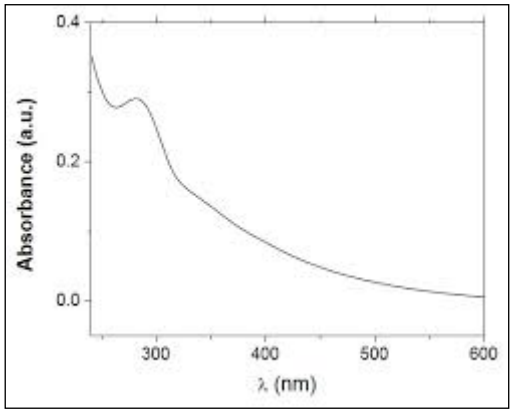

Figure 3: The absorbance spectrum of Copper nanoparticles showing maximum absorbance near 280nm

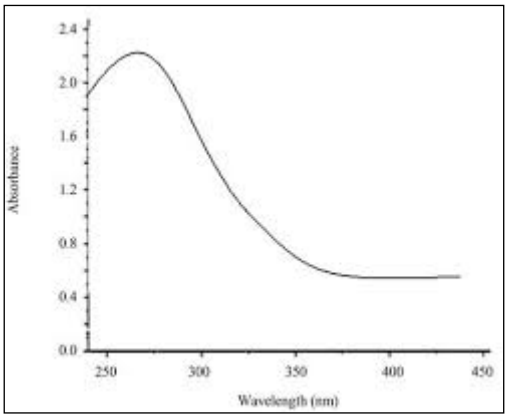

Figure 4: The absorbance spectrum of Zinc nanoparticles showing maximum absorbance near 275nm

The UV-Vis spectra of CuO NP prepared with 5mM solution of Copper sulphate was shown in Figure 3. The absorption peak of the prepared nano CuO was found at around 280 nm. The UV-Vis absorption spectra of the ZnNP were shown in Figure 4. Absorption spectra of Zn nanoparticles formed in the reaction media has absorbance maxima at 275nm. The colour change i.e pale blue to light brown for copper nanoparticle and a transparent liquid to dark brown solution was oberserved in the UV- vis spectrophotometer and is due to the Plasmon resonance phenomenon which is the collection of oscillation of electrons.

FTIR analysis:

This FTIR gives information on the vibrational and rotational modes of motion of a molecule and hence an important technique for identification and characterisation of a substance. The Infrared spectrum of an organic compound provides a unique fingerprint, which is readily distinguished from the absorption patterns of all other compounds; only optical isomers absorb in exactly the same way. Hence FTIR is an important technique for identification and characterization of a substance. The results of FTIR of the prepared extract and biosynthesised CuNP and ZnNP were shown in Figures 5-7 respectively.

The FTIR spectrum of the bark extract showed a large sharp peak at 3450 cm-1 strongly indicates the presence of alcohols and phenols whereas the band broadens with the spectra of the biosynthesised nanoparticles showing the utilisation of these phytochemicals for the reduction of metal ions. The FTIR spectrum of Copper nanoparticles [Figure: 6] showed a sharp band at 3400 cm-1 whereas the ZnNP spectra [Figure: 7] showed the band between 3000-3500 cm-1. These correspond to O-H stretching H-bonded alcohols and phenols. For both the nanoparticles, the peak found around 1350-1550 cm-1 showed a stretch for C-H bond and peak around 1150-1500 cm-1 showed the bond stretch for N-H for the presence of aliphatic amines. Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional

groups. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles (i.e.; capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. Carbonyl groups proved that flavanones or terpenoids absorbed on the surface of metal nanoparticles. Flavanones or terpenoids could be adsorbed on the surface of metal nanoparticles, possibly by interaction through carbonyl groups or π-electrons in the absence of other strong ligating agents in sufficient concentration. The presence of reducing sugars in the solution could be responsible for the reduction of metal ions and formation of the corresponding metal nanoparticles. It is also possible that the terpenoids play a role in reduction of metal ions by oxidation of aldehydic groups in the molecules to carboxylic acids.

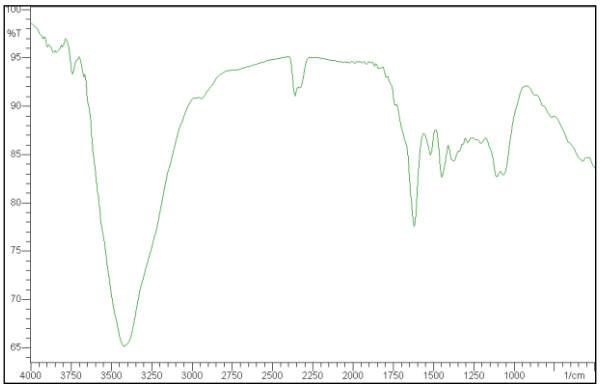

Figure 5: The FTIR absorbance spectrum of aqueous bark extract

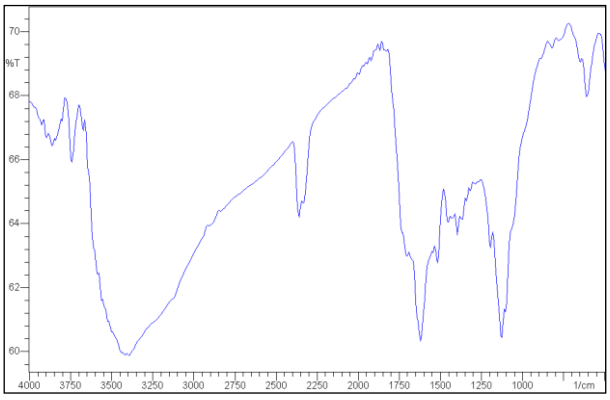

Figure 6: The FTIR absorbance spectrum of Copper nanoparticles

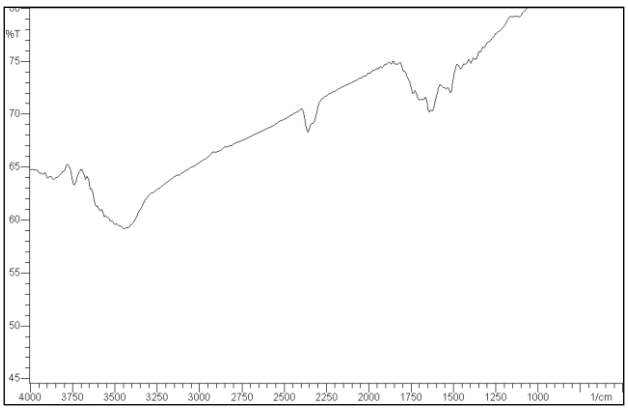

Figure 7: The FTIR absorbance spectrum of zinc nanoparticles

Scanning electron microscopy:

Scanning Electron Microscopy is done for revealing the surface morphology of particles. The electron beam is generally scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. SEM can achieve resolution better than 1 nanometer.

The surface morphology of the prepared Cu and Zn nanoparticles was examined by FESEM. The above figure shows the SEM image of freshly synthesized Copper and zinc nanoparticles respectively (Figure 8-11). It can be observed that the copper nanoparticles are in spherical form with size ranging from 10-26nm. It can be seen from figure that the copper nanoparticles aggregate to form rod like structure. The diameter of the rod is varied from as thin as 293.6nm to 464.9nm suggesting the formation of rods by spherical particles. This linear orientation is nearly due to the magnetic properties of copper species being in a transition state. The Zinc particles are also in the form of nanospheres of size ranging from 15-25nm.

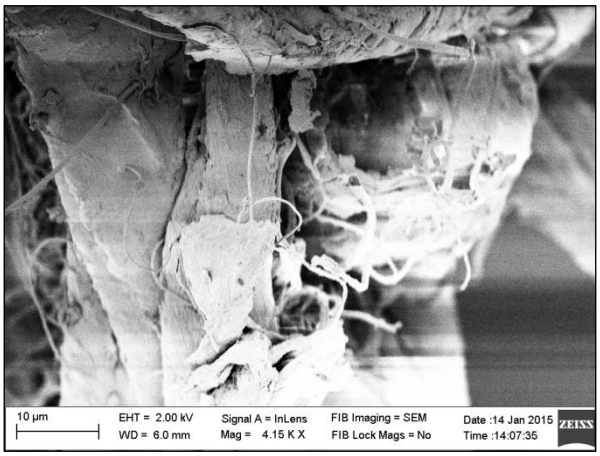

Figure 8: SEM image showing surface morphology of the copper nanoparticles

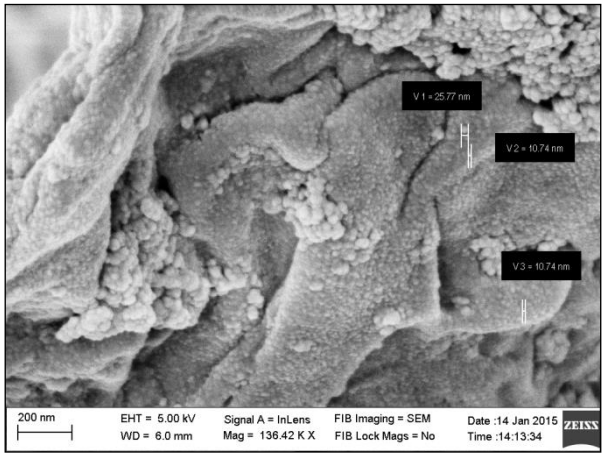

Figure 9: SEM image showing Particle size of the Copper nanoparticles

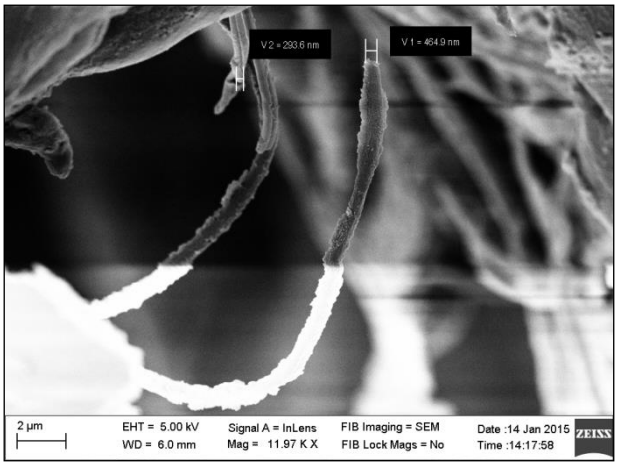

Figure 10: SEM image showing size of the copper NPs aggregate as nanorod

The surface morphology of the prepared Cu and Zn nanoparticles was examined by FESEM. The above figure shows the SEM image of freshly synthesized Copper and zinc nanoparticles respectively (Figure 8-11). It can be observed that the copper nanoparticles are in spherical form with size ranging from 10-26nm. It can be seen from figure that the copper nanoparticles aggregate to form rod like structure. The diameter of the rod is varied from as thin as 293.6nm to 464.9nm suggesting the formation of rods by spherical particles. This linear orientation is nearly due to the magnetic properties of copper species being in a transition state. The Zinc particles are also in the form of nanospheres of size ranging from 15-25nm.

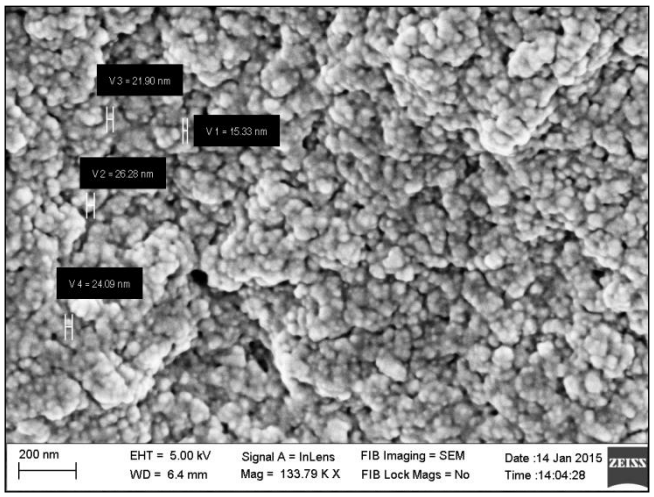

Figure 11: SEM image showing surface morphology and particle size of zinc nanoparticle

X-ray diffraction (XRD) analysis:

Diffraction pattern gives information on translational symmetry - size and shape of the unit cell from Peak Positions and information on electron density inside the unit cell, namely where the atoms are located from Peak Intensities. It also gives information on deviations from a perfect particle, if size is less than roughly 100 – 200 nm, extended defects and micro strain from Peak Shapes & Widths.

Further, the synthesized particles are subjected to XRD which has given a clear picture on the presence crystalline cubic phase of monoclinic Cupric oxide (CuO) exhibiting 2θ values 32˚,23˚, 33.21˚, 35.59, 38.60, 46.22, 54.93.57.93 (Figure 12) which are closely matched with the values of monoclinic phase CuO reported by [13-15].

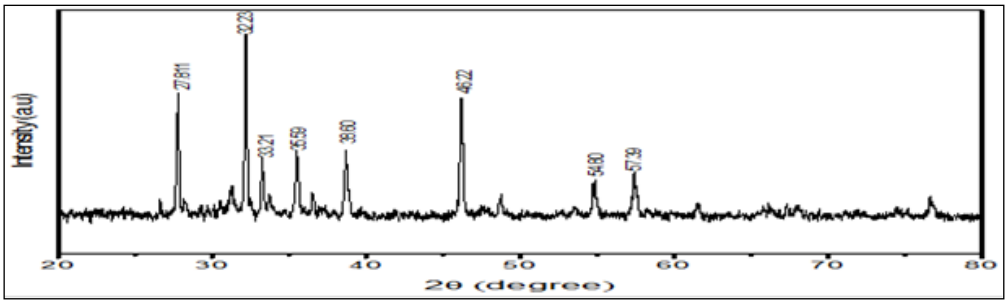

Figure 12: XRD of Cu nanoparticles using *terminalia* bark extract

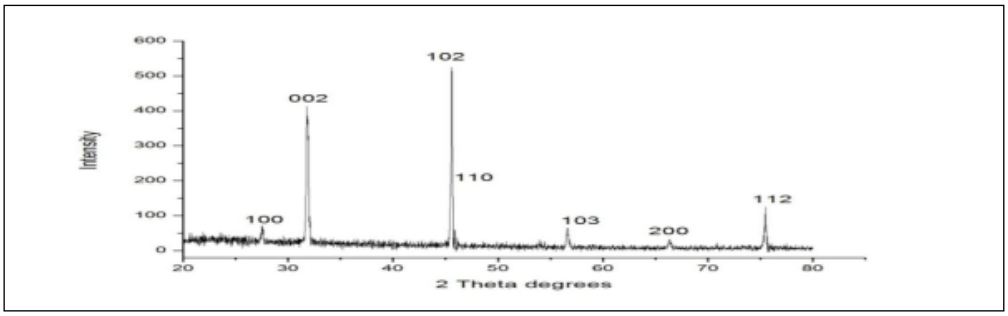

Figure 13: XRD of Zn nanoparticles using *Terminalia* bark extract

Figure 13 shows the distinct diffraction peaks around 46˚. The XRD study confirms the wurtzite structure of ZnO nanoparticles and the formation of narrow peak with the Bragg's angle of 2θ=102˚ suggesting the Face centered cubic nature of the ZnO nanoparticle. Stabilization of the nanoparticles occurs by some capping agents which are confirmed by the sharp peaks. The intense Bragg's angle reflection suggests the strong X-ray scattering centres in the crystalline phase which is due to the capping agents.

Antibacterial activity of the biosynthesised copper and zinc nanoparticles

The characteristic features of nanoparticles namely the larger aspect ratio renders greater surface area of contact with the microbial pathogens and provides enhanced reactivity. Additionally the smaller size of NPs facilitates easy entry into the microbial cell membrane and enables inhibition mechanisms to occur inside the bacterial cell. The antibacterial activity of the copper and zinc nanoparticles synthesised by bioreduction method was given in Figure 14. The zone of inhibition is given as mean value between the triplicates. The antibacterial activity of the biosynthesised nanoparticles varied proportionally to the concentration. Copper nanoparticle exhibited maximum antibacterial efficacy than the zinc nanoparticle against all the four organism tested when compared to the positive control [Streptomycin]. Further, *K.pneumoniae* showed high resistivity to both the biosynthesised nanoparticle when compared to other tested organism. It is quite interesting to note that all bacterial species tested in this study showed resistance to the synthetic antibiotic drug which in turn indicates the better antibacterial activity of the ZnO NPs than the commercially available synthetic drug. The plates showing zone of inhibition with Copper and Zinc nanoparticle against all the bacterial isolates is given in Figures 15 and 16 respectively. The MIC and MBC determined was given in Table 1 and 2 respectively which indicates that bacterial growth was inhibited.

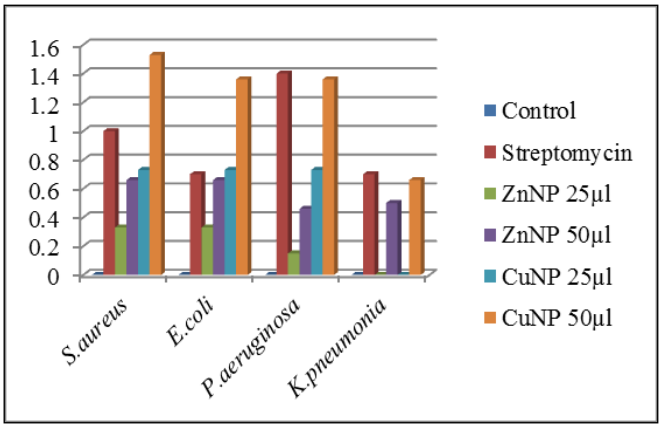

Figure 14: Antibacterial activity of biosynthesised nanoparticles

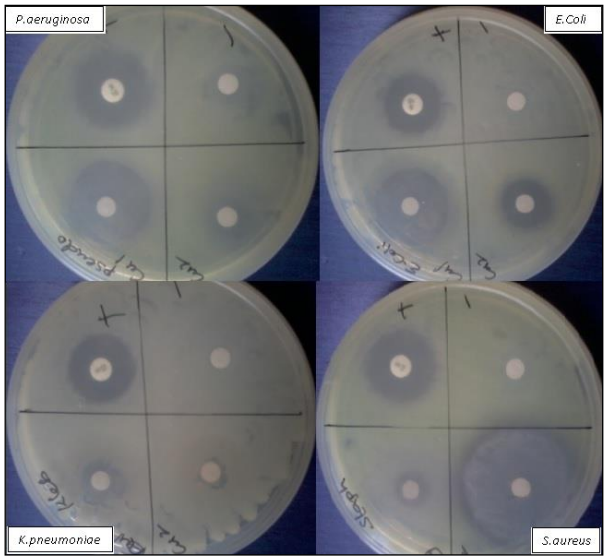

Figure 15: Plates showing zone of inhibition by copper nanoparticle

Table 1: The minimum inhibitory concentration (MIC) of *Terminalia arjuna* bark were measured by Micro dilution method (in µg/ml)

Test Organisms Bacteria	ZnNP (25µl)	ZnNP (50µl)	CuNP (25µl)	CuNP (50µl)	Streptomycin
S. aureus	7.4	13.3	15	48.9	18.2
E. coli	7.2	12.9	14.9	39.8	15.8
P. aeruginosa	3.1	11.6	17.6	42.9	34.2
K. Pneumoniae	1.5	10.2	1.8	16.9	16

Table 2: The minimum bactericidal concentration (MBC) of *Terminalia arjuna* bark were measured by Micro dilution method (in µg/ml)

Bacteria	ZnNP (25µl)	ZnNP (50µl)	CuNP (25µl)	CuNP (50µl)	Streptomycin
S. aureus	12.5	25.6	28.5	96.2	35.7
E. coli	11.9	23.8	27.2	75.3	28.8
P. aeruginosa	5.3	21.1	32.8	82.2	66.5
K. pneumoniae	2.6	19.4	3.1	30.8	30.2

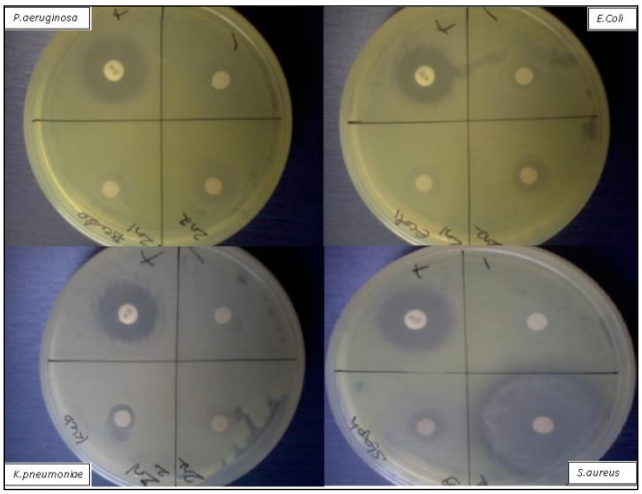

Figure 16: Plates showing zone of inhibition by zinc nanoparticle

In this way, the results identified that growth of all the test organisms were inhibited by the biosynthesised nanoparticles. Among the tested bacterial pathogens, gram-positive bacterial strain has been found to be more susceptible than gram-negative bacterial strains. This may be attributed to the fact that the cell wall in gram-positive bacteria consists of a single layer, whereas, the gram-negative cell wall is a multilayered structure bounded by an outer cell membrane. Thus our findings suggest the use of nanoparticles biosynthesised from *Terminalia arjuna bark extract* against bacterial infections and application for wound dressing. Copper used as

an antimicrobial agent for decades has revealed a strong antibacterial activity and was able to decrease the microorganism concentration by 99.9% [16], [17].

Assay of membrane stabilisation

It is evidence that the aqueous bark extract and the biosynthesised nanoparticle protected the human erythrocyte membrane against lysis induced by hypotonic solution and heat. During inflammation, lysosomal enzymes and hydrolytic components are released from the phagocytes to the extracellular space, which causes damages of the surrounding organelles and tissues and also assists a variety of disorders [18]. It was found that NSAIDs act either by inhibiting these lysosomal enzymes or through stabilization of lysosomal membranes. Again, RBC exposure to harmful substances such as hypotonic medium, heat, etc results in the lysis of the membranes, accompanied by the oxidation and the lysis of hemoglobin [19].

The membrane lytic activity of the nanoparticles with hypotonic solution is shown in Figure 17 and protection against heat induced hemolysis is given in Figure 18. It was observed that copper nanoparticles exhibited maximum haemolytic activity [69%] when compared to ZnNP [63%]. Similar efficacy was also seen with heat induced hemolysis where CuNP showed 74.8% haemolytic activity and ZnNP showed 70% activity. Both the nanoparticles exhibited better haemolytic activity with heat induced hemolysis that with hypotonic solution induced hemolysis. Both the biosynthesised nanoparticle showed comparable haemolytic activity with the positive control drug, Acetyl salicylic acid.

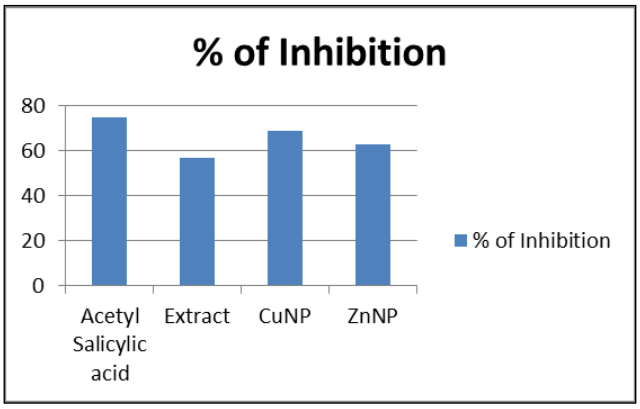

Figure 17: Antihemolytic activity of Biosynthesised Nanoparticles against Hypotonic solution induced hemolysis of erythrocyte membrane

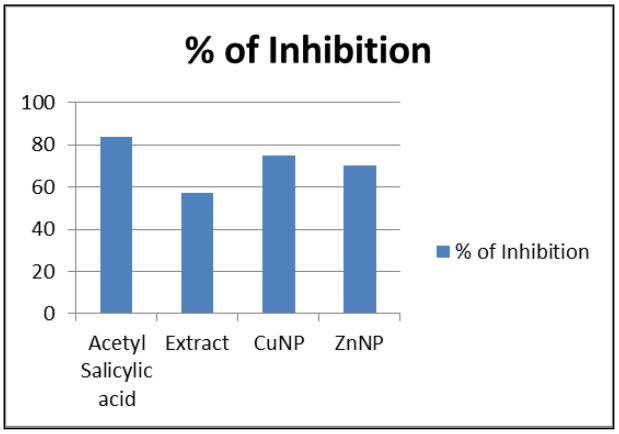

Figure 18: Antihemolytic activity of Biosynthesised Nanoparticles against heat induced hemolysis of erythrocyte membrane

The inhibition of hypotonicity and heat induced RBC membrane lysis was taken as a measure of the mechanism of anti-inflammatory activity of the plant extract, because human RBC membranes are considered similar to lysosomal membrane components [20]. One can say that the possible mode of action of the extract, fractions and standard anti-inflammatory drugs may be connected with binding to the erythrocyte membranes with consequent alteration of surface charges of cells. This could have prevented physical interaction with agents of aggregation or promote dispersion by mutual repulsion of the charges as being involved in the hemolysis of RBCs. In some

research, it has been reported that some chemical components present in the extracts can have the same mechanism, which are well known for their anti-inflammatory activity [21]. Both in vitro and in vivo studies in experimental animals showed that the flavonoids exert stabilizing effects largely on lysosomes [22] as tannin and saponins are capable of binding cations and other biomolecules, and are capable of stabilizing the erythrocyte membrane [23]; and report says that the bark extract of *T.arjuna* has tannins, saponin, and lots of flavonoids [24]. Our research reveals that nanoparticles synthesised through green approach showed potent RBC membrane stabilization activity with a good protection against both hypotonic solution and heat-induced lysis.

CONCLUSION

Highly pure Cu and Zn NPs were prepared by a bioreduction method. XRD spectrum revealed that CuO NPs were monoclinic crystals with space group C2/c. FESEM showed the surface morphology of Cu and Zn NPs. CuO NPs showed excellent antimicrobial activity against bacterial strains when compared to ZnNP. Consequently, CuO NPs have potential for external uses as antibacterial agents in surface coatings on various substrates to prevent microorganisms from attaching, colonizing, spreading, and forming biofilms in indwelling medical devices. It is evident that metal based nanoparticles due to their biological and physiochemical properties are promising as antimicrobials and therapeutic agents. They can be used to address a number of challenges in the field of nanomedicine. But it must be remembered that they can also possibly cause adverse biological effects at the cellular and subcellular levels. Therefore, after the cytotoxicity and clinical studies of the nanoparticles can find immense application as antimicrobials in the consumer and industrial products.

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