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Synthesis, Characterization, And Antimicrobial Properties of Copper Nanoparticles Using Citrus Limon Fruit Extract

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Abstract

Green synthesis of metallic nanoparticles has been expanding due to its potential in development of new technologies. Synthesis of copper nanoparticles (CuNPs) was carried out using aqueous extract of Citrus limon using a novel green route for the synthesis and stabilization. The nanoparticles was investigated to Citrus limon was confirmed by UV-visible spectroscopy, FTIR spectra, X-ray diffraction (XRD) and scanning electron microscopy (SEM). The FTIR spectra of CuNPs indicated that the being of flavonoids and phenolic functional groups could reduce the Cu2+ ions to CuNPs. The CuNPs nanoparticles were investigated using UV–vis absorption spectra for surface Plasmon resonance (~579 nm). Crystalline diffraction peaks with nanospheres distributed through the surface. The X-ray diffraction (XRD) pattern of the CuNPs agrees with Cu metal with the reported and the crystallite average size is ~30 nm Scanning electron microscopic (SEM) show uniform spherical particles obtained by this biological method. In addition, CuNPs and the aqueous extract of the plant demonstrated suitable antimicrobial activity is found to be effective of CuNPs. In results confirmed that the use of aqueous extract of Citrus limon has a significant potential for developing clean, easy, cost effective and efficient method for green synthesis of copper nanoparticles and significant biological activity, suggesting their potential as therapeutic agents.

Keywords: Citrus limon fruit, Copper nanoparticles, Characterization, Anti-bacterial activity

1. Introduction

Nanotechnology is a field of science with vast potential in medicine plants. Being like with nature, the combination of nanoscience and biology will not only strengthen the fight against pathogenic microorganisms but can also result in a change in slant towards struggling infectious disease**s** **(Janardhanan R 2009)** Metal-based NPs, in specific, have found wide-ranging efficacy in the creation of cosmetics and textiles (**Shende 2015).**. Researchers have sightsaw different methods and sources for creating NPs, leading to the innovation of their remarkable biological properties such as antimicrobial, anticancer, antioxidant, antidiabetic and drug delivery competence **(Tshireletso, Pitso 2021, Jahan,**

Israt 2021). Of these biological activities, the anticancer potential of NPs has gained significant interest due to the upsetting occurrence of cancer worldwide [Kieran, 2017]. Green synthesis is a set of principles that eliminates the use of hazardous substances in manufacturing and applications of chemical products.

Nanoparticles are tiny solid materials with a structural length of a few nanometers. They are small and have a large surface to volume ratio; their size and shape-dependent properties are of interest in applications such as bio-sensing and optics. There are many metal nanoparticle applications, mainly in pharmacy and medical sciences, especially in counteracting antimicrobial properties. Copper nanoparticles (CuNPs) have recently attracted special attention because of their low-cost and novel optical, mechanical, catalytic, electrical and thermal conduction properties, which are different from that of their bulk metals. Copper nanoparticles (CuNPs) were synthesized under ambient temperature by wet chemical method and evaluated their antibacterial activity by the means of well diffusion method. In this study, we have analyzed the effect of NaBH4 concentration on the synthesis of CuNPs. Surface plasmon resonance (SPR) was occurred at 575 nm (**Lalit yadav et al., 2017**Green synthesis of nanoparticles using citrus peel extracts is known to be environmentally friendly and non-toxic when compared to chemical methods. In this study, different citrus peels extracts obtained with the solvents acetone and distilled water were used to synthesize copper oxide nanoparticles (CuONPs) **) (Tshireletso et al., 2021)**.

Green synthesis is a set of principles that eliminates the use of hazardous substances in manufacturing and applications of chemical products. There is a need for such methods since there are increasingly popular concerns associated with the environment and therefore we need "green" substances that are environmentally friendly. The usage of plant extracts in green synthesis of nanoparticles is easy to perform and rapid and production on the large scale is recently an emerging area of research. This plays an essential role in nanotechnology, as using plant extracts is eco-friendly, cost-effective and is a better alternative over chemical methods. Moreover, plant extracts are known to have a great significance in the treatment of microbial infections and have been used as a valuable source for maintaining human health. He present study focuses on photosynthesis of copper oxide nanoparticles (CuONPs) using the aqueous leaf extracts of Gomphrena globosa (G. globosa) and Gomphrena serrate (G. serrate) for the first time.

Majid Asri et al., 2023 Copper oxide nanoparticles (CuONPs) are listed as metal oxide semiconductors with chemical and chemical properties. CuONps are further analyzed for antibacterial effect with Gram-positive and Gram-negative bacteria, and in both cases, high antibacterial activity is observed. Therefore, these nanoparticles are an interesting material for electrochemical and biological studies. Various microbiological tests were performed using varying concentrations of green synthesised Cu NPs with sizes 40 and 25 nm respectively. Effectiveness of nanoparticles increases with increasing particle dose, treatment time and synthesis method. In addition

Presently, only a few works on green synthesis of CuNPs have been reported. To the best of our knowledge, no works have been reported on the effect of type of copper salts and reducing agents on the shape and size of CuNPs. Therefore, the objective of the present study was to test the effect of type of copper salts (copper acetate, copper chloride, and copper sulfate) and reducing agents (sodium hydroxide and ascorbic acid) on the shape and size of CuNPs. The synthesized CuNPs were characterized by UV–visible spectrophotometer, energy dispersive X-ray spectra (EDS), field emission scanning electron microscopy (FE-SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction crystallography (XRD), and the antimicrobial activity of CuNPs was evaluated (**Fig 1)**.

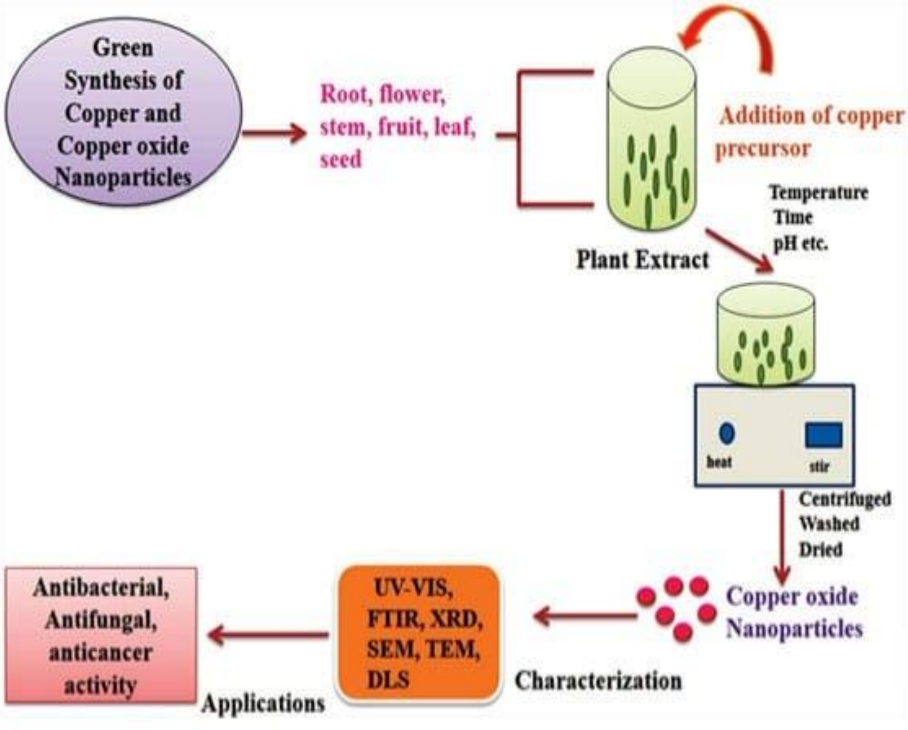


Figure1.Development and characterization copper oxide nanoparticles

Figure1. CITRUS LIMON

2. Material and Methods

Materials

Chemical and reagents Copper sulfate pentahydrate ($CuSO_4$. $5H_2O$, ≥ 98%) purchased from Sigma-Aldrich, Germany and used without further purifications. Citrus limon fruits were purchased from local market. De-ionized and distilled water was obtained from our laboratory.

Preparation of Citrus limon fruits extract

Fresh samples of Citrus Lemon fruits feel were exposed to washing with distilled water and cutting to a small piece and dropped into 100 ml de-ionized water in 250 ml glass beaker at 80ºC for 10 min **(Mohammad W. Amer 2021).** Then, the extract was filtered on filter paper (What man No. 41) to obtain Citrus limon fruits aqueous extract. The clear extract to filtrate was kept in a glass flask in a refrigerator for the experimental work.

Green synthesis of copper nanoparticles (CuNPs)

Citrus limon fruits aqueous extract (100 ml) was mixed with 4g of copper sulphate pentahydrate under magnetic stirring at room temperature (27 °C) for 4 h. The blue colour of copper sulphate pentahydrate changed to brown colour within 10 minutes, indicating the formation of CuNPs due to reduction of copper ions from Cu (II) ions to Cu metal. The samples were then centrifuged at 3000 rpm for 10 min to get a clear supernatant at room temperature. The copper nanoparticles obtained were dried in an oven at 80-90 °C for further studies (FT-IR, SEM and XRD analysis).

Characterization of CuNPs

Characterization of CuNPs to Ultraviolet-visible spectra for the deep red dispersions was measured using Shimadzu UV-1601 spectrophotometer. Crystalline metallic copper nanoparticles were examined by Powder X-ray diffraction experiments were carried out on a 6000 X-ray diffraction instrument (Shimadzu), and the pat terns were recorded at a scan speed of 4° per minute with Cu Kα1 radiation (λ =1.54060 Å) operating at 40 kV and 40 mA. Fourier transform infrared (FTIR) was used to identify the functional groups that were present in citrus limon fruits aqueous extract was obtained in the range 4000 400 cm^{-1} with IR-Prestige-21 Shimaduz FTIR spectrophotometer, using KBr pellet method. The morphological investigation was done using Scanning electron microscopy (SEM) analysis of copper nanoparticles analysis was done using Hitachi S-4500 SEM machine.

Antibacterial activity

The antimicrobial activity of the CuNPs against the selected microorganisms was investigated by two methods, ie, qualitative evaluation and quantitative evaluation The assay was conducted against bacterial species, including E. coli, Staphylococcus aureus, Bacillus subtitles, Bacillus cereus, Klebsiella Pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Streptococcus pneumoniae were assayed by agar well diffusion method and disc diffusion method **(Bauer 1959).** After 24 h of incubation at 37 °C, the zone of inhibition (ZOI) around the disc was measured.

Statistical analysis

All of the data from three independent replicate trials were subjected to analysis using Statistical package: Statistics 8.0. The data are reported as the mean ± SD and significant differences between mean values were determined. In this study involved the utilization of one-way analysis of variance (ANOVA).

3. Results

UV-Vis analysis

In the present study, the formation of CuNPs was initially confirmed by the characteristic color change from light blue to deep brown, followed by UV-Vis spectroscopic analysis. **Fig 2** shows the time dependent UV-Vis spectra of the reaction mixture using Citrus limon fruits peel using, results revealed the conversion of copper ions to copper nanoparticles with almost 100% bio reduction of metal ions as evidenced by qualitative testing of supernatant after the purification of copper nanoparticles. The difference in the rate of bio reduction observed may be assign to the differences in the activities of the amino acids present in Citrus Limon fruits aqueous extract (Yugandhar 2017). The entire reaction mixture is turned to brown colour, and exhibit an absorbance peak around 579 nm characteristic of CuNPs, due to its surface Plasmon resonance absorption band **(Fig 4).** It was observed that the The CuNPs have spherical shape with an average diameter of 28 nm (Pradnya Zambare 2023). The spectrum of copper oxide nanoparticles synthesized using acetone exhibited distinct peaks at wavelengths ranging from 285–293 nm.(Saranyaadevi 2014)These findings align with previous reports in which the UV-Vis spectra for copper oxide nanoparticles ranged from 280–1000 nm.In the present investigation, the reaction mixtures showed a single SPR band revealing spherical shape of copper nanoparticles, which was further confirmed by SEM images Scanning electron microscopy (SEM) of the synthesized of copper nanoparticles is illustrated in images.

Scanning electron microscopy (SEM)

SEM visualizes the surface morphology of the sample. Scanning electron microscopy (SEM) of the synthesized of copper nanoparticles is illustrated in **Fig. 3** It was observed that the The CuNPs have spherical shape with an average diameter of 28 nm. . Scanning electron microscopy (SEM) analysis (Fig. 5) revealed that CuNPs particles have the average particle size 20nm. The particle size distribution of the synthesized copper nanoparicles is ranging between 5 nm and 28 nm. A narrow size distribution of the copper nanoparticles (CuNPs) was observed with average size 28 nm. There are many examples of greenly synthesized CuNPs characterized by SEM. SEM analysis of CuNPs showed spherical shape CuNPs with size range of 6–70 nm. Similarly, CuNPs prepared using Leaf extract of Ricinus communize showed particles diameter as 20–30 nm and 21.22 nm respectively (Mohammad 2021, Jakhmola 2019, Ji 2015).

Antibacterial activity

Antibacterial activity showed the green synthesized copper nanoparticles displayed high antibacterial activity towards minimum inhibitory concentration (MIC) values were obtained the 8 tested pathogenic strains of **E. coli, Staphylococcus aureus, Bacillus subtitles, Bacillus cereus, Klebsiella Pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Streptococcus pneumoniae and Citrus lemon** fruits aqueous extract showed very low effect against microbial strains **(Fig 6).** The Citrus lemon peel extract was found to be highly efficient for the synthesis of CuNPs at nanoscale and the prepared NPs showed promising antimicrobial activity, which could have practical application to control bacterial infections. In view of advantages offered by green synthesis route **(Varghese 2021);** it can be used for the preparation of NPs for different applications. These results may refer to differences in the cell wall of each strain; the cell wall of Gram-negative strains (E. coli) is wider than the cell wall of Gram-positive strains (S. aureus). This is probably of the toxicity of copper ions on E. coli includes a rapid DNA degradation, followed by a reduction of bacterial respiration; it is also known that copper ions inhibit certain cytochromes in the membrane for tested against E. coli (ATCC 25922) and S. aureus (ATCC 29213). The results are presented in **Table 1**, where it can be seen that the copper nanoparticles present the best antibacterial activity against the two strains tested in comparison with Citrus limon fruits aqueous extract and the reference drug. The MIC of the copper nanoparticles is higher when it is tested against E. coli than when tested against S. aureus.

Figure 2. preparation of copper nanoparticles using citrus fruits

S. No	Test Organisms	Zone of inhibition in (mm) against different Concentration (50 µg/ml)				
		PC	25µl	50µl	75 µl	100µl
1	E. coli	17	-	-	10	14
2	Staphylococcus aureus	24	-	13	17	19
3	Bacillus subtitles	23	20	24	27	29
4	Bacillus cereus	30	10	17	20	24
5	Klebsiella Pneumoniae	27	-	10	12	13
6	Pseudomonas aeruginosa	28	9	13	15	20
7	Proteus vulgaris	30	13	15	18	20
8	Streptococcus pneumoniae	28	-	10	11	13

Table 1. The antibacterial activity of copper nanoparticles synthesized by using leaf extract of citrus lemon

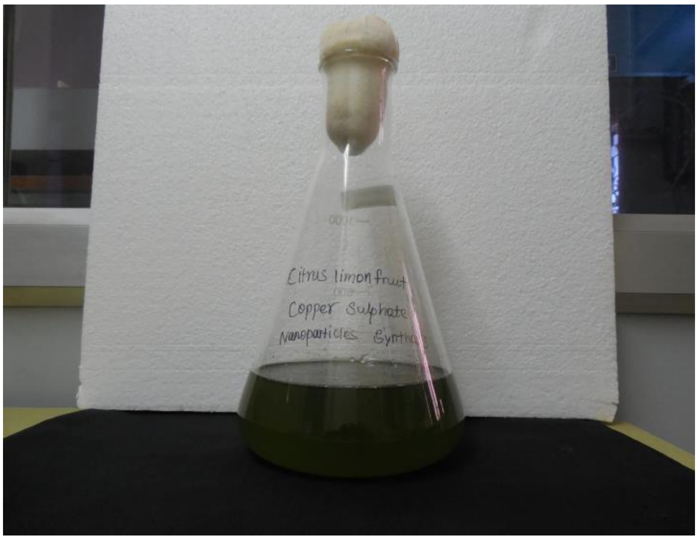
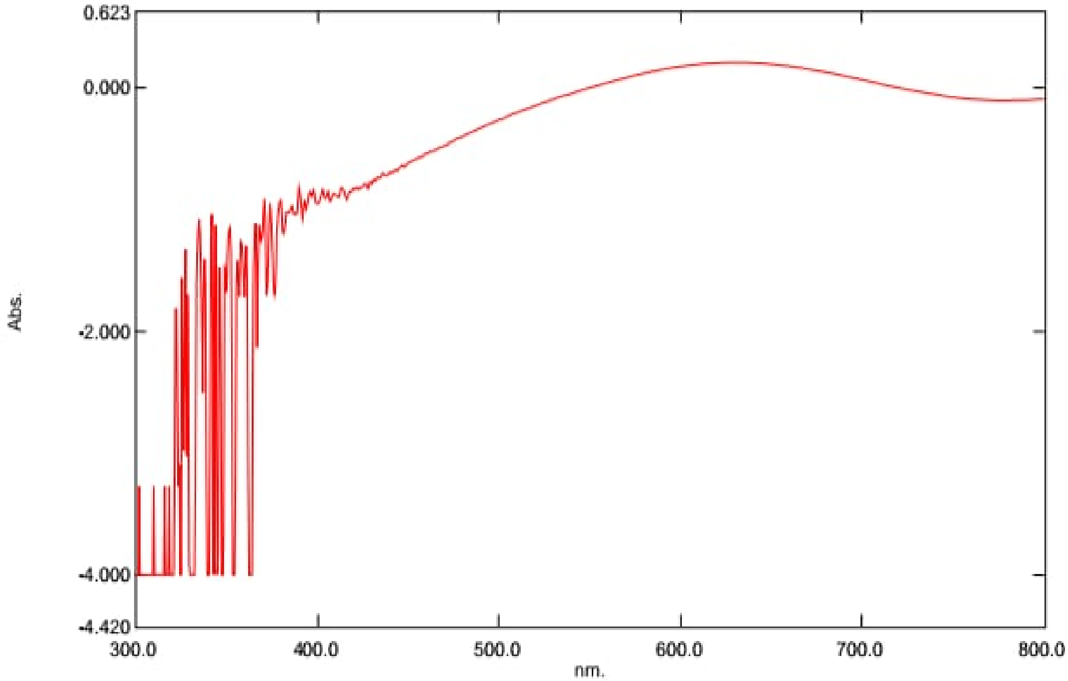


Figure 3.Synthesis of copper nanoparticles from citrus lemon



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Auto Sampling Interval: Enabled
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Measuring Mode: Absorbance
Slit Width: 1.0 nm
Light Source Change Wavelength: 340.0 nm
S/R Exchange: Normal

[Attachment Properties]
Attachment: None

[Operation]
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InterPolate: Disabled
Average: Disabled

[Sample Preparation Properties]
Weight:
Volume:
Dilution:
Path Length:
Additional Information:

No.	P/V	Wavelength	Abs.	Description
1		785.0	-0.106	
2		629.0	0.203	
3		436.0	-0.703	
4		413.0	-0.824	
5		398.0	-0.844	
6		390.0	-0.822	
7		374.0	-0.955	
8		358.0	-1.273	
9		342.0	-1.041	
10		335.0	-1.082	
11		318.5	-3.273	
12		310.0	-3.273	
13		787.0	-0.107	
14		777.0	-0.110	
15		438.0	-0.723	
16		416.0	-0.920	
17		400.0	-0.955	
18		392.0	-1.074	
19		376.5	-1.697	
20		363.5	-4.000	
21		353.5	-4.000	
22		340.0	-4.000	
23		325.0	-4.000	
24		314.0	-4.000	

Figure 4 . UV-vis absorption spectra of copper nanoparticles using Citrus limon fruits extract

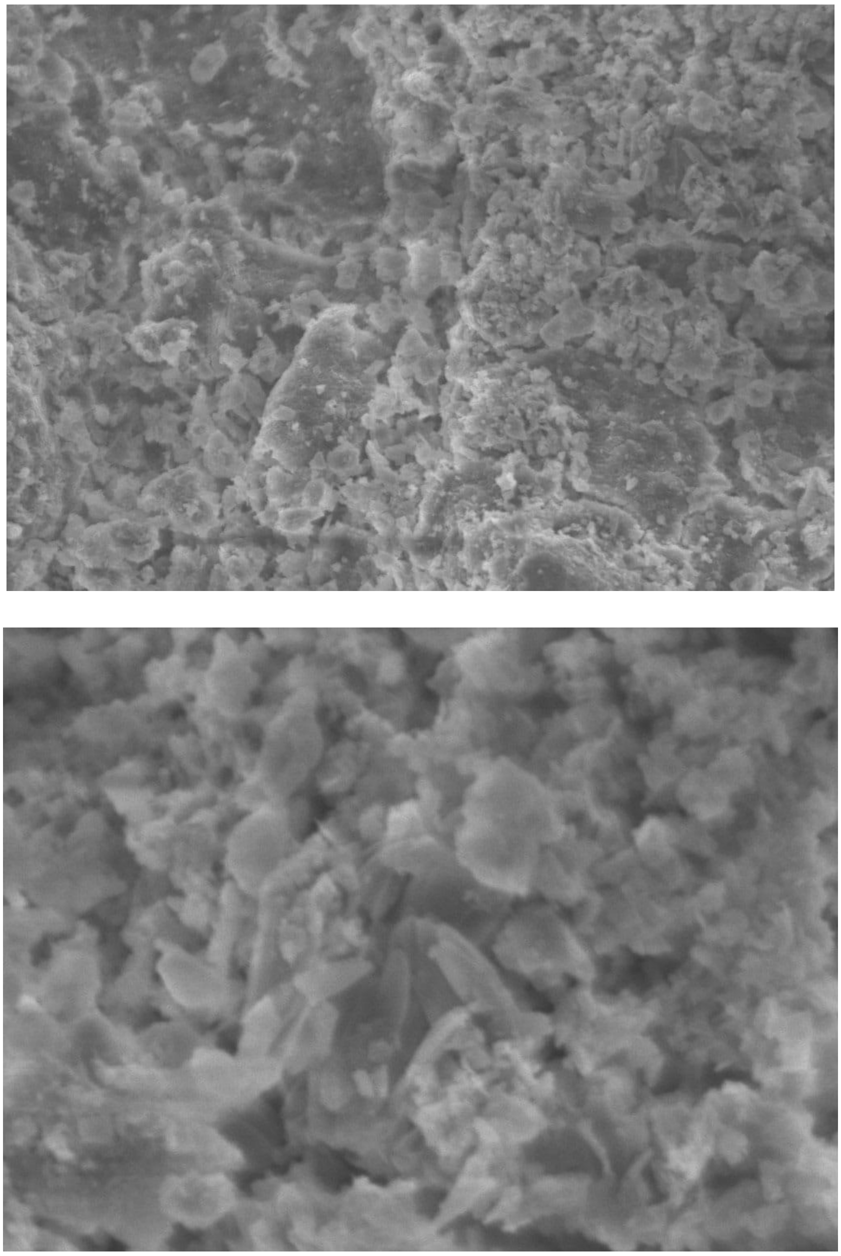
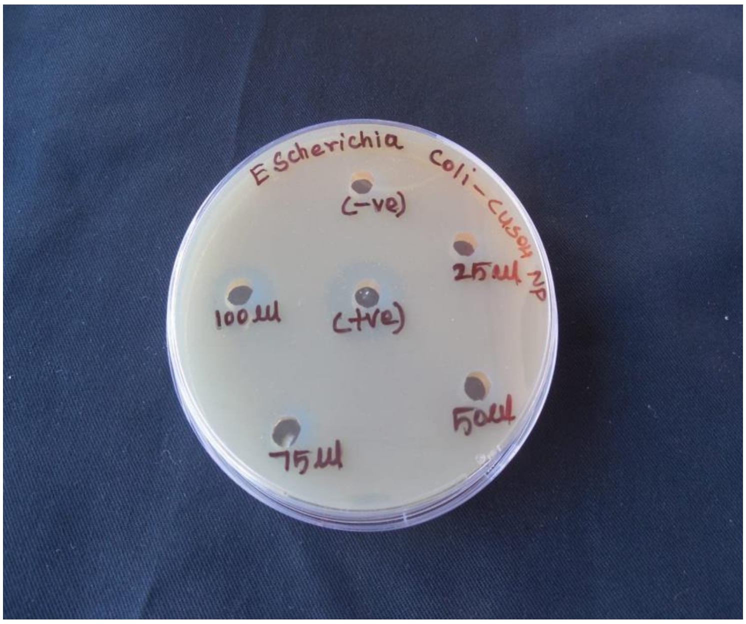
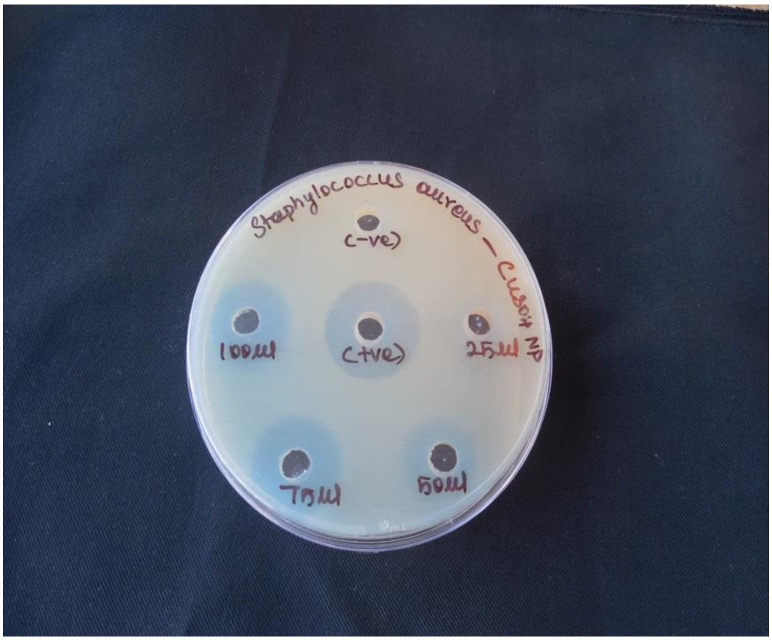


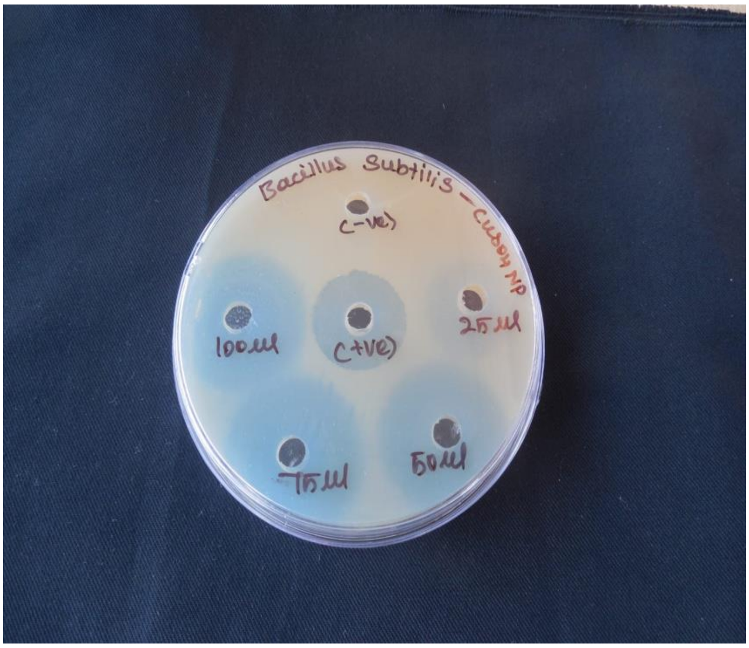
Figure 5.SEM of the green synthesized copper nanoparticles



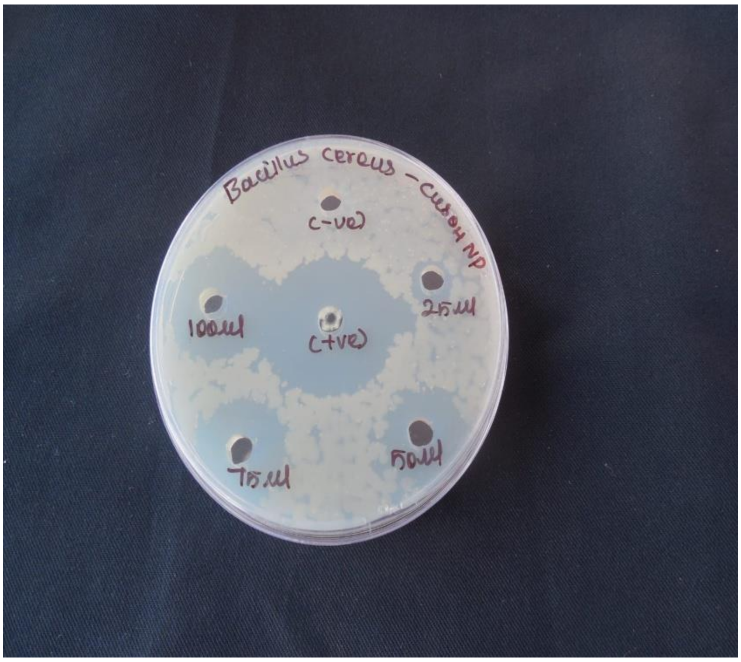
Escherichia Coli



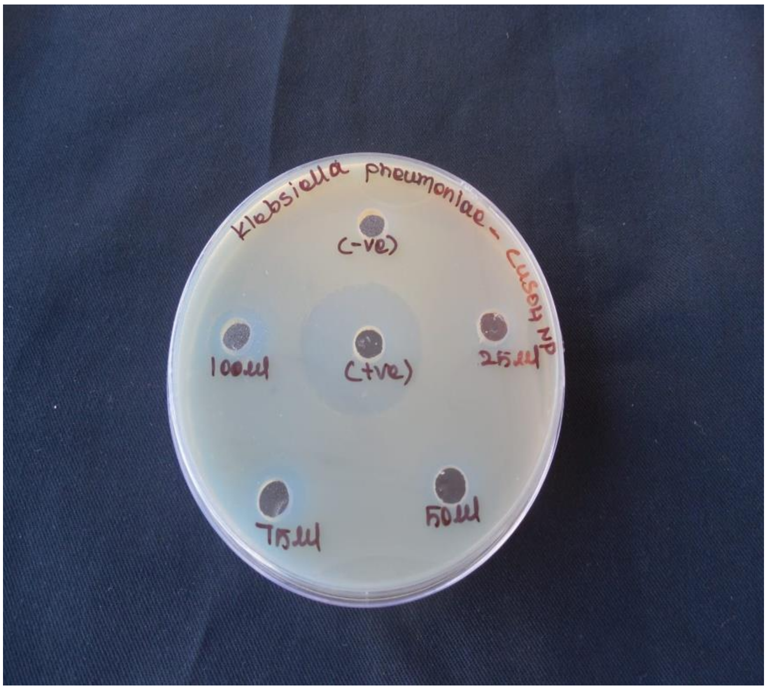
Staphylococcus aureus



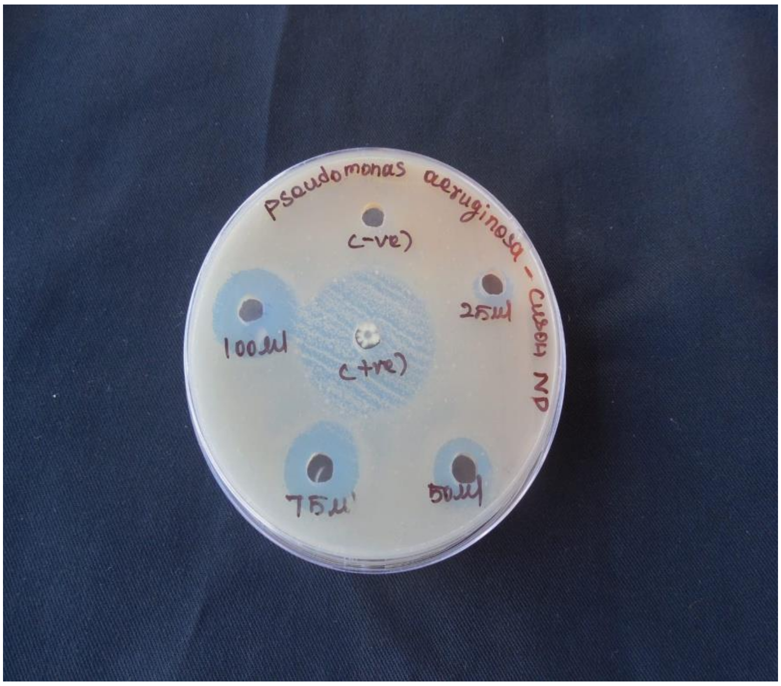
Bacillus subtitles



Bacillus cereus



Klebsiella pneumoniae



Pseudomonas aeruginosa

Figure 6. Zones of inhibition (well diffusion assay for copper nanoparticles against microbial strains)

Proteus vulgaris

Streptococcus Pneumoniae

4. Conclusion

This study reports on the green synthesis of CuONPs prepared by broth by a novel biological synthesis technique which is simple and environmentally benign. It is an easy, cost-effective and doesn't involve any harmful and toxic chemicals. The characterization of synthesized copper nanoparticles elucidated by microscopic and spectroscopic techniques which includes SEM, FTIR, XRD and UV Visible confirm the formation of copper nanoparticles. The UV-Vis absorption spectra show that copper oxide nanoparticles synthesized from selected peel extracts exhibit characteristic peaks at the wavelengths 280–320 nm. CuNPs exhibited an excellent antibacterial activity against E. coli and S. aureus pathogenic bacteria. SEM images reveal that the particles appear to be almost rod-like and sphere-shaped. SEM images showed spherical shapes and the size estimation, and the subsequent presentation in different histograms revealed an average size ranging between 48–76 nm. So it can concluded that green synthesized copper nanoparticles using Citrus limon fruits extract as environment-friendly with low-cost and excellent antibacterial activity. It is an easy, cost-effective and doesn't involve any harmful and toxic chemicals. The biomolecules present in the leaf broth not only reduce the metal ions and also stabilize the metal NPs.

5. Acknowledgements

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