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Antimicrobial efficiency of garlic (*Allium sativum*) leaf derived copper nanoparticles against pathogenic bacteria

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Abstract

Present study deals with synthesizing CuNPs from *Garlic* (*Allium sativum*) plants leaf and investigates its antibacterial activity against different bacteria. CuNPs was synthesized by Herbal method. The characterization of nanoparticles was done by XRD, FTIR and UV-spectroscopy method. Antibacterial activity was determined by disc diffusion, well diffusion method and MIC.

This green synthesis method is alternative to chemical methods, since it is cheap, pollutant free and eco-friendly. Thus the obtained CuNPs demonstrated remarkable antibacterial activity against three human pathogenic bacteria when used in combination with commercially available antibiotics. CuNPs have become an important approach for applications in nanobiotechnology in the development of antibiotic treatment of different bacterial infections.

Keywords: Cu Nps nanoparticles; Antibacterial activity; *S. typhi*; *E. coli*; *S. aureus*

1. Introduction

Nps are synthesized by various techniques such as chemical methods lithography and laser ablation. These techniques are very expensive, time consuming and not good for environment. The different plants have potential of for agglutination of Nanoparticles were found in the earlier century. Since then, several plants and plant products have been used for the synthesis of Nanoparticles. The presence of enzymes, phytochemical, protein and other components are commonly use in the synthesis of Nanoparticles by plant extracts [1-5].

Cu Nps are very susceptive to O_2, so there are many issues related to its oxidation resilience and stability. Nanoscale structures of copper have a resemblance in oxidizable property. So, many methods are available for synthesis of Cu Nps. Cu are successfully synthesized by reduction in micro emulsions and reverse micelles, thiol-induced reduction in supercritical water [6], radiolysis [7], vapor depositions, [8] thermal decomposition, [9] and laser irradiation, sonoelectro chemical [10].

Copper nanoparticles are synthesize from radiolysis reduction [7], thermal decomposition [11], vapor deposition [12], electrochemical reduction [13], copper Nps are synthesis using hydrazine hydrate and starch [14] green synthesis of Cu nanoparticles was achieved by using microorganisms [15].

Cu Nanoparticles are obtained at low cost, high conductivity and easily available other than metal Nanoparticles. Synthesis of Copper nanoparticles was done by two methods and their comparison on the based size observed by [16]. Synthesis of plants extract mediated copper nanoparticles and their impact on pathogenic bacteria was studied by [17],

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they reported that synthesis of Cu Nps Garlic (*Allium sativum*) plants and investigate its antibacterial activity against different bacteria.

Shobha studied the biological synthesis of copper nanoparticles and its impact [18]. They observed Copper (Cu) nanoparticles have wide applications in agricultural, industrial engineering and technological fields. In agriculture much effort has been made in recent years to ascertain the necessity of certain minor elements in the economy of plants.

Synthesis of silver nano-particles by chemical methods was toxic and various hazardous chemicals were used for their synthesis. Green synthesis is preferred over conventional synthesis because it is cost-effective, eco-friendly, single-step method that can be easily scaled up for large scale synthesis and does not require high energy, temperature, pressure and toxic chemicals.

2. Material and methods

2.1. Bacterial genera

The following three bacterial genera were used for present investigation: *Escherichia Coli, Staphylococcus aureus, Salmonellae typhi* etc.

2.2. Plants

The following plant was used for synthesis of nanoparticles: Garlic leaf (*Allium sativum*).

2.3. Synthesis of Silver nanoparticles

Syntheses of nanoparticles from plants were done by method described by [19]

2.4. Antibacterial test

- Antibacterial test was done by disc diffusion and well diffusion [20].
- Antibacterial test was done by minimum inhibitory concentration (MIC) [21].

2.5. Media

The following media were used for present research work:

- Nutrient agar for *E. coli*.
- Manittol salt agar for *S. aureas* and *S. typhi*

2.6. Characterization of nanoparticles:

Characterization of nanoparticles was done by XRD, FTIR and UV spectroscopy method of [22-24].

3. Results and discussion

Characterizations of copper nanoparticles synthesis from Garlic leaf (*Allium sativum*)

3.1. XRD (X-ray diffraction)

Characterizations of Cu nanoparticles by X-ray diffraction were done to confirm the crystalline nature of the Cu Nps synthesis from garlic leaf (*Allium sativum*). The Cu Nps were used as dry powers for XRD analysis. Diffracted intensity was observed from 20º to 80º at 2θ angles. Compared the XRD spectrum with the standard spectrum for confirm that the formed Cu Nps were in the form of nanocrystals. Various diffraction lines were observed at 2θ angle 29.4, 36.22, 40.33, 44. 52, 62. 86 (Fig1).

The particle size of the Cu Nps synthesized through herbal method may be calculated by Debye-Scherrer's equation.

$$D = \frac{K\lambda}{\beta \, COS \, \theta}$$

Were
D is the crystal size,
k is the Scherrer's constant with the value 0.94
λ is the wavelength of the X-ray
β is the full width at half maximum
θ is the Bragg angle.

Calculations using Scherrer's equation showed that the average particle size was in the range of 6 to 8 nm.

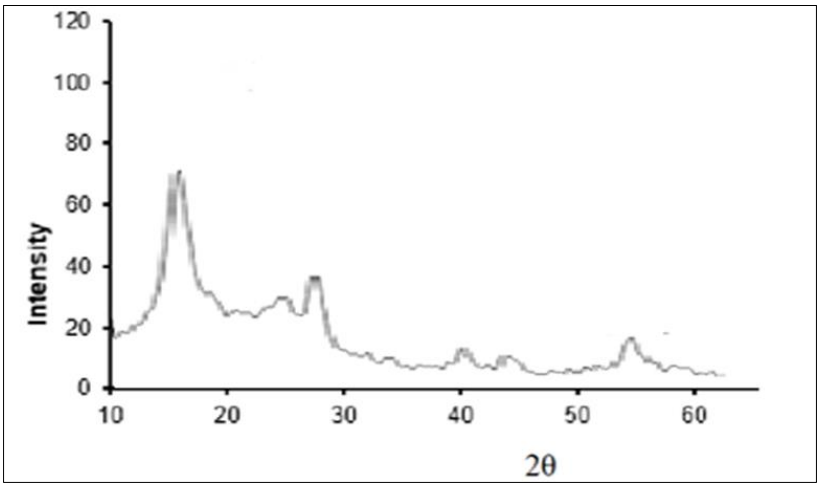

Figure 1 X-ray diffraction pattern of Cu Nps prepared from *Allium sativum* leaf

3.2. FTIR (Fouries Transform Infrared Spectroscopy)

FTIR observation was done for identification of the biomolecules which responsible for capping and stabilization of the Cu nanoparticles synthesized from *Allium sativum* leaf extract. The FTIR spectrum of Cu Nps leaf extract mediated Cu nanoparticles showed the different peaks at 1025, 1199, 1380, 1486, and1810cm^{1} (Figure2).

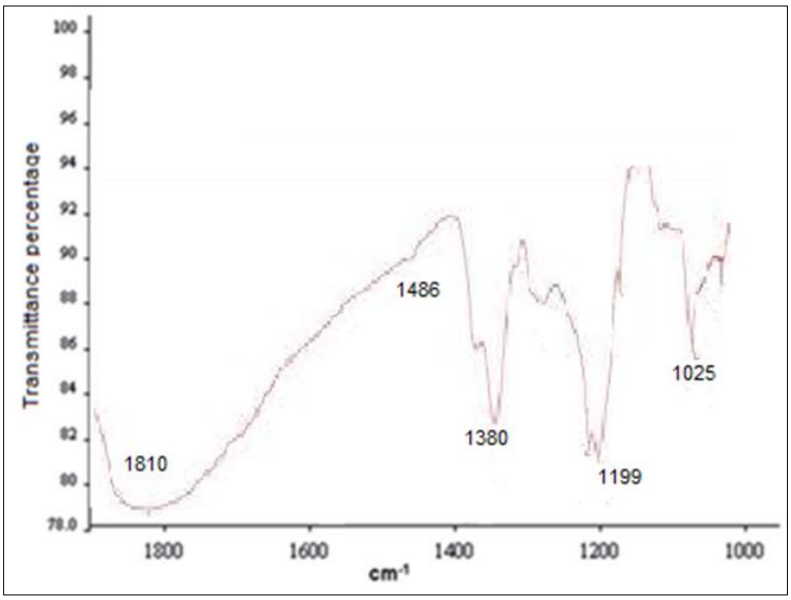

Figure 2 FTIR spectrum of Cu Nps prepared from *Allium sativum* leaf

The FTIR spectrum of *Allium sativum* leaf extract mediated Cu nanoparticles reveal the presence of various functional groups like the presence of (- OH) stretching of phenolic group, (>C=O) and (>NH) stretching. In the infrared region of the electromagnetic spectrum, the presence of flavanones or terpenoids absorbed on the surface of Cu nanoparticles, Cu NPs gave rise to the well-known signatures.

3.3. UV- Visible Spectroscopy

The UV- Vis spectroscopy used to examine the size and shape controlled nanoparticles in aqueous suspensions. The absorption spectrum of aqueous solution *Allium sativum* leaf extract mediated Cu nanoparticles was observed in the range of 300-800 nm and maximum absorbance was recorded at 630 nm, results suggesting the stability and size of Cu nanoparticles.

The sharing of peak indicates the particle shape. The results indicate that there is no aggregation in UV-Vis absorption spectrum. The result reveal that the reduction of Cu ion into Cu NPs. Reduction of Cu ions present in the aqueous solution of the Cu complex during the reaction with the ingredients present in the *Allium sativum* leaf extract observed by the UV-Vis spectroscopy revealed the presence of Cu nanoparticles may be correlated with the UV-Vis spectra

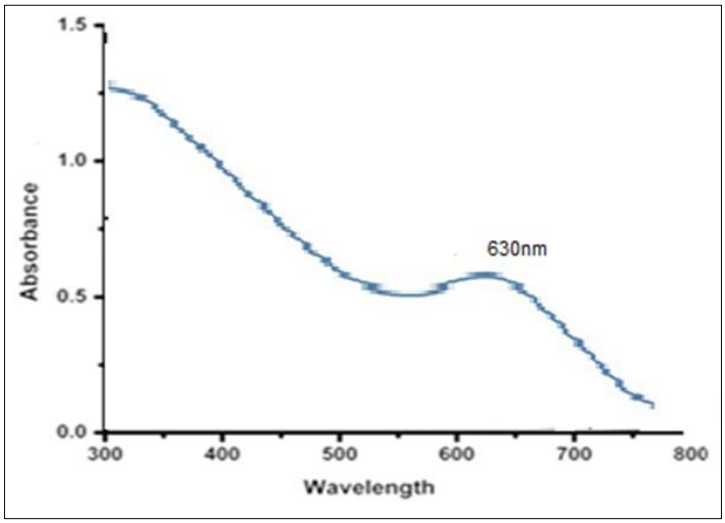

Figure 3 UV- Vis spectroscopy of Cu Nps prepared from *Allium sativum* leaf

3.4. Antibacterial activity of copper nanoparticles

3.4.1. Disc diffusion method

In negative control water was applied in disc of cultured petriplates. There was no inhibition zone against *E. coli, S. typhi* and *S. aureas* bacteria.

Table 1 Antibacterial activity of water + copper nanoparticles of *Allium sativam* different bacteria by disc diffusion method

Sr. No	Organism (pathogens)	Water (-)ve Control	Inhibition zone (Radius) in cm				
			0.1 gm/ml	0.2 gm/ml	0.3 gm/ml	0.4 gm/ml	0.5 gm/ml
1	*E. coli*	-	1.2±0.13	1.4±0.16	1.2±0.19	1.5±0.21	1.6±0.32
2	*S. aureus*	-	0.9±0.15	1.0±0.17	1.1±0.27	1.2±0.28	1.2±0.24
3	*S. typhi*	-	0.6±0.18	0.9±0.20	0.9±0.23	1.0±0.32	1.0±0.36

In positive control (experiment) the *Allium sativum* copper nanoparticles used with different solvent (such as water) and different concentration (0.1 gm, 0.2 gm, 0.3 gm, 0.4 gm and 0.5 gm) applied over on bacterial culture plate. Both solvent with nanoparticles showed the zone of inhibition. The results are summarized in table 1 and Figure 4 and 6.

In disk diffusion *Allium sativum,* copper nanoprticles + water showed the zone of inhibition against different bacteria. The zones on inhibition were 1.2 cm, 1.4 cm, 1.2 cm, 1.5 cm and 1.6 cm against *E. coli* and 0.9 cm, 1.0 cm, 1.1 cm, 1.2 cm and 1.2 cm against *S. aureus* and 0.6 cm, 0.9 cm, 0.9 cm, 1.0 cm and 1.0 cm against *S. typhi* with different concentration (0.1 gm, 0.2 gm, 0.3 gm, 0.4 gm and 0.5 gm) respectively.

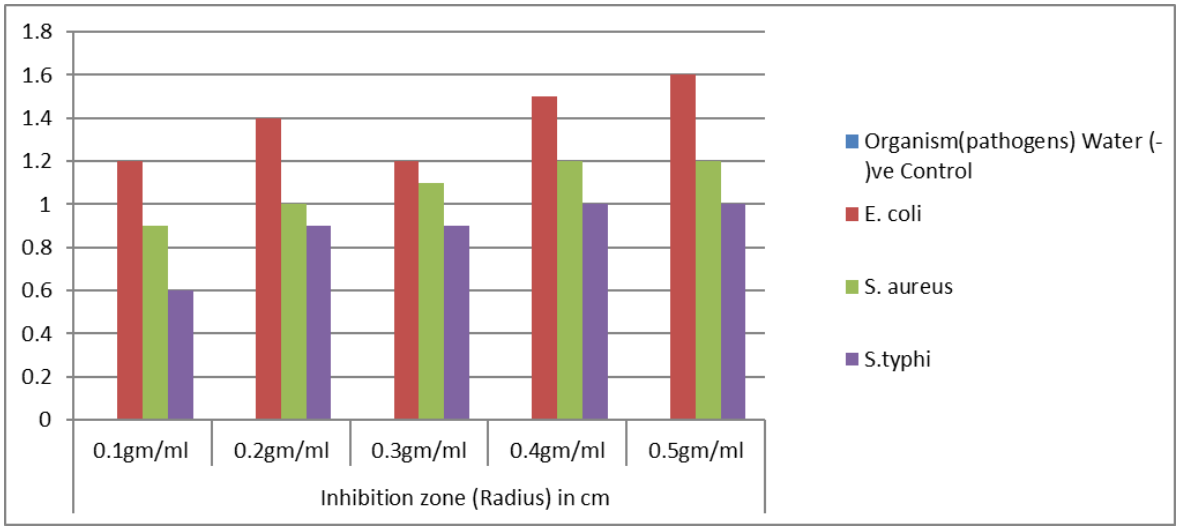

Figure 4 Antibacterial activity of water + copper nanoparticles of *Allium sativam*differentbacteria by disc diffusion method

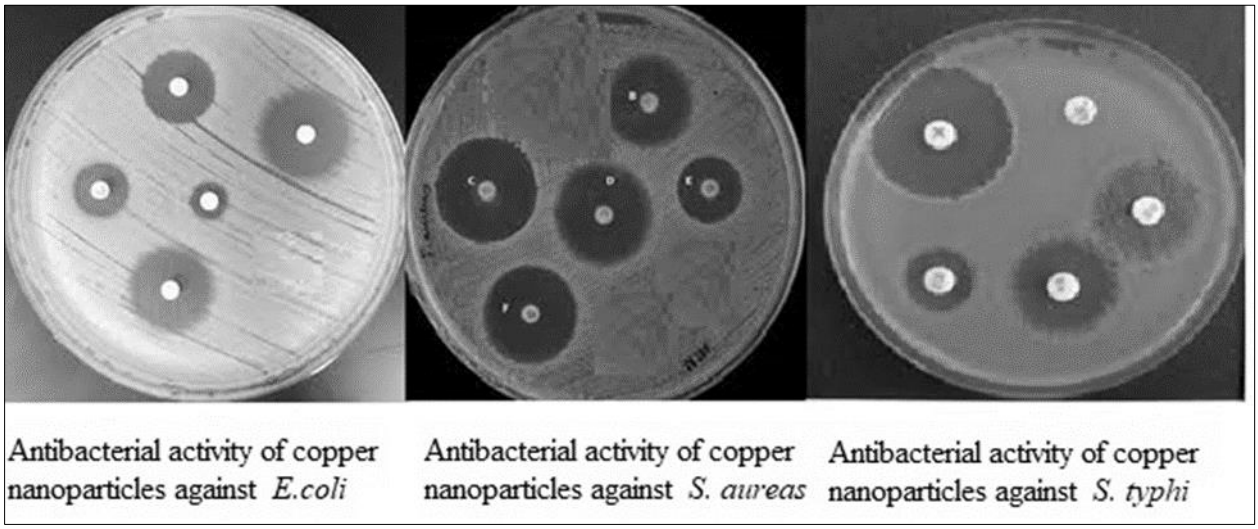

Figure 5 Antibacterial activity of water + copper nanoparticles of *Allium sativam* different bacteria by disc diffusion method

3.5. Well diffusion method

In negative control water was applied in well of cultured petriplates. There was no inhibition zone against *E. coli, S. typhi* and *S. aureas* bacteria.

In positive control (experiment) the *Allium sativum* copper nanoparticles used with different solvent (such as water) and different concentration (0.1 gm, 0.2 gm, 0.3 gm, 0.4 gm and 0.5 gm) applied over on bacterial culture plate. Solvent with nanoparticles showed the zone of inhibition. The results are summarized in table 2 and fig. 6 and 7.

In well diffusion *Allium sativum,* copper nanoprticles + water showed the zone of inhibition against different bacteria. The zones on inhibition were 1.4cm, 1.5cm, 1.6cm, 1.7cm and 1.9cm, against *E. coli* and 1.1cm, 1.2cm, 1.3cm, 1.6 and

1.6cm against *S. aureus* and 1.0cm, 1.1cm, 1.2cm, 1.6 and 1.6cm against *S. typhi* with different concentration (0.1 gm, 0.2 gm, 0.3 gm, 0.4 and 0.5 gm) respectively.

Table 2 Antibacterial activity of water + copper nanoparticles of *Allium sativum* different bacteria by well diffusion method

Sr. No	Organism(pathogens)	Water (-)ve Control	Inhibition zone (Radius) in cm				
			0.1 gm/ml	0.2 gm/ml	0.3 gm/ml	0.4 gm/ml	0.5 gm/ml
1	*E. coli*	-	1.4±0.12	1.5±0.14	1.6±0.16	1.7±0.18	1.9±0.20
2	*S. aureus*	-	1.1±0.24	1.2±0.26	1.3±0.28	1.6±0.17	1.6±0.19
3	*S. typhi*	-	1.0±0.10	1.1±0.11	1.2±0.18	1.6±0.24	1.6±0.25

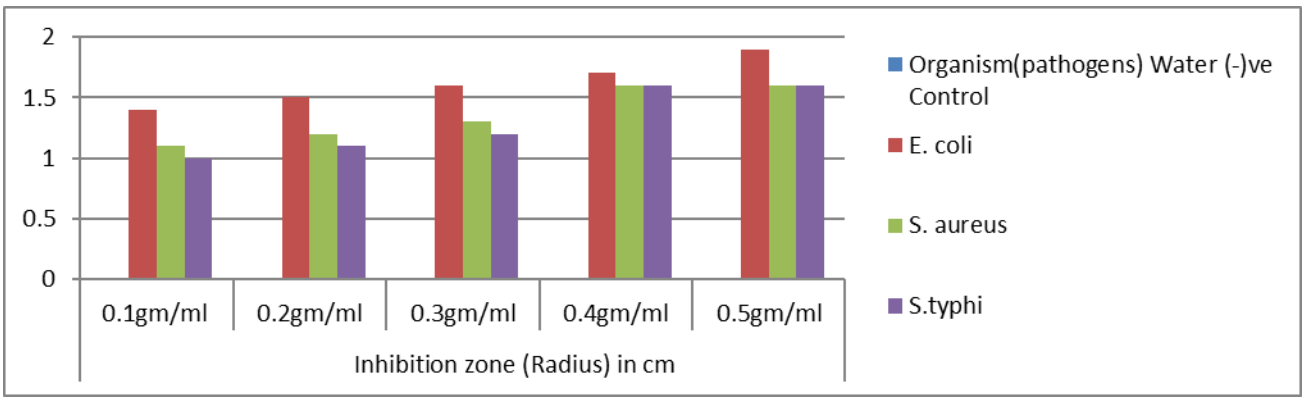

Figure 6 Antibacterial activity of water + copper nanoparticles of *Allium sativum* different bacteria by well diffusion method

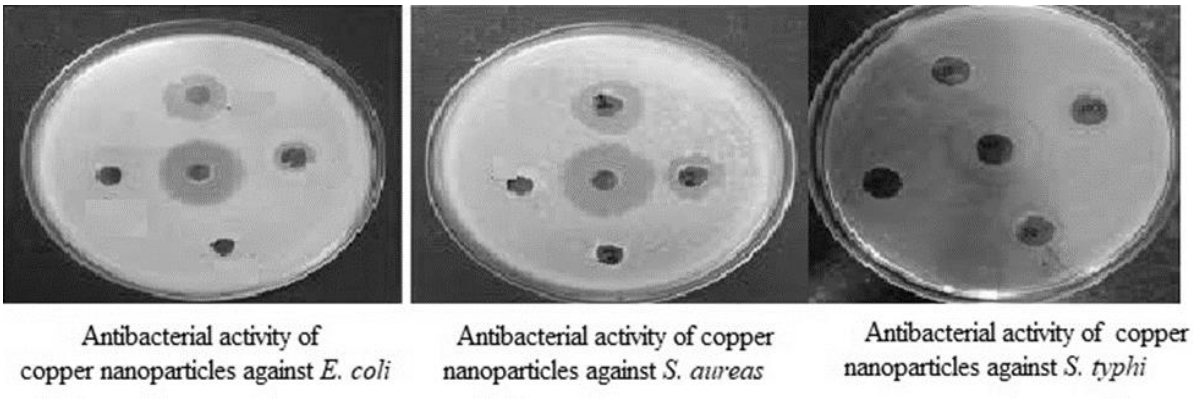

Figure 7 Antibacterial activity of water + copper nanoparticles of *Allium sativum* different bacteria by well diffusion method

3.6. MIC effect of Cu Nanoparticles on different bacteria

Table 3 MIC effect different Nanoparticles of *Allium sativum* on *E. coli*, *S. aureus* and *S. typhi* bacteria at different time interval.

S. No.	Sample	Con. Of Nps	OD at 630 nm at different time intervals			
			24hrs.	48 hrs.	72hrs.	96 hrs.
1	Blank	Plan Media	00	00	00	00
2	Cu Nps against *E. coli*	1 gm/100ml	0.22±0.12	0.21 ±0.14	0.12±0.17	0.09±0.17
3	Cu Nps against *S. aureus*	1 gm/100ml	0.19±0.16	0.12±0.19	0.08±0.20	0.08±0.22
4	Cu Nps against *S. typhi*	1 gm/100ml	0.18±0.20	0.12±0.22	0.06±0.24	0.05±0.26

The efficiency of Cu Nanoparticles synthesis form *Allium sativum* was assessed on the basis on O.D at different time interval against *E. coli, S. typhi* and *S. aureas* at 1 gm/100ml concentration and at different time interval i.e., 24, 48, 72 and 96hours. The results were summarized in table 3 and figure 8.

After 24 hours O.D was found decreased. The Nanoparticles inhibit the growth of *E. coli, S. typhi* and *S. aureas* after 48, 72 and 96 hours. The O.D of Cu Nanoparticles was more effective against *E. coli* followed by *S. aureus* than *S. typhi*.

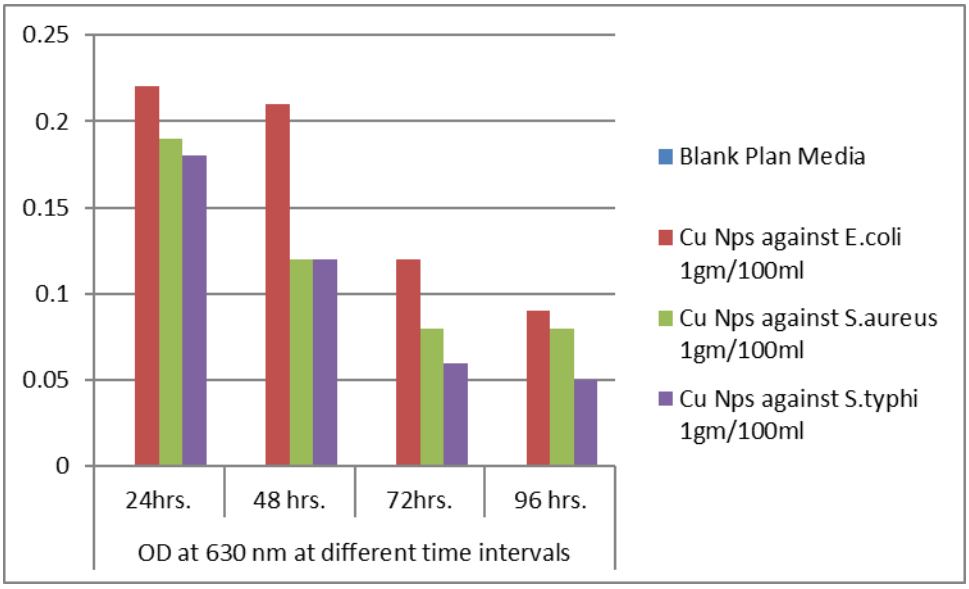

Figure 8 MIC effect different Nanoparticles of *Allium sativum* on *E. coli,S. aureus* and *S. typhi* bacteria at different time interval**.**

Nanoparticles have large surface area available for interaction, which enhances bactericidal effect than the large sized particles; hence, they impart cytotoxicity to the microorganisms. The mechanism by which the nanoparticles are able to affected the bacteria is not understood completely, but studies suggest that when bacteria were treated with nanoparticles, changes took place in its membrane morphology that produced a significant increase in its permeability affecting proper transport through the plasma membrane, leaving the bacterial cells incapable of properly regulating transport though the plasma membrane, resulting into cell death [25] .Many scientists worked on the nanoparticles and their antibacterial activity. They found that nanoparticles have effective antibacterial activity Schacht, [28-29] reported antibacterial activity of zinc nanoparticle.

It was observed that nanoparticles have penetrated inside the bacteria and have caused damage by interacting with phosphorus and sulfur containing compounds such as DNA [30]. It is believed that DNA may have lost its replication

ability and cellular proteins and become inactive after treatment with nanoparticles. In addition, nanoparticles prevent the growth and division. The nanoparticles have an additional contribution to the bactericidal efficacy [31].

Plant-mediated biological synthesis of nanoparticles is gaining importance due toits simplicity and eco-friendliness. These biosynthesis of gold nanoparticles by plants such as *alfalfa* [32, 33] *Aloe Vera* [34] *Cinnamomum camphora* [35] *Azadirachta indica* [36] *Embica officinal's* [37] *lemongrass* [38]. *Tamarinds indica L/n* [39] have also been reported. In the present investigation *Allium sativum* CuNps showed antibacterial activity against *E. coli, S. typhi* and *S. aureus.* Thus, corroborate with finding of previous authors that plant mediated nanoparticles may be good antibacterial agent.

4. Conclusion

In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. Cu Nps have been widely used for antimicrobial and biomedical products. Present study concluded that, the Cu Nps has strong antibacterial activity. The significance of over results is that Cu Nps solutions have a significant antibacterial activity against *S. aureus, S. typhi* and *E. coli.* These nanoparticles may capable of entering into the bacterial cell through cell wall and cell membrane, where they might inhibit the enzymatic activity and finally stopped the growth and division of bacterial cell. Hence use of our nanoparticles may help in inhibiting the studied bacterial growth.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to disclose.

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