SYNTHESIS OF PLANTS EXTRACT MEDIATED COPPER NANOPARTICLES AND THIR IMPACT ON PATHOGENIC BACTERIA

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ABSTRACT *This study was aimed to synthesize CuNP from Ocimum sanctum and Ficus benghalenis plants and investigate its antibacterial activity against different bacteria. In the present study CuNP was synthesized by herbal method. The characterization of nanoparticles was done by XRD and FTIR. Antibacterial activity was determined by disc diffusion and well diffusion method. It was found that CuNPs showed antibacterial activity in the form of zone of inhibition. Copper nanoparticles (CuNPs) were dissolved in two different solvent like water and 70% ethanol. Both solvent with Copper nanoparticles show the zone of inhibition against bacteria like E. coli and S. aureus. Maximum zone of inhibition (1.9 cm) was observed at 0.4 gm concentration of Ocimum sanctum CuNPs + 70 % ethanol against S. aureus with well diffusion method. Minimum zone of inhibition (0.5 cm) observed at 0.1 gm concentration of Ficus benghalenis CuNPs + water against E. coli with disk diffusion method.*

Keywords: *CuNPs nanoparticles, Antibacterial activity, E. coli, and S. aureus.*

Introduction

Nanoparticles possess high surface area to division ratio. Nanoparticles such as that silver, gold, cadmium sulfide, zinc sulfide, and zinc oxide play important role in various fields [1- 4]. Copper nanoparticles are known to be vastly susceptive to oxygen and so there are many problems related to the stability and oxidation resistance. The synthesis of copper nanoparticles has not been as widely found out as that of many. Other metals in view of the easily oxidizable nature of copper, which is increased in nanoscale structures. Yet, many methods have been reported. Copper nanoparticles have been successfully synthesized, for example, by radiolysis [5], laser irradiation [6], thermal decomposition [7-8], thiol-induced reduction in supercritical water [9], and reduction in micro emulsions [10], reverse micelles [11], vapor depositions [12], sonoelectrochemica [13], flame spray [14] and chemical reduction [15-17] methods. The Cu nanoparticles are synthesize from vapor deposition[18], electrochemical reduction[19], radiolysis reduction [20] thermal decomposition [21] copper metal salt [22] and room temperature synthesis using hydrazine hydrate and starch [23] in recent, green synthesis of Cu nanoparticles was achieved by using microorganisms [24].

Material and Method

Bacterial genera

The following two bacterial genera were used in present investigation:-

- *Escherichia coli*
- *Staphylococcus aureus.*

Plants

The following two plants were used for synthesis of herbal based nanoparticels:-

- *Ocimum Sanctum* (Tulsi) [leaves]
- *Ficus benghalensis* (banyan) [leaves]

Synthesis of copper nanoparticles.

Syntheses of nanoparticls from plants were done by method discovered by [25].

Antibacterial test

Antibacterial test was done by disc diffusion and well diffusion method discovered by [26].

Media:

The following two media were used in the present research work:-

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

Characterization of nanoparticles:-

Characterization of nanoparticle was done by XRD and FTIR method [27- 29].

Results and Discussion:

XRD of *Ficus benghalensis* CuNPS

Fig.1: showed the XRD pattern of the compacted Cu-NPs sample. The diffraction pattern mainly exhibited peaks at 37.97˚, 39.13˚, 43.47˚, 49.71˚and 53.72˚ in a 2θ scale, which can be indexed to (111) (111), (200), (210), and (211) reflections of fcc copper, indicating cubic phase of copper metal. Other CuO or Cu_2O impurity peaks were observed in the spectra, suggesting that the synthesized particles were not of highly purity prepar. It is known that copper nanoparticles rapidly oxidize on exposure to the atmosphere, which can result in particle aggregation and could affect the antimicrobial properties of CuNPs. Scherer equation was used to calculate crystallite size giving approximately 39.02 nm and lattice constant a was1.39Å.

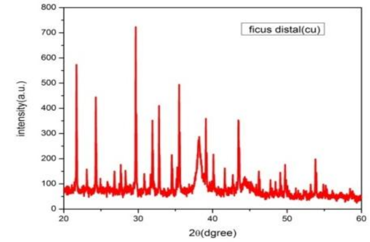


Fig 1- XRD of ficus *benghalensi* distal CuNPS

XRD of *Ocimum sanctum* CuNPS

Fig.2 showed the XRD pattern of the compacted CuNPs sample. The diffraction pattern mainly exhibited peaks at 35.55˚, 39.13˚, 43.35˚, 49.57˚and 53.8˚ in a 2θ scale, which can be indexed to (111), (111), (200), (210), and (211), reflections of fcc copper, indicating cubic phase of copper metal. Other CuO or Cu_2O impurity peaks were observed in the spectra, suggesting that the synthesized particles were not of highly purity preparing with herbal method. It is known that copper nanoparticles rapidly oxidize on exposure to the atmosphere, which can result in particle aggregation and could affect the antimicrobial properties of CuNPs. Scherer equation was used to calculate crystallite size giving approximately87.92 nm and lattice constant was 4.07Å.

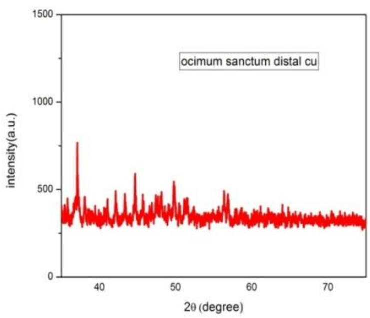


Fig 2- XRD of *Ocimum sanctum* distal CuNPS:-

FTIR OF Copper Nanoparticles *Ficus* Leaf extract

The FTIR spectra of copper nanoparticles are shown in the fig.3. The peak were observed in spectrum at 3675.126 cm^{-1} assigned to O-H (s) stretching, 2084.81532 cm^{-1} assigned to S-H vibration stretching,1625.17 cm^{-1}assigned to C=C stretching and bending and 1109.75cm^{-1} assigned to C-O stretching. These results were found to be in line with the previous reported data.

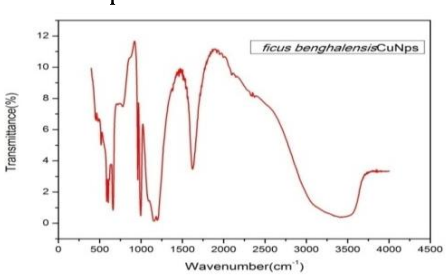


Fig:-3 *Ficus benghalensis* CuNps.

FTIR OF Copper Nanoparticles *Ocimum sanctum* Leaf extract

The FTIR spectra of copper nanoparticles are shown in the fig.4. The peak were observed in spectrum at 3605.12 cm^{-1} assigned to O-H (s) stretching, 2104.30917 cm^{-1} assigned to S-H vibration stretching,1635.430 cm^{-1}assigned to C=C stretching and bending and 1116.05cm^{-1} assigned to C-O stretching. These results were found to be in line with the previous reported data.

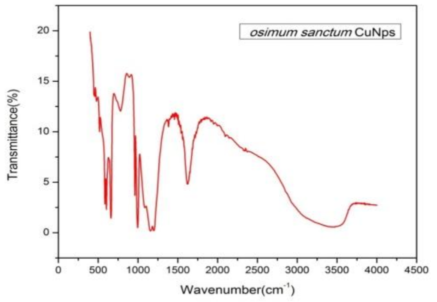


Fig: - 4 *Ocimum sanctum* CuNps

Antibacterial activity of Copper nanoparticles

The impact of antibacterial activity of Copper nanoparticles tested on the basis of disc diffusion and well diffusion methods were summarized in table 1-4.

The water and 70% ethanol was applied in control disc and cultured petriplates. There were no inhibition zone was observed against *E. coli* and *S. aureas* bacteria.

Table:-1 Antibacterial activity of *Ocimum sanctum* derived copper nanoparticles against different bacteria.

S. No.	Name of Bacteria	Zone of inhibition under Disk diffusion method							
		Con. of water + copper nanoparticles in gm/ml				Con. of 70% & Ethanol + copper nanoparticles in gm/ml			
		0.1gm	0.2gm	0.3gm	0.4gm	0.1gm	0.2gm	0.3gm	0.4gm
1.	*E. coli*	0.8	1.0	1.2	1.5	0.5	1.5	1.6	1.8
2.	*S. aeurus*	0.9	1.3	1.5	1.8	1.5	1.6	1.7	1.8

Graph 1:- Antibacterial activity of *Ocimum sanctum* derived copper nanoparticles against different bacteria.

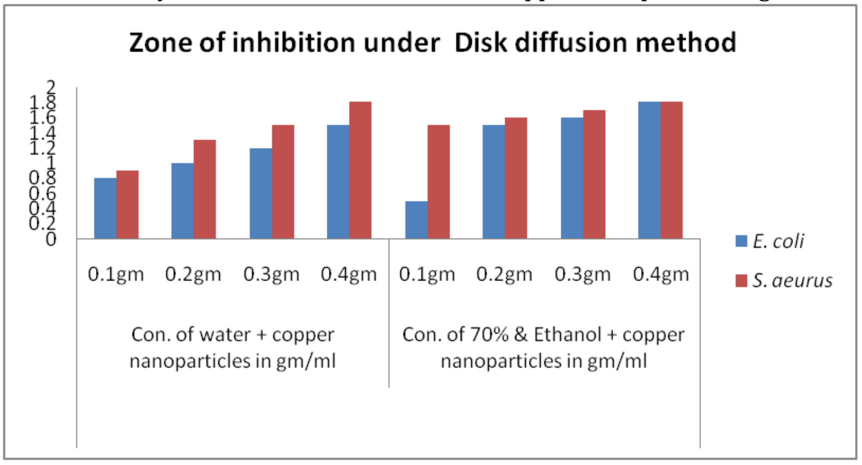


Table:-2 Antibacterial activity of *Ocimum sanctum* derived copper nanoparticles against different bacteria.

S. No.	Name of Bacteria	Zone of inhibition under well diffusion method							
		Con. Of water + copper nanoparticles				Con. Of 70% & Ethanol + copper nanoparticles			
		0.1gm	0.2gm	0.3gm	0.4gm	0.1gm	0.2gm	0.3gm	0.4gm
1.	*E. coli*	1.2	1.4	1.6	1.8	0.5	0.8	1.0	1.4
2.	*S. aeurus*	1.3	1.5	1.7	1.9	1.5	1.6	1.8	1.9

Graph 2:- Antibacterial activity of *Ocimum sanctum* derived copper nanoparticles against different bacteria.

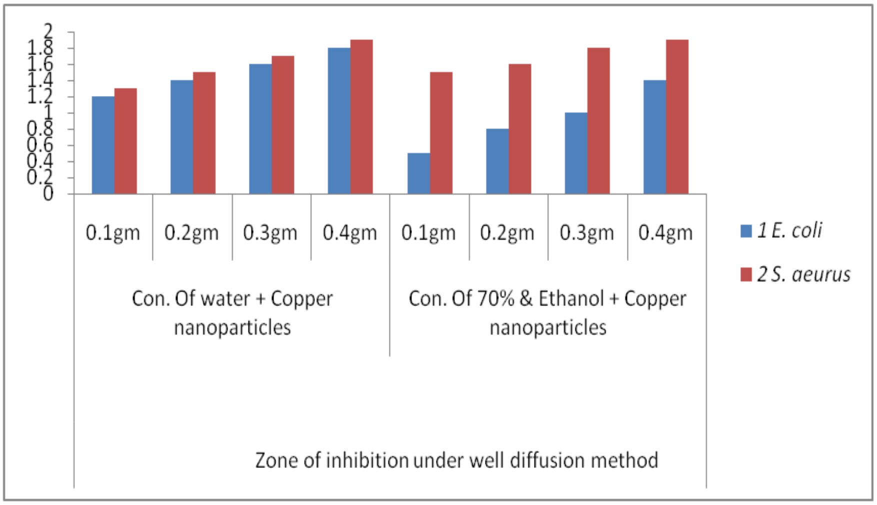


Table:-3 Antibacterial activity of *Ficus bghanensis* derived copper nanoparticles against different bacteria.

S. No.	Name of Bacteria	Zone of inhibition under Disk diffusion method							
		Con. of water + copper nanoparticles in gm/ml				Con. of 70% & Ethanol + copper nanoparticles in gm/ml			
		0.1gm	0.2gm	0.3gm	0.4gm	0.1gm	0.2gm	0.3gm	0.4gm
1.	*E. coli*	0.8	1.0	1.2	1.5	0.5	1.5	1.6	1.8
2.	*S.aeuru*	0.9	1.3	1.5	1.8	1.5	1.6	1.7	1.8

Graph 3:- Antibacterial activity of *Ficus benghalensis* derived copper nanoparticles against different bacteria.

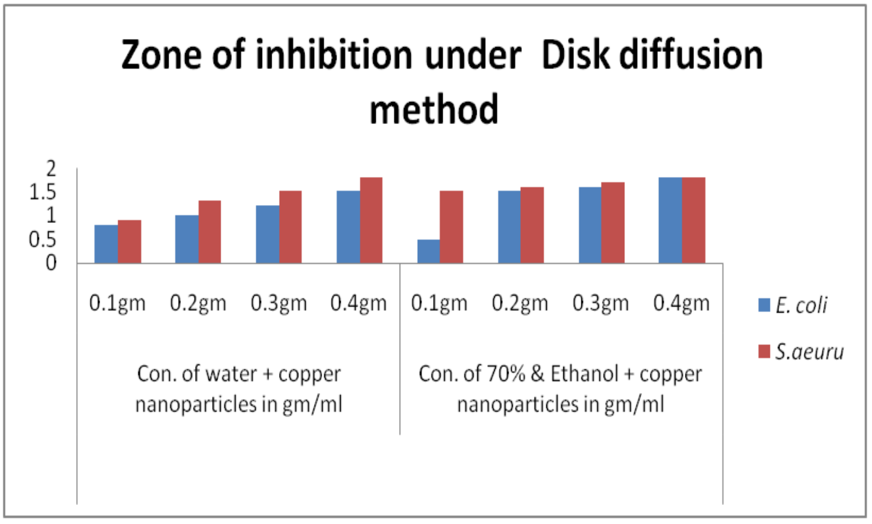
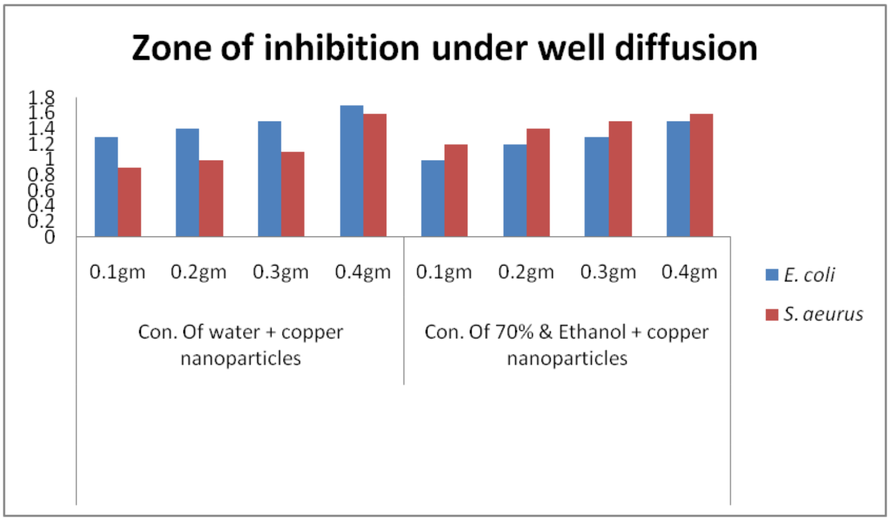


Table:-4 Antibacterial activity of *Ficus benghalensis* derived copper nanoparticles against different bacteria.

S. No.	Name of Bacteria	Zone of inhibition under well diffusion							
		Con. Of water + copper nanoparticles				Con. Of 70% & Ethanol + copper nanoparticles			
		0.1gm	0.2gm	0.3gm	0.4gm	0.1gm	0.2gm	0.3gm	0.4gm
1.	*E. coli*	1.3	1.4	1.5	1.7	1.0	1.2	1.3	1.5
2.	*S. aeurus*	0.9	1.0	1.1	1.6	1.2	1.4	1.5	1.6

Graph 4:- Antibacterial activity of *Ficus benghalensis* derived copper nanoparticles against different bacteria.



In group the copper nanoparticles used with different solvent (such as water and 70 % water) and with different concentration (0.1gm, 0.2gm, 0.3gm and 0.4). They were applied over bacterial culture plate. Both the solvents showed the zone of inhibition. The results obtained are summarized in Table 1, 2, 3 and 4.

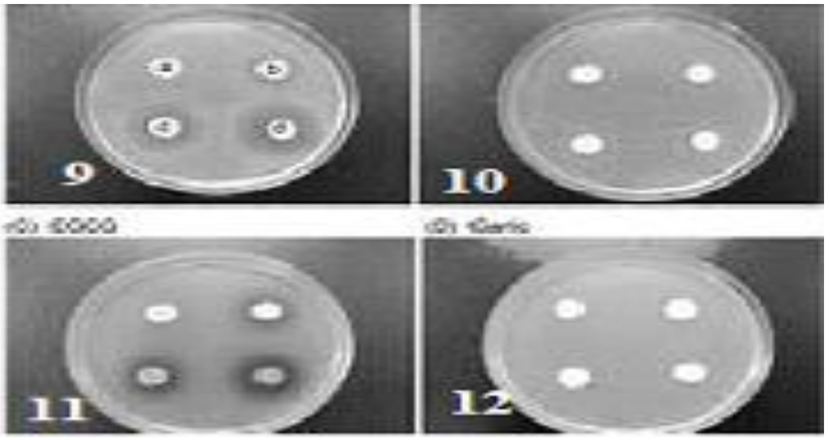
Disk diffusion method:-

In disk diffusion *Ocimum sanctum* copper nanoprticles + water showed the zone of inhibition against different bacteria. The zone on inhibition were 0.8cm,1.0cm,1.2cm and 1.5cm against *S. aureus* and 0.9cm, 1.3cm, 1.5cm and 1.8cm against *E. coli* with different concentration (0.1gm, 0.2gm, 0.3gm and 0.4gm) respectively.

In disk diffusion *Ocimum sanctum* copper nanoparticles + 70% ethanol showed the zone of inhibition against different bacteria. The zones of inhibition were 0.5cm, 1.5cm, 1.6cm and 1.8cm, against *S. aureus* and 1.5cm, 1.6cm, 1.7cm and 1.8cm against *E. coli* with different concentration (0.1gm, 0.2gm, 0.3gm, and 0.4gm respectively.

In disk diffusion *Ficus benghalensis* copper nanoprticles + water showed the zone of inhibition against different bacteria. The zone of inhibition were 0.8cm,1.0cm,1.2cm and 1.5 against *S. aurues* and 0.9cm, 1.3cm, 1.5cm and 1.8cm against *E. coli* with different concentration (0.1gm, 0.2gm, 0.3gm and 0.4gm) respectively.

In disk diffusion *Ficus benghalensis* copper nanoparticles+70% ethanol showed the zone of inhibition against different bacteria. The zone of inhibition were 0.5cm,1.5cm,1.6cm and 1.8cm against *E. coli* and 0.5cm, 1.6cm, 1.7cm and 1.8cm against *S. aureus* with different concentration (0.1gm, 0.2gm, 0.3gm and 0.4gm) respectively.



Figs: - 09, 10, 11 and 12 shows antibacterial activity of *Ficus benghlensis* copperr nanoparticles against bacteria disk diffusion method.

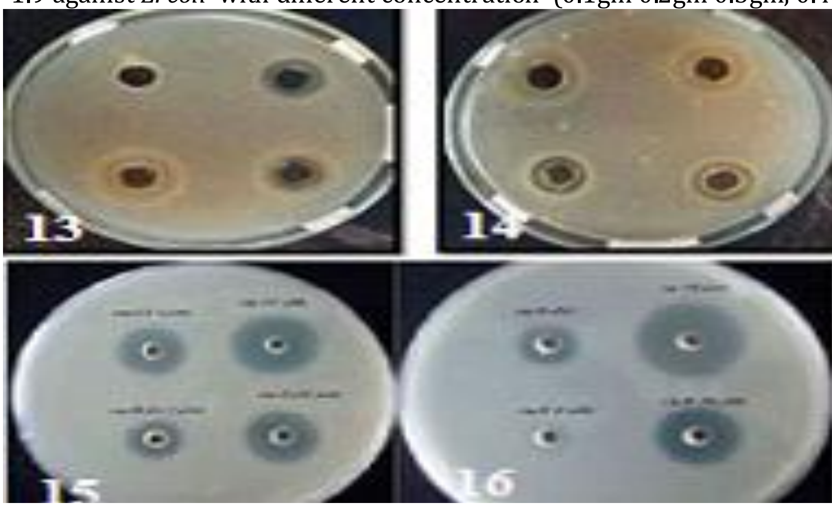
Well diffusion method:-

In well diffusion *Ficus benghalensis* copper nanoprticles + water showed the zone of inhibition against different bacteria. The zone on inhibition were 1.3cm, 1.4cm, 1.5cm, 1.7cm against *S. aureus* and 0.9cm, 1.0cm,1.1cm, 1.6cm .against *E. coli* with different concentration (0.1gm, 0.2gm, 0.3gm and 0.4gm) respectively.

In well diffusion *Ficus benghalensis* copper nanoparticles+70% ethanol showed the zone of inhibition against different bacteria. The zone of inhibition were 1.0cm,1.2cm,1.3cm,1.5cm against *S. aureus* and 1.2cm,1.4cm,1.5cm,1.6cm against *E. coli* with different concentration (0.1gm 0.2gm 0.3gm0.4gm) respectively.

In well diffusion *Osimum sanctum* copper nanoprticles + water showed the zone of inhibition against different bacteria. The zone of inhibition were1.2cm, 1.4cm, 1.6cm, 1.8cm against *S. aureus* and1.3cm, 1.5cm, 1.7cm, 1.9cm against *E. coli* with different concentration (0.1gm 0.2gm 0.3gm, 0.4gm) respectively.

In well diffusion *Osimum sanctum* copper nanoparticles + 70% ethanol showed the zone of inhibition against different bacteria. The zone of inhibition were 0.5cm, 0.8cm, 1.0cm and 1.4cm against *S. aureus* and 1.5cm, 1.6cm, 1.8cm and 1.9 against *E. coli* with different concentration (0.1gm 0.2gm 0.3gm, 0.4gm) respectively.



Figs: - 13, 14, 15 and 16 shows antibacterial activity of *Ficus* and *Osimum* copper nanoparticles against bacteria well diffusion method.

Both the solvent used in the present experiment (well and disk diffusion method) with copper nanoparticles showed the zone of inhibition against bacteria like *E. coli* and *S. aureus.* Maximum zone of inhibition 1.9 cm has observed at 0.4 gm concentration of *Ocimum sanctum* CuNPs + 70 % Ethanol against *S. aureus.* While minimum zone of inhibition 0.5 cm has observed at 0.1 gm concentration of *Ficus benghalenis* and *Osimum sanctum* CuNPs + 70 % ethanol against *E. coli*.

Nanoparticles prepared for the present experiments showed effective antibacterial activity. It was observed that nanoparticles have penetrated inside the bacteria and have caused damage by interacting with phosphorus and sulfur containing compound such as DNA [30].may have lose its replication power and cellular proteins and become inactive after treatment with nanoparticles. In addition, nanoparticles may be preventing the growth and cell division. The nanoparticles have an additional contribution to the bactericidal efficacy. Heavy metals are toxic and react with proteins, therefore they bind protein molecules, as a result cellular metabolism is inhibited causing death of microorganism [31].

Copper nanoparticles are generally immediately available in most volumes. Researchers have also recommended the use of silver and copper ions as superior disinfectants for waste water generated from hospitals containing infectious microorganism. Previously reported antibacterial activity of copper nano particle, it was found that it has significant potency to act as bactericidal agent than gold, silver, zinc, nano particles. Combination of different nano particles such as silver & copper may show more significant effect on bacterial growth. Surfaces of copper nano particles interact straight with the bacterial cell wall &outer membranes, leads to breakage of cell wall &destroy bacteria [32].

In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. These biosynthesis of gold nanoparticles by plants such as *alfalfa* [30, 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 *Ocimum sanctum* and *Ficus* *benghalenis* CuNPs showed antibacterial activity against *E. coli* and *S. aureus.* Thus the results of present study corroborate with finding of previous authors that plant mediated nanoparticles may be good antibacterial agent.

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