Original Research Article

Studying the potential of Copper Nanoparticles synthesized from *Staphylococcus aureus* against drug-resistant bacteria

Abstract

Copper nanoparticles (CuNPs) have gained a lot of interest due to their tendency to combat various bacterial strains, making them potential candidates against antibiotic resistant bacteria. Green synthesis of CuNPs eradicates the potential drawbacks of other traditional methods such as high cost, toxic chemicals and adverse conditions. The preparation of CuNPs from the culture supernatant of *Staphylococcus aureus* and the analysis of antibacterial potential of prepared CuNPs. **Study design:** Experimental study to analyze the synthesis of CuNPs from the culture supernatant of *Staphylococcus aureus* under various optimization conditions including pH, temperature, incubation time, and concentration of salt. This research study focuses on the preparation of CuNPs by the reduction of Cu^{2+} ions using the culture supernatant of clinical isolate of *Staphylococcus aureus* as the feasible and cost effective methodology to produce the fine and well defined nanoparticles. The prepared nanoparticles underwent the various characterization techniques including Ultraviolet Visible Spectrophotometry, Dynamic Light Scattering and Scanning electron microscopy. The prepared copper nanoparticles were studied for their antibacterial potential by the Agar Well Method against drug resistant and drug sensitive bacteria. The surface plasmon resonance of copper nanoparticles was maximum around the visible range wavelength of 380 nm which is the characteristic absorbance peak of copper nanoparticles while the dynamic light scattering analysis recorded the maximum percentage intensity of particle size distribution around 295 nm. SEM analysis showed the amorphous nature of particles with no distinct size and shape. CuNPs showed an appreciable zone of inhibition against of diamtere 29mm against drug resistant *Salmonella typhimurium* and zone was produced against drug sensitive *Bacillus subtillis* had a diameter of 25mm. The antibacterial potential of copper nanoparticles was found to be appreciable against drug resistant *Salmonella typhimurium, Staphylococcus epidermis* and *Staphylococcus aureus*.

Keywords: *Staphylococcus aureus*, CuNPs, drug resistance, antibacterial, Green synthesis

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1. Introduction

Today, the most important field of modern research emphasizes on the miniaturization of bulk metallic and non-metallic materials to the size of 1-100nm by various synthetic methodologies, which is termed nanotechnology. This technology is capable of controlling and changing the physical and biological properties of nano-materials, thus presenting the advantageous transformation of applications in various fields including biomedicine, pharmaceutics, optical devices, electrical field and cosmetics industry. Moreover, this technology has been supportive enough to introduce nano-medicines with targeted drug delivery and potential bioavailability [1]. Metallic nanoparticles show remarkable biological and physical properties depending on their size and shape, thus, copper nanoparticles have gained much importance due to their potential physical and chemicals properties for being the transition 3D metal. Moreover, copper is the most abundant metal in nature and offers less expensive, less toxic and easy methodologies for the preparation of nanoparticles. Copper plays functional role as enzymes at low concentration and supportive role in metalloproteins. Many different oxidation states can be acquired by copper based materials enhancing the reactivity of copper, thus plays role in the production of hyperperoxide free radicals, inactivation of enzymes, disturbing the membrane stability and capping of the vital functional groups of microbes making the copper nanoparticles as successful combatant against the harmful microbial agents [2]. Various methods for the preparation of copper nanoparticles have been investigated which are differentiated as chemical, physical and biological methods. Chemical reduction method was utilized by Ostaeva *et al.* to produce the stable copper nanoparticles by the reduction of copper ion in acrylic acid blend solution to produce the stable copper nanoparticles of size less than 10nm [3]. Other methods including in the category of chemical synthesis processes are microemulsion, photochemical, Thermal decomposition and electrochemical methods. Chemical methods are used frequently but they possess the major drawback of utilizing the toxic reducing agents which are not safe for disposal as well offering the environmental toxicity. Copper nanoparticles have also been synthesized from various physical methods including ball Milling method, laser ablation method, pulse Wire Discharge method, inert gas condensation and exploding wire method but have a major disadvantage of their expensive nature of methodology as they mostly require costly vacuum systems [4].

Due to the above mentioned limitations of chemical and physical methods for the synthesis of copper nanoparticles, green synthesis processes have gained much importance due less toxic reagents and methodology. The main goal to be achieved by green synthesis is to synthesize the nanomaterials to prevent the possible toxic effects of the pollutants or introduction of the nanomaterials capable of cleaning the pollution in environment. That is why, green synthesis is considered much safer method of preparing nanoparticles because they don't utilize the toxic chemicals and reagents, less expensive because of no vigorous utilization of energy sources and produce biocompatible products by using the biological components as reducing agents and capping of nanoparticles [5]. Bacteria are considered preferably for the metallic nanoparticles synthesis because they offer tremendous advantages including rapid multiplication, capability to withstand the extreme conditions, incidence in environment in larger population and easy methods of cultivation. Bacteria growth is monitored by various optimizing conditions

including pH, temperature, incubation time and oxygenation [6]. Various bacteria have been used for the synthesis of copper nanoparticles including *Pseudomonas fluorescens* [7] *Salmonella typhimurium* [8] *Enterococcus faecalis* [9] *Bacillus cereus* [10] *Klebesiella pneumoniae* [11] *Schewanella loihica* [12] *Rhodococcus species* [13] and *Morganella morgana sp.* [14]. Various suppositions for the mechanism of microbial synthesis of nanoparticles have been reported which suggest the bacteria as the potential environment friendly catalysts for reducing metal and preparing various biomaterials, moreover, bacteria have the tendency to produce wide range of metal oxides through respiratory processes [15]. The anaerobic respiration of bacteria causes the electrons to transfer from the reduced organic metallic form to oxidized inorganic component in order to produce the nanocrystals as a result of the bioremediation process. Bacteria have also developed the mechanism to detoxify the cellular environment having higher concentration of toxic metallic salt by producing the metal nanoparticles, moreover, bacteria release the stabilizing and capping agents for the nanoparticles synthesis [16]. It has been reported that the reductase enzymes released from *Streptomyces sp*. play a dominant role in the reduction of metal ions and converting them to metal nanoparticles [17]. Copper nanoparticles show remarkable biological applications including antimicrobial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella enterica* and *Micrococcus luteus,* anticancerous, antifungal and antiviral activities. The prominent antiviral potential of CuNPs has been reported as it can reduce the half-life and viability of COVID-19 virus and influenza virus [18]. Copper nanoparticles have been proved as anticancerous agents for initiating the apoptotic pathway for the death of various cell lines including human breast cancer cell lines (MD A-MB-231)[19], colon cancer cell lines(Caco-2) [20] and hepatic cancer cell lines(MCF-2) [21]. Copper has been prominently used to eradicate the oral microbes including *Porphyromonas gingivalis* and *Streptococcus mutans*. Copper nanoparticles destroy the microbial cells by producing the reactive oxygen species (ROS) which destabilize the cell membrane and cell wall, and then damage the DNA and proteins. By various studies it has been suggested that CuNPs cause the production of ROS by leaching of ions and the release of singlet oxygen species by the destabilization of mitochondrial membrane potential resulting in the destruction of DNA.

In this study, *Staphylococcus aureus* was considered as the most efficient bacterial source for the green synthesis of copper nanoparticles with magnificent applications as discussed previously. There are very few publication reporting the consideration of *Styaphylococcus aureus* as the reducing source of metal ions. Nanda *et al.* reported the synthesis of silver nanoparticles from the culture supernatant of *Staphylococcus aureus* and their antibacterial potential against several bacterial isolates including methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermis* [22]. Similarly, Medina Cruz *et al.* reported the appreciative antibacterial efficacy of selenium nanoparticles (SeNPs) produced by the selenium reductase enzymes of the *Staphylococcus aureus.* The zone of inhibition formed by the prepared NPs against *Staphylococcus aureus* was much dominant as compared to the selenium nanoparticles produced by other bacterial strains. This research validated the strong antibacterial activity of nanoparticles synthesized from the *Staphylococcus aureus* against the clinical isolate of

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Staphylococcus aureus as compared to the Se nanoparticles synthesized from the other bacterial strains [23]. This conclusion motivated us to analyze the effect of copper nanoparticles synthesized from *Staphylococcus aureus* against the clinical drug resistant isolate of *Staphylococcus aureus* which has not been reported before by any research article. Moreover, copper nanoparticles have been proved beneficial for their ecological safety, biocompatibility and most cost effective [24] and hence, can be used as replacement of antibiotics which on repeated exposure can develop the antibiotic resistance but it requires the well planned in vitro experimental design for rats followed by the dose compatibility on humans for the commercialization of Nano medicine.

2. Materials and Methods

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2.1. Isolation and purification of *Staphylococcus aureus (S. aureus)*

Three isolates of *S. aureus* were collected from clinical diagnostic lab and two isolates were community-acquired *S. aureus.* They were cultured on Luria Bertani agar as prepared per standard recipe having 1g of Tryptone, 0.5g of yeast, 1g of NaCL and 1.5g of agar dissolved to 100ml of distilled water. The media was autoclaved, poured on sterilized petri plates to solidify on which bacterial isolates were cultured and incubated at 37^0C to obtain the pure bacterial colonies for the synthesis of copper nanoparticles. The pure cultured bacterial colonies were stored at 4^0C in refrigerator [25].

2.2. Morphological and biochemical characterization of *S. aureus*

S. aureus isolates were identified by growing them on Luria Bertani agar and observing their growth pattern on agar along with shape, size and color of colonies. Moreover, shape of bacterial colonies was identified under light microscope after gram staining [26]. The biochemical screening of S.aureus was done by growing them on mannitol salt agar, catalase and coagulase assay [27][28, 29]. 13.3g of mannitol salt agar was dissolved to 120ml distilled water for the culturing of five bacterial isolates. Catalase assay was performed by adding few drops of 15% hydrogen peroxide in 5ml of freshly grown broth culture in test tube while it was also performed by adding a drop of 15% H_2O_2 on the bacterial colony taken on glass slide. While the coagulase test was performed by adding freshly prepared culture in 1ml of 1:10 dilution of human blood plasma in test tube.

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2.3. Synthesis of copper nanoparticles

Preparation and collection of bacterial culture supernatant

The bacterial culture supernatant was prepared by adding a few colonies of bacterial isolate in autoclaved Mueller Hinton Broth prepared by adding 0.7g of meat extract, 0.6g of starch and 6g of peptone in 350ml of distilled water with pH adjusted to precisely 7. The inoculated media was placed in rotatory shaker at 150 rpm and 37^0C for about 48 hours to get maximum optical density. After acquiring the maximum

bacterial growth, the culture broth was added in 50ml Falcon tube and subjected to the centrifugation of 4500 rpm for about 15 minutes. The supernatant was collected in other clean Falcon tube [30].

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Bacterial synthesis of copper nanoparticles

Different concentrations of copper nitrate trihydrate $Cu(NO_3)_2$ $.3H_20$ solution were prepared as 1mM, 2mM, 3mM, 5mM, 5mM, 7mM, 9mM, 11mM, 13mM and 15mM were prepared from 1M stock solution and added in the bacterial culture supernatant in the same ratio. The pH of the reaction mixture was adjusted to 7 pH and the prepared reaction mixture was incubated at 37^0C for about 48 hours. After the completion of incubation time, absorbance of the reaction mixture was studied in the wavelength range of 100-600nm ultraviolet visible spectrophotometry to analyze the copper nanoparticles synthesis [23].

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Optimization parameters

To study the effect of physiological parameters on the optimal production of copper nanoparticles, the reaction mixture was exposed to a set of varying pH (5, 7 and 9), incubation time (24, 48 and 72 hours) and temperature (27^0C, 37^0C and 40^0C), moreover the bacterial culture supernatant was subjected to the various salt concentration in various ratios of 1:1, 1:2, 1:4, 1:6, 1:8, 2:1 and 4:1. Their effect was analyzed by the ultraviolet visible spectrophotometry [31].

2.4. Characterization of CuNPs

UV-visible spectral analysis

The reduction of copper ions to the copper nanoparticles was analyzed by T-80 UV-Visible spectrophotometer while the wavelength range was selected from 200-600nm [32] .

Dynamic Light scattering analysis (DLS analysis)

Malvern Zetasizer (version 7.11) was used for the accurate measurement of particle size using the method of multiple diffractions and Mie scattering theory as principle. The zeta potential was also measured to analyze the stability of nanoparticles in colloidal solution [33].

Scanning electron microscopy (SEM analysis)

The prepared copper nanoparticles were perfectly dried to a powdered form to analyze the accurate morphology of particles. The morphological characterization of CuNPs was done at various magnifications and resolution in Agriculture University, Faisalabad [34].

2.5. Biological activity

Antibacterial activity of CuNPs

Antibacterial activity of copper nanoparticles was performed against drug resistant and drug sensitive bacterial isolates. The collected drug resistant isolates were identified by growing them on differential media. Drug resistant *Escherichia coli* and *Klebesiella pneumonia* were grown on MacConkey agar [35], *Salmonella typhimurium* was grown on EMB agar [36], Methicillin resistant *Staphylococcus aureus* was grown on blood agar and *Staphylococcus epidermis* was grown on mannitol salt agar [37]. After the identification of bacterial isolates, they were grown in nutrient broth. The optical density of prepared culture broths was adjusted to that of 0.5% Macfarland solution. The agar well antibiotic susceptibility testing was performed by pouring Mueller Hinton agar onto the sterilized plates and wells were formed after solidification. The culture broth was spread on agar by the autoclaved spreader after which 100ul of CuNPs solution was added in one well, 100ul solution of salt in other and the standard drug solution in the third well. The prepared plates were incubated for 24 hours at 37^0C [38].

3. Results

3.1. Morphological and biochemical characterization

After the incubation of 24 hours, the Luria Bertani agar plates showed round and scattered colonies with yellowish golden color grown in the same pattern without any contamination. Microscopic observation of the gram stained culture on glass slide showed the pink colored bacterial cells which were arranged in the form of bunch of grapes. As the prefix “coccus” means round so the round shape of bacterial cells was the clear indication of *Staphylococcus aureus*. Also, bunch like arrangement of bacterial cells differentiates the *Staphylococcus aureus* form *Staphylococcus epidermis which* occur in the form of chain. Biochemical characterization done by mannitol salt agar showed the yellow bacterial colonies turning the color of agar from red to yellow which occurred by the acidic due to fermentation of mannitol. Catalase test proved to be positive due to evolution of oxygen in the form of bubbles in the test tube. While the coagulase test showed the turbidity in the end of test tube due to coagulation in plasma by the release of coagulase enzyme present in bacteria, so all the collected bacterial isolates were proved *Staphylococcus aureus.*

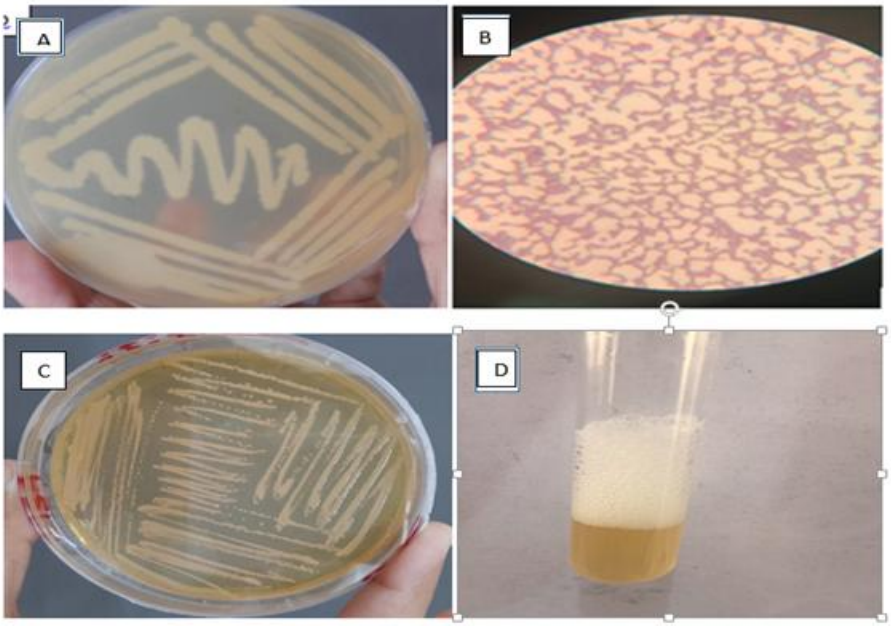

Fig 1. Morphological and Biochemical characterization of Bacterial isolates. A) *Staphylococcus* aureus colonies on Luria Bertani Agar B) Microsopic examination of gram stained bacterial sample C) *Staphylococcus aureus* colonies on Mannitol Salt agar D) Positive catalase test for *Staphylococcus aureus* showing the bubbles formulation

3.2. Bacterial synthesis of CuNPs

All the collected bacterial samples of *S.aureus* were grown in the Mueller Hinton broth and their capability to reduce the copper ions of salt solution to copper nanoparticles was observed after the proper incubation by the change in color of reaction mixture and the UV-Visible Spectrophotometer in the wavelength range of 200-600nm. The analysis of results obtained at the end of screening process concluded the incidence of copper reducing ability in only one bacterial sample that was subjected to various optimization conditions to obtain the well-defined CuNPs in higher volume.

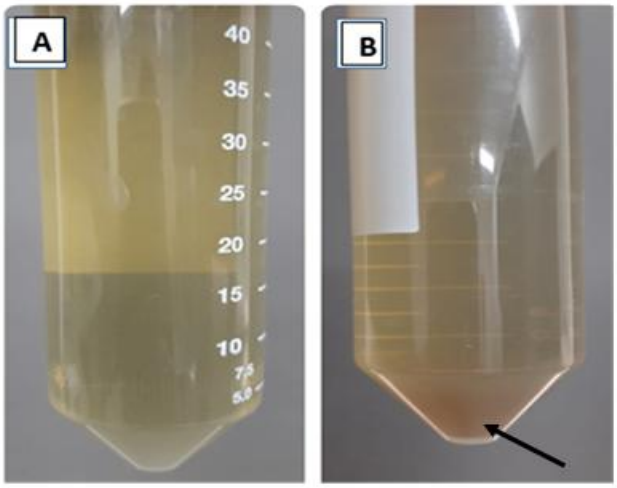

Fig 2. Color change in reaction mixture after incubation A) Reaction mixture with no color change B) reaction mixture with color change and Light brown colored CuNPs settled at bottom

3.3. Optimization of nanoparticle's synthesis process

Effect of pH

Synthesis of nanoparticles requires the monitoring of pH for optimum production of nanoparticles. Initially the reaction mixture possessed pH 6 which was brought to pH 7 and pH 9 by adding drop of 1N NaCl and to pH 5 by adding a drop of 1N HCl. pH 7 proved to be the optimum pH for the activity of reducing enzymes of bacteria causing the change in color of reaction mixture indicating the production of CuNPs.

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Effect of temperature

Nanoparticles synthesis was obvious at optimum temperature of 37 ^{0}C by the prominent change in color while the variation in temperature didn't generate any positive results due to the possibility of instability and inactivity of bacterial reducing enzymes.

Effect of Cu $(N0_2)_3.3H_2O$ concentration

The enzymes of bacteria were capable to reduce the copper ions of salt at concentration of 2mM and 11mM as concluded by the change in color of reaction and the UV Visible Spectrum analysis recording the peak absorption at wavelength of 380nm, however, there was not even a slight change in color at any other concentration of salt.

Effect of bacterial culture supernatant to salt ratio (Bacterial supernatant:salt)

The optimum ratio was selected after performing the reaction in all possible ratios. Synthesis of nanoparticles was obvious in the reaction where bacterial supernatant was higher in volume than that of salt solution. The analysis of results by UV spectrophotometer concluded the ratio of 4:1 (bacterial supernatant: salt) as the best performing ratio for the reduction of copper ions to copper nanoparticles.

Effect of incubation time

The synthesis of nanoparticles occurred on the completion of required incubation time while the results were recorded right after the reaction as well as 24 hours, 48 hours and 72 hours. The whole reduction process took minimum 48hours and maximum 72 hours to produce well defined and stable nanoparticles as observed by UV Visible spectrum

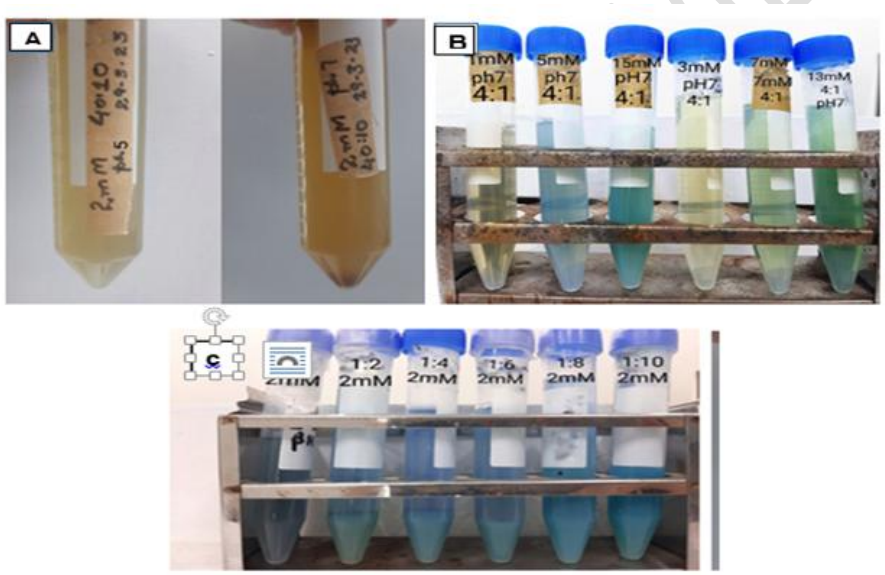

Fig 3. A) Effect of pH B) Effect of salt concentration C) Effect of bacterial supernatant: salt solution

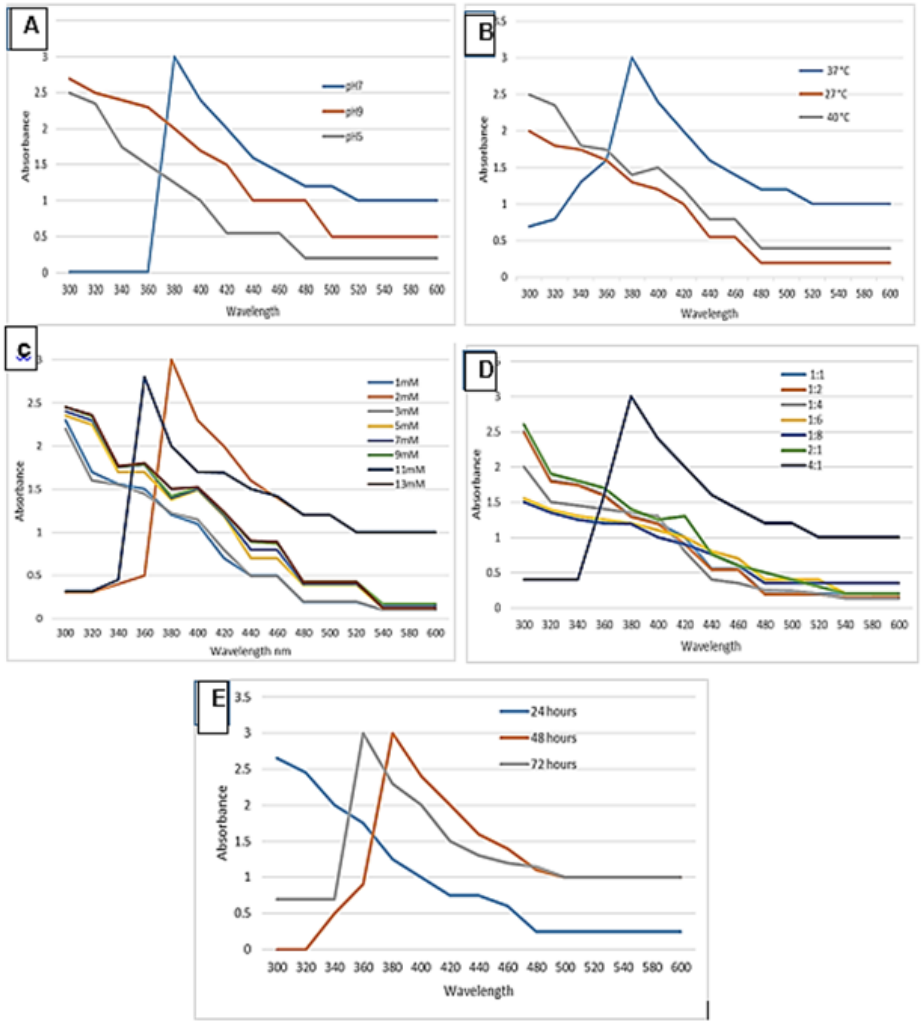

Fig 4. Graphical Insight of various Optimization conditions A) Effect of pH B) Effect of temperature C) Effect of salt concentration D) Effect of bacterial supernatant : Salt E) Effect of incubation time

3.4. Characterization of nanoparticles

The Ultraviolet visible spectrophotometry of prepared copper nanoparticles showed the non-liner spectrum with no prominent peak, however the absorption was seen maximum at the wavelength 380nm. The absorption of light at the wavelength in UV-visible range of 350-400 nm caused the excitation of

copper nanoparticles exhibiting the phenomenon of surface Plasmon resonance. The bacterial supernatant showed a broad range sharp peak at 300nm wavelength due to the absorbance of light by bacterial components as well as constituents of Mueller Hinton broth (Fig 5). The size of CuNPs was measured by DLS analysis. The only sharp peak was obtained which presented the maximum intensity of particle size distribution around 295nm (Fig 6). SEM analysis of copper nanoparticles was performed at magnification of X100 and X200 with resolution of 500um and 50um respectively. SEM Micrographs showed the amorphous morphology with no distinct dimensions.

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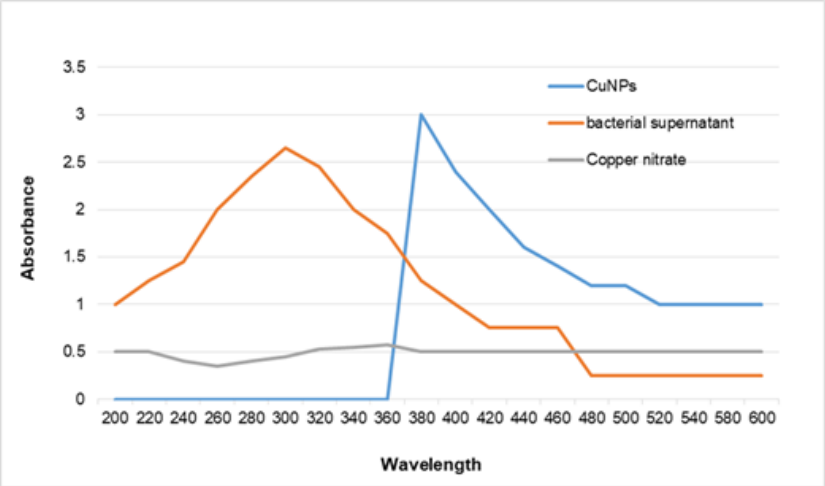

Fig 5. Ultraviolet Visible spectrum of CuNPs

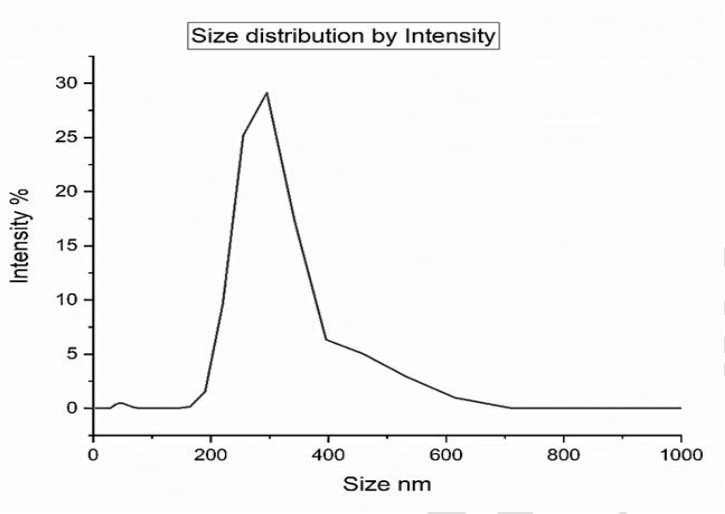

Fig 6. Graphical analysis of the results from DLS analysis showing the maximum % intensity of the particular size distribution

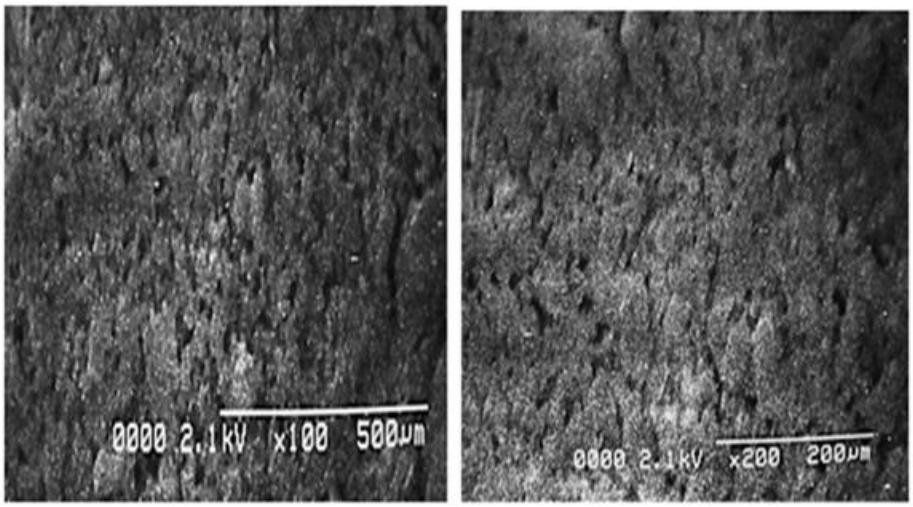

Fig 7. Scanning electron Micrographs of CuNPs at the magnification of X100 and X200

3.5. Biological activity of CuNPs

Identification and scrutinization of drug resistant strains

The drug resistant bacterial isolates produced the characteristic colored colonies on their respective

differential media as shown in the table. However, their scrutinization was done for the sensitive and resistant antibiotics by the disk diffusion method and the results were recorded as shown in Table 1.

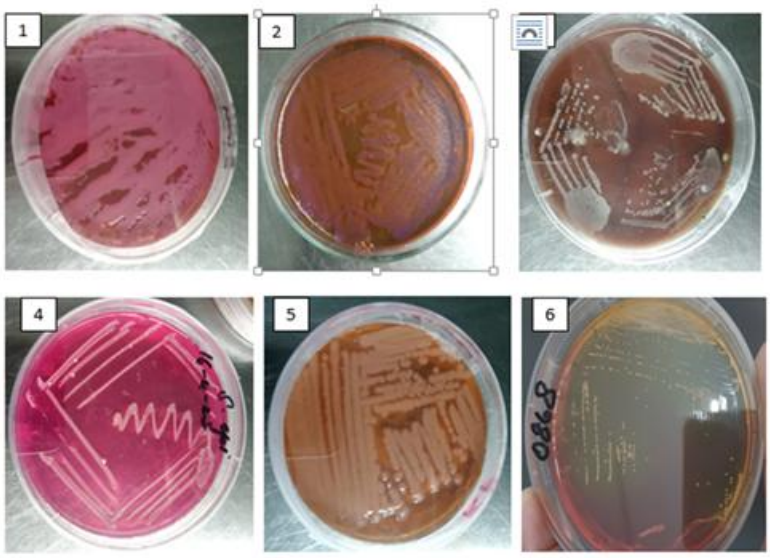

Fig 8. Identification of collected drug resistant isolates on differential Medias

Table 1 Differentiation of bacterial isolates on basis of their colonies color on the respective differential media

	Bacterial strain	**Agar medium**	**Colonies**
1	*Escherichia coli*	MacCkonkey agar	Pink colonies due to fermentation of lactose
2	*Salmonella typhimurium*	EMB agar	colorless colonies due to no lactose fermentation
3	*Methicillin resistant staphylococcus aureus*	Blood agar	Whitish yellow colonies due to beta hemolysis of blood cells.
4	*Staphylococcus epidermis*	Mannitol salt agar	White colonies due to no manitol fermentation
5	*Klebesiella pneumoniae*	MacConkey agar	Brownish pink colonies due to lactose fermentation

6	*Staphylococcus aureus*	Mannitol salt agar	Yellow colonies due to mannitol fermentation

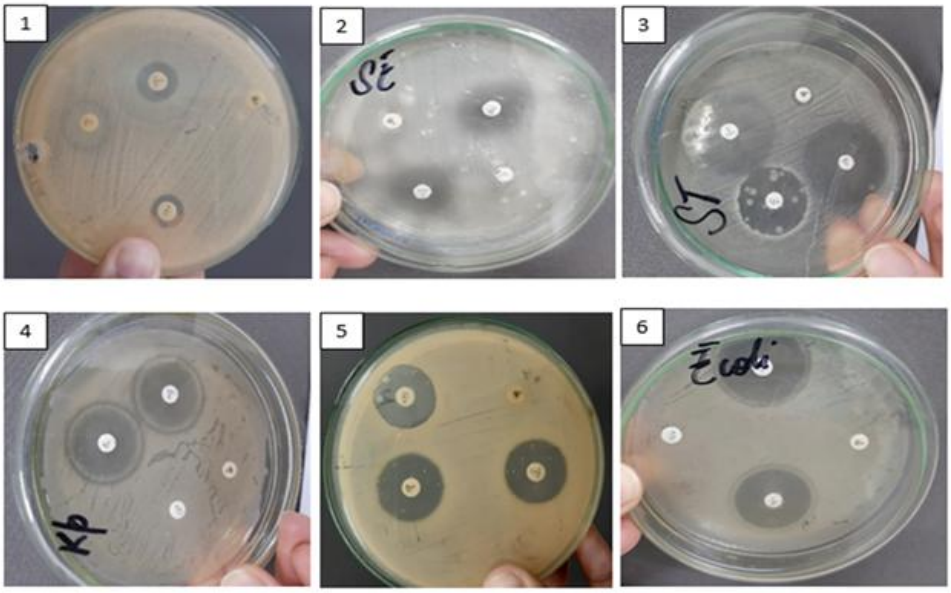

Fig 9. Scrutinization of drug resistant bacterial isolates showing the zones of inhibition against sensitive drugs

Table 2 Scrutinization of sensitive and resistant drugs for each bacterial isolate

	Bacterial isolate	**Sensitive drug**	**Resistant drug**
1	*Staphylococcus aureus*	CIP , P	LEV , MET
2	*Staphylococcus epidermis*	CIP , LEV	P , MET
3	*Salmonella typhimurium*	CIP, LEV, P	MET
4	*Klebsiella pneumoniae*	CIP, LEV	P , MET
5	*Methicillin resistant Staphylococcus aureus*	CIP, LEV,P	MET
6	*Escherichia. coli*	MET, P	CIP , LEV

Penicillin P, Levofloxacin LEV, Methicillin MET, Ciprofloxacin CIP

3.6. Antibacterial activity against drug resistant bacterial isolates

The antibacterial potential of CuNPs was analyzed against drug resistant *Salmonella typhimurium, Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli*, Methicillin-resistant *Staphylococcus aureus and Klebsiella pneumonia.* The maximum zone of inhibition was produced by CuNPs against *Salmonella typhimurium* having diameter of 29mm. Staphylococcus aureus was found sensitive to Ciprofloxacin ad produced a zone of diameter 11mm against the antibiotic however, the zone of inhibition produced by CuNPs was much greater than that of antibiotic with the diameter of 19mm. CuNPs didn't show any bacterial efficacy against some of the bacterial isolates as well.

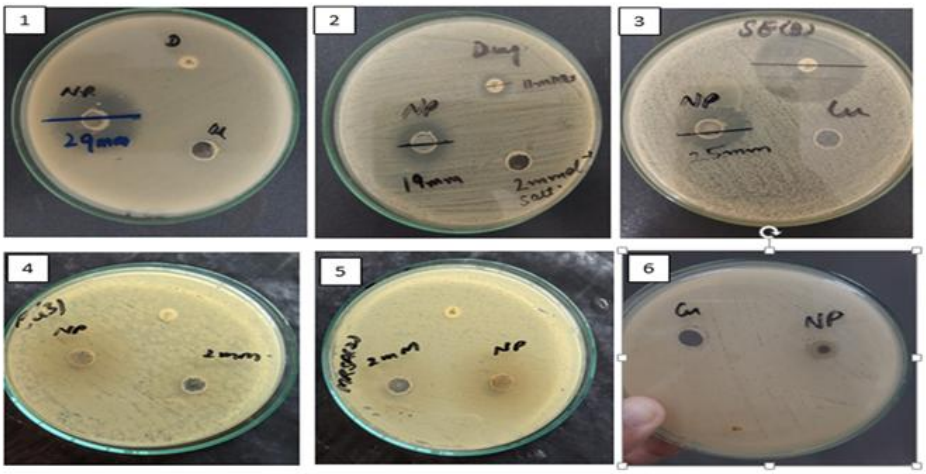

Fig 10. Antibacterial activity of CuNPs against drug resistant bacterial isolates

Table 3 Zones of inhibition produced by CuNPs against drug resistant bacteria

	Bacterial strain	ZOI of CuNPs	ZOI of antibiotic
1	***Salmonella typhimurium***	29mm	0mm
2	***Staphylococcus aureus***	19mm	CIP 11mm
3	***Staphylococcus epidermis***	25mm	0mm
4	***Escherichia coli***	0mm	0mm
5	**Methicillin-resistant *Staphylococcus aureus***	0mm	0mm
6	***Klebsiella pneumoniae***	0mm	0mm

Antibacterial activity against drug sensitive bacterial strains

The microbial method of nanoparticle's synthesis produced the CuNPs with efficient antibacterial potential against drug sensitive bacterial strains including *Pseudomonas aeroginosa, Salmonella enterica*, *Escherichia coli* and *Bacillus subtilis*, while the growth of *Bacillus subtilis* was prominently reduced in presence of CuNPs with maximum zone of inhibition of 25mm.

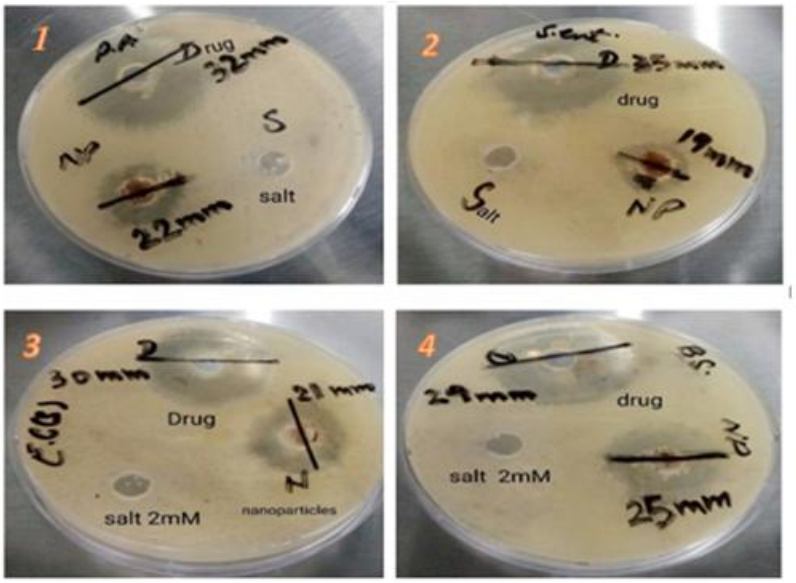

Fig 11. Antibacterial activity of CuNPs against drug sensitive bacterial strains

Table 4 Zones of Inhibition produced by CuNPs against drug sensitive bacterial strains

	Bacterial strain	Zone of inhibition CuNPs (100ul)	Zone of inhibition Antibiotic (100ul)	Zone of inhibition Salt (100ul)
1	*Pseudomonas aeruginosa*	22mm	32mm	0mm
2	*Salmonella enterica*	19mm	35mm	0mm
3	*Escherichia coli*	21mm	30mm	0mm
4	*Bacillus subtilis*	25mm	29mm	0mm

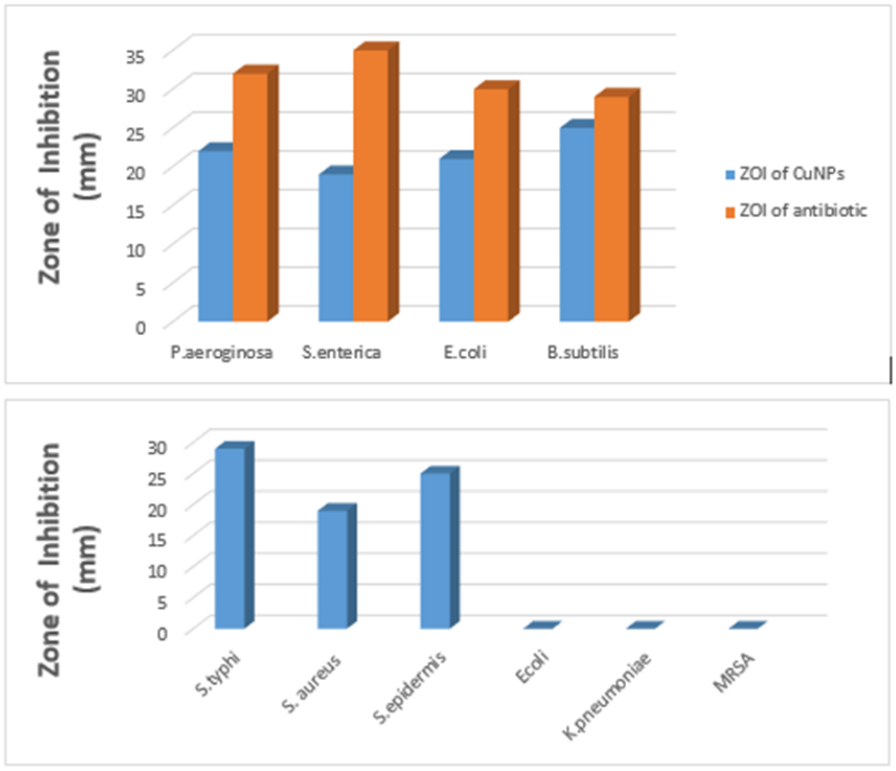

Fig 12. Graphical representation of antibacterial activity against drug sensitive and drug resistant bacteria

4. Discussion

Copper nanoparticles (CuNPs) hold many advantageous attribute including large surface and greater penetration power which enhances their effect in any application, moreover, CuNPs possess remarkable optical property and enhanced catalytic potential with low cost of fabrication [34]. Two synthesis approaches which are considered while the production of metallic nanoparticles include Top Down and Bottom Up approach constituting of various methodologies. Though, top down methods are easy to perform but they have major limitations in producing particularly small sized particles with characteristic physiochemical properties as they can render some changes in the surface chemistry of nanoparticles [39]. Bottom up methods include chemical reduction methods which make use of expensive reducing agents associated with toxic effects, high cost and impurities [40]. The easiest and beneficial replacement to such methods is green synthesis which can efficiently deal with complications of traditional methods. Some positive features associated with green synthesis include their cost effective methodology, ecofriendly technique with no toxic reducing agents and large scale production of nanoparticles,

moreover, it doesn't require high temperature, pressure and energy [41]. The biological sources used for the green synthesis of metal nanoparticles include plants, fungus and micro-organisms. Plants being the economical source offer the simple method of production but involve the utilization of flavonoids and terpenoids which produce the polydispersed nanoparticles while, microbial method is devoid of any such kind of limitation and is considered as inexpensive Nano factory to produce the nanoparticles with precisely small size, less chances of polydispersity and higher tendency of safe degradation [42]. Considering these beneficial aspects of microbial green synthesis of nanoparticles, copper nanoparticles have been prepared from the culture supernatant of *Staphylococcus aureus*, because the studies have supported the incidence of reducing potential in gram positive bacteria such as selenite reduction to produce the elementary selenium. This reducing capability has been emerged as detoxification mechanism to get rid of metal ions, thus reducing them to metal nanoparticles. Till now two research studies have supported the use of *Staphylococcus aureus* to produce the metal nanoparticles and proved their antibacterial potential against the resistant bacterial strains as well [23][22]. The reduction of copper ions to copper nanoparticles was analyzed by the color change of reaction mixture as well as Ultraviolet visible spectrum which showed the peak at 380nm as the characteristic wavelength of copper nanoparticles in the range of 200-600nm. Different studies have confirmed the peak for copper nanoparticles in range of 300-400nm[34] while the peak for bacterial supernatant was retrieved at precisely 300nm and for $Cu(NO_2)_3.3H_2O$, there appeared somewhat a flat line spectrum. The DLS analysis concluded the 295nm size of CuNPs which possessed the amorphous morphology with no clear dimension and shape as shown by SEM micrographs. The possible reason behind the size greater than the CuNPs from any other source is the capping of bacterial components such as lipids or liposomes on the nanoparticles leading to the amorphous shape of particles as well.

CuNPs were studied for their antibacterial efficacy against the drug resistance bacteria considering the antibacterial resistant as the global health concern. The multidrug resistant bacterial isolates were scrutinized for their resistance to several antibiotics. The prepared copper nanoparticles showed appreciable efficacy against the resistant bacteria. However, some of the bacterial isolates were not inhibited by CuNPs, might be they could better respond towards the higher concentration of copper nanoparticles. Notably, the CuNPs prepared from *Staphylococcus aureus* produced the zone of inhibition larger in diameter than that of sensitive antibiotic which supported the conclusion of Medina Cruz *et al.* as the synthesis of selenium nanoparticles prepared from *Staphylococcus aureus* reduced the bacterial growth phase predominantly than the SeNPs prepared from any other bacteria.

There is no confirmation for any particular mechanism for the antibacterial mechanism of nanoparticles but several studies have suggested the interaction of CuNPs with the cell membrane and the disturbance of overall integrity of membrane, denaturation of proteins and interaction with the sulfur containing compounds such as DNA thus leading to the death of bacteria [43]. Researches performed on the antibacterial activity of CuNPs against *Escherichia coli* have suggested the production of reactive oxygen

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species from bacteria which cause the denaturation of DNA, peroxidation of lipids and oxidation of proteins ultimately causing the bacterial cells to die [44][45].

5. Conclusion

The major health concern faced by public health today is the antibacterial resistant which can be eradicated by the synthesis of biocompatible nanoparticles having antibacterial potential. Microbial method of green synthesis has been used as the environment friendly and the cost effective method for the synthesis of non-toxic copper nanoparticles with appreciable antibacterial potential against multidrug resistant bacteria. *Staphylococcus aureus* was used to analyze its potential to reduce the copper ions resulting in the nanoparticles and then studying their antibacterial potential against drug resistant and drug sensitive bacteria. This research can be proved helpful to utilize the properties of pathogenic as well as non-pathogenic microbe as the efficient source of producing the advantageous nano-products with appreciable and enhanced biological activities.

Comment [Ma15]: as efficient sources of producing advantageous

References

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