RESEARCH PAPER

Copper–Magnesium Oxide Nanocomposites: A Dual-Functional Nanomaterial For Antimicrobial And Breast Cancer Therapeutics

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ABSTRACT

Nanoparticle particle and nanomaterials are increasingly being explored for their potential application in medicine. One of the most promising areas of application is drug delivery, where nanoparticle can be used as carriers to deliver drugs to specific cells or tissues in the body. The cancer is the major cause of morbidity and mortality and still today there isn't any promising therapeutics for treating cancer.The nanocomposite of copper and magnesium oxide nanoparticle is synthesized from copper sulphate and magnesium sulphate as starting material. Sodium hydroxide act as a reducing and capping agent in the nanoparticle synthesis. After synthesis, the nanoparticles were characterized by UV-spectrophotometer, FT-IR. The crystalline morphology and size of the nanoparticles were determined by DLS and SEM analysis. Furthermore, anti-microbial activities were evaluated. The average size of the nanoparticle was 80 nm. The cytotoxicity of the different concertation of the nanocomposite of copper and magnesium oxide nanoparticle is evaluated. The minimum inhibitory concentration of the nanocomposite is 53.06±0.9 µg.

Keywords : Copper oxide nanoparticles (CuO NPs), Magnesium oxide nanoparticles (MgO NPs), CuO–MgO nanocomposite, Nanoparticle synthesis, Nanomedicine, Targeted therapy, Breast Cancer

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INTRODUCTION

2.3 million women were diagnosed with breast cancer in 2022, and 670000 deaths occur globally every year. Breast cancer is mostly affected by mutations in the BRCA1, BRCA2, and PALB2 genes. If a particular woman has a mutation in this gene, it can be cured by removing both breasts or by chemoprevention. To treat breast cancer, the strategies available are surgery to remove the breast, chemotherapy, and radiotherapy. If the cancer cell is oestrogen receptor positive (ER) or progesterone receptor (PR), then it can be treated with endocrine therapy. But if the cancer is ER or PR-negative, then it can only be cured with chemotherapy if it is in the early stages. The above-mentioned treatments not only target the cancer cell itself but also kill normal cells. Nanotechnology plays a major role in the treatment of cancer. Due to their smaller size and charged surface, bio-compatibility nanoparticles are used to treat cancer.

The copper oxide nanoparticle has photoconductive and photothermal applications. It is used in power-saving batteries. Copper nanoparticles have a lot of applications in the healthcare industry. It has the ability to kill 99% of both grammeme-positive and grammeme-negative bacteria.

MgO nanoparticles are the most promising candidate compared to other metal oxide nanoparticles. Because it has excellent optical, thermal, mechanical, and electrical properties. Its different shapes, such as platelets, flowers, stars, spheres, rods, and cubes, make these nanoparticles suitable for different applications. MgO nanoparticles are also used in the fields of tissue regeneration and wound healing.

A similar study was used with the copper oxide nanoparticle to inhibit autophagy in the MCF-7 cell line by autophagosome formation. It can be confirmed with different tests, and a treated nanoparticle was observed using TEM. Like that, the magnesium-doped copper spinel ferrite superparamagnetic nanoparticles can be tested in the MCF-7 cell line with different concentrations under UV irradiation. In a recent research study, the magnesium oxide nanoparticle synthesised with Saussureacostus was tested in the

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MCF-7 cell line. It shows the prominent cytotoxicity and efficient dye degradation of methylene blue dye.

In this study, the copper and magnesium oxide nanoparticles were synthesised by the sol-gel method, and both nanoparticles were characterised by different methods: FTIR, SEM, and UV-visible spectrophotometer. The antimicrobial activity of copper and magnesium oxide was studied in grammeme-positive and grammeme-negative bacteria. Finally, the nanoparticles of copper and magnesium oxide were mixed in a 1:1 ratio, and then they were tested in the MCF-7 breast cancer cell line. The cytotoxicity of the nanoparticle mixer showed better activity.

MATERIALS AND METHODS

SYNTHESIS OF COPPER OXIDE NANOPARTICLE

For the synthesis of copper oxide and magnesium oxide nanoparticle the copper sulphate pentahydrate and magnesium oxide heptahydrate act as a starting material. 0.1 M copper sulphate pentahydrate was weighted and dissolved in 100 ml of distilled water. Using magnetic stirrer to make a homogenous solution. 0.1 M of sodium hydroxide prepared separately. The sodium hydroxide solution was filled in the burette, it was added to drop wise into the copper sulphate solution. The entire process was kept at 80°C. stop the reaction once the blue colour solution was change into black colour and it was stand to settle at room temperature.

It was sonicated to reduce the size of the nanoparticle and washed with methanol 3 times to remove the ironic contaminants. Then it was dried at 60°C and calcined at 400°C for 2 hrs.

SYNTHESIS OF MAGNESIUM OXIDE NANOPARTICLE

Magnesium oxide nanoparticle was synthesized by sol-gel method. 0.1M sodium hydroxide solution was dropwise into 0.1 M magnesium sulphate heptahydrate solution.The white colour precipitate was started to form. Stop the reaction once the pH of the solution reaches 10-12. The formed magnesium hydroxide was sonicated and washed with methanol to remove the ironic impurities and it was dried and calcined in muffle furnace at 400 °C for 2 hrs.

CHARACTERISATION

Standard analytical methods are used to study the morphological features of the synthesised copper and magnesium oxide nanoparticles. The initial confirmation of the formation of the nanoparticles can be done using a UV-visible spectrophotometer ( ). For the analysis of the sample in the UV-visible spectrophotometer, the samples were ultrasonicated to disperse the particles before scanning. The size, PDI, and zeta potential of the nanoparticle can be identified using (). The surface morphology of the formed nanoparticles can be studied by (). The functional group present in the sample and molecular interactions are studied using ().

ANTIMICROBIAL ACTIVITY

It is a test used to determine the zone of inhibition and measure the antimicrobial activity of the nanoparticle. In a 250-ml conical flask, add 100 ml of distilled water and 2.8 g of nutrient agar. Keep it for sterilisation for 20 minutes at 120 °C and 15 psi. The autoclaved nutrient agar medium was poured into the sterile Petri dish. After solidification of the agar, add 100 µl of both E. coli (grammeme-negative) and Bacillus subtilis (grammeme-positive) overnight culture, then spread with an L rod. Using a well puncher, the well was punched on the plate. Add 50 µl of different concentrations (20, 30, 40 mg/ml) of copper and magnesium oxide nanoparticles and a 1:1 ratio of both nanoparticles in the well. Once the samples were added, the plates were placed in the incubator at 37 °C.

CYTOTOXICITY ASSAY

The monolayer cell culture was trypsinized, and using the appropriate media containing 10% FBS, the cell count was adjusted to 1.0 x 105 cells/ml. In the 96-microtiter plate, add 100 µl of cell suspension (50,000 cells/well). After 24 hours of incubation, the monolayer was formed. Remove the supernatant and wash it once with media. Then 100 µl of different concentrations of nanoparticle samples were added to each well. All the plates were incubated at 37ºC for 24 hours in a 5% CO_2 incubator. 100 µl of MTT reagent (5 mg/10 ml in PBS) was added to the well. The plates were incubated for 4 hours at 37 ºC in a 5% CO_2 atmosphere. After incubation, 100 µl of DMSO was added to solubilize the formazan. A microtiter reader was used to measure the absorbance at 590 nm. The procedure was repeated three times, and the average value was taken.

RESULTS AND DISCUSSION

Copper Oxide Nanoparticle

Once the sodium hydroxide was added to the copper sulphate pentahydrate solution, the blue colour changed to b black. The metal precursor solution is shown in Figure 1. Figure 2 shows the copper hydroxide solution after adding NaOH to the copper sulphate solution. This black precipitate was further washed, dried, and calcined at 400 °C in a muffle furnace.

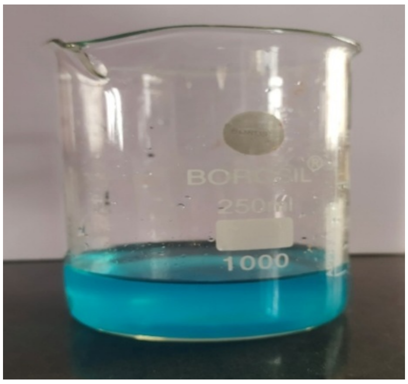


Figure 1. Copper sulphate solutions (black precipitate)


Figure 2. Copper hydroxide pentahydrate solution


Figure 3. Copper oxide nanoparticle after calcination

MAGNESIUM OXIDE NANOPARTICLE

The white precipitate formation indicates the formation of magnesium hydroxide nanoparticles. It was further dried and calcined at 400 °C in a muffle furnace to reduce the magnesium hydroxide to magnesium oxide. The white magnesium hydroxide precipitate is shown in Figure 4.

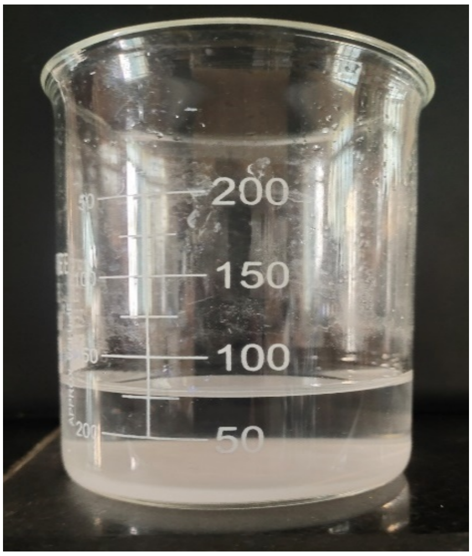


Figure 4. Magnesium hydroxide nanoparticle after calcination

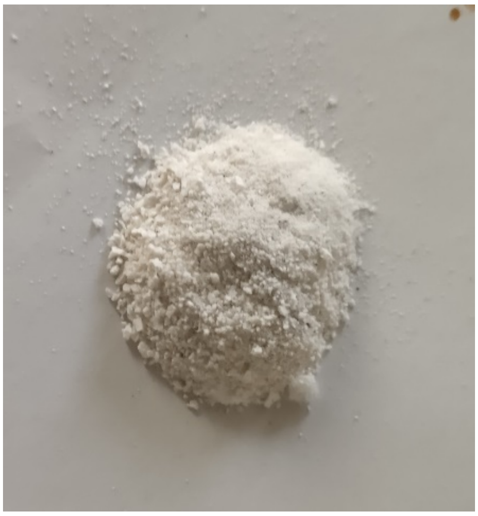


Figure 5. Magnesium oxide solutions (white precipitate)

UV VISIBLE SPECTRAL ANALYSIS

Initial confirmation was done using UV-visible analysis. The absorption peaks were obtained at 300 nm and 220 nm for copper oxide and magnesium oxide nanoparticles, respectively. Figures 6 and 7 show the UV-visible spectra of copper and magnesium oxide nanoparticles.

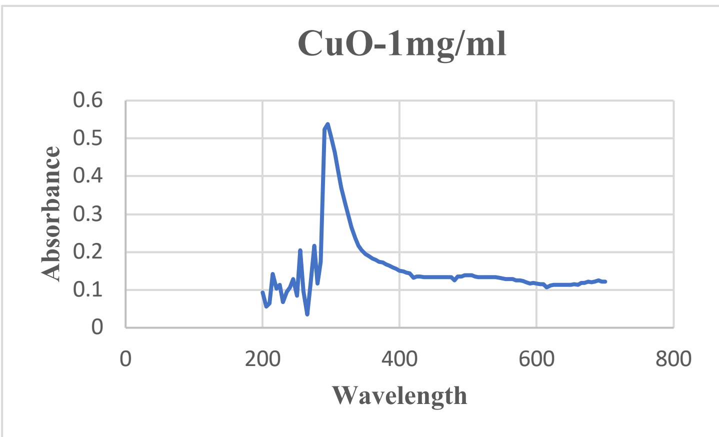


Figure 6. UV-Visible spectrum of copper oxide nanoparticle

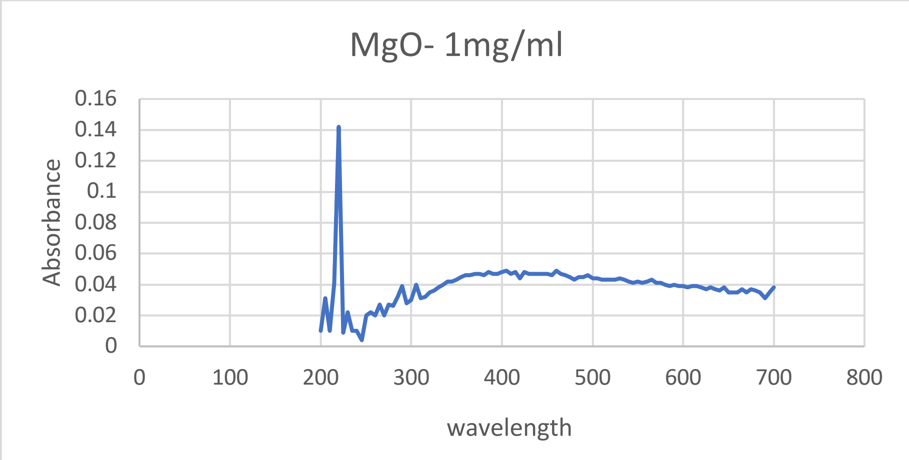
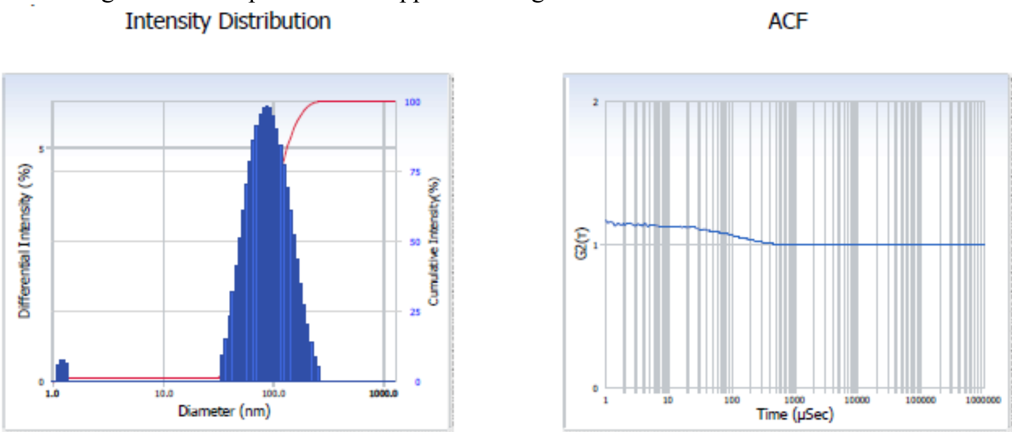


Figure 6. UV-Visible spectrum of magnesium oxide nanoparticle

PARTICLE SIZE ANALYSIS

In the dynamic light scattering instrument, the particles are scanned in the range of 3 nm to 10 nm by flctuation in the intensity of scattered light. The nanoparticles of copper and magnesium oxide are 80 and 83 nm.



Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	1.2	0.1
2	99.7	44.6
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	98.0	46.0
Residual :	2.783e-003	(O.K)

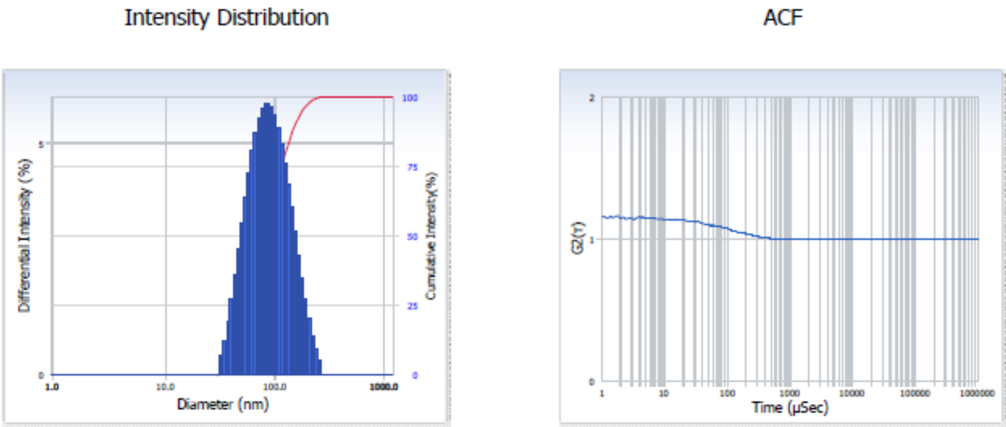
Cumulants Results

Diameter	(d)	: 80.1	(nm)
Polydispersity Index	(P.I.)	: 0.196	
Diffusion Const.	(D)	: 6.140e-008	(cm²/sec)

Measurement Condition

Temperature	: 25.0	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8878	(cP)
Scattering Intensity	: 31944	(cps)
Attenuator 1	: 24.38	(%)

Figure 7. DLS pattern of copper oxide nanoparticle



Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	100.3	45.3
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	100.3	45.3
Residual :	2.645e-003	(O.K)

Cumulants Results

Diameter	(d)	: 83.1	(nm)
Polydispersity Index	(P.I.)	: 0.160	
Diffusion Const.	(D)	: 5.918e-008	(cm²/sec)

Measurement Condition

Temperature	: 25.0	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8878	(cP)
Scattering Intensity	: 28669	(cps)
Attenuator 1	: 19.57	(%)

Figure 7. DLS pattern of magnesium oxide nanoparticle

ZETA POTENTIAL ANALYSIS

The synthesised nanoparticles of copper oxide and magnesium oxide had charges of -13.4 mV and -11.7 mV, respectively, indicating moderate stability. The Zeta potential is used to measure the charge of the particle in order to determine the stability of the colloid or dispersion. The dispersion is stable when the charge of the particle ranges from -25 to 25 mV.

FTIR ANALYSIS

The intensity peak between400 and 600 cm^{-1} corresponds to the stretching vibrations of the Cu-O bonds. But based on the crystal structure, the exact wavenumber can vary. A strong peak around 500–600 cm^{-1} is considered characteristic of Cu-O bond formation in CuO nanoparticles. The O-H stretching vibrations are observed around 3400cm^{-1}. It is associated with atmospheric moisture in the surroundings. The band observedat1638.6 cm^{-1} is due to amide bond.

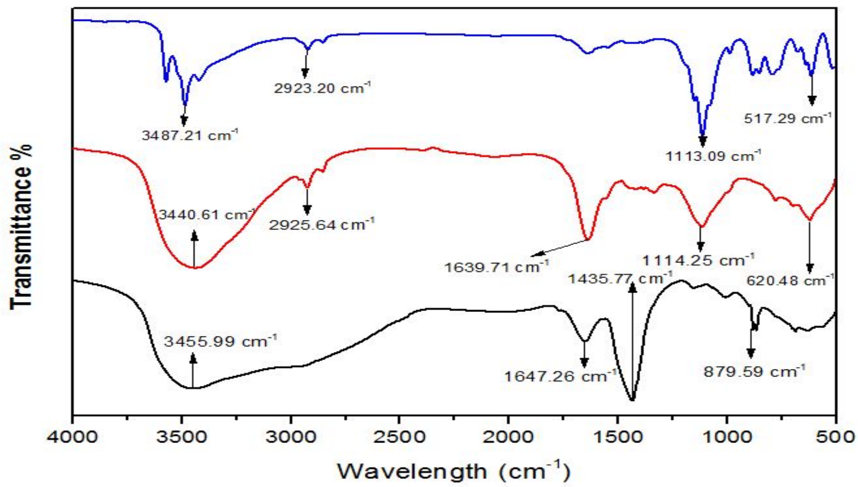


Figure 8. FTIR pattern ofCuO nanoparticle

For magnesium oxide nanoparticles, the O-H stretching vibration is observed around 3400 cm^{-1}. The peak between 600-900 cm^{-1} associated with Mg-O stretching. 1400–1600 cm–1 Peaks in this range may be related to carbonate ions or carboxylate groups, among other functions. These could result from air pollution or leftover organic compounds used in the production.

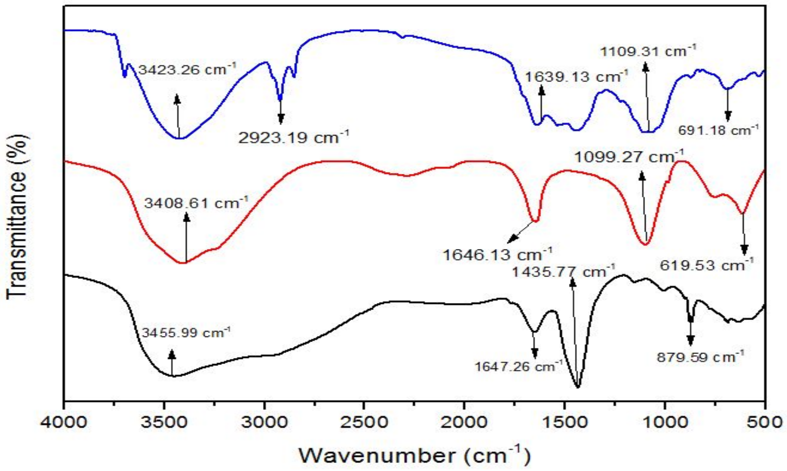


Figure 9. FTIR pattern ofMgO nanoparticle

Scanning electron microscopy is used to study the surface morphology and size of the nanoparticles. The size distribution of the synthesised nanoparticles ranges from 70 to 100 nm. The average size of the nanoparticles was 80 nm. In the below images, the nanoparticles were agglomerated and colloidal in nature due to the drying and calcination processes.

SEM ANALYSIS

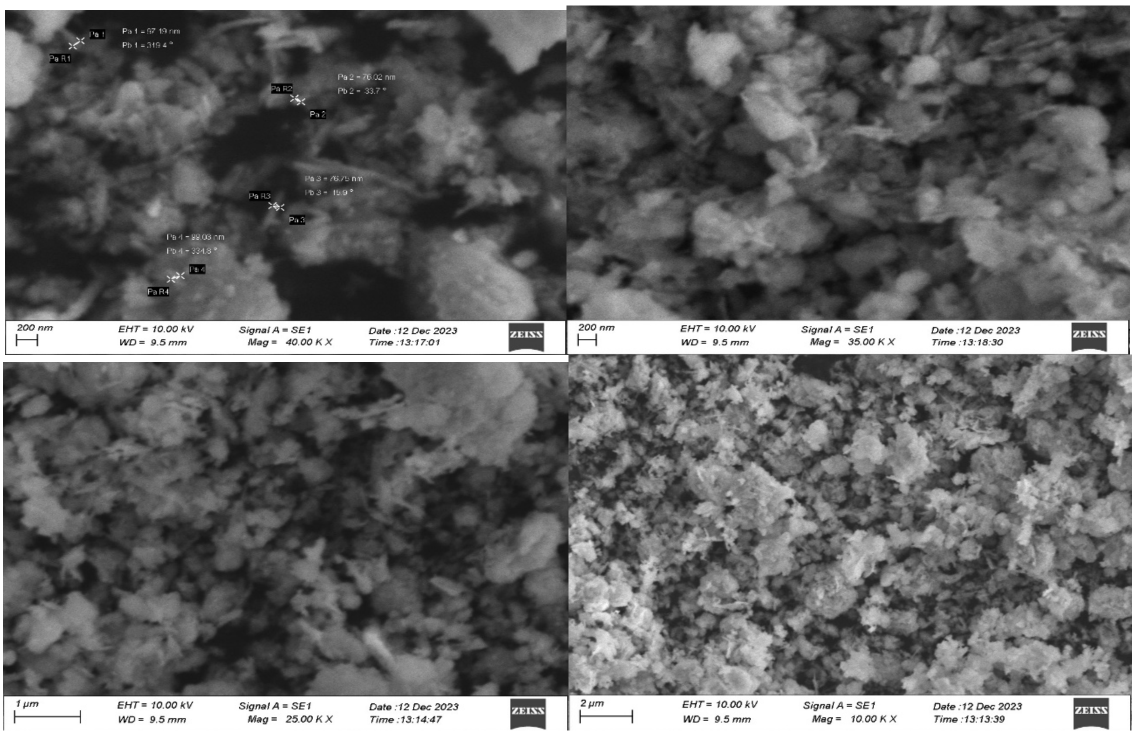


Figure10. SEM image of CuO nanoparticle

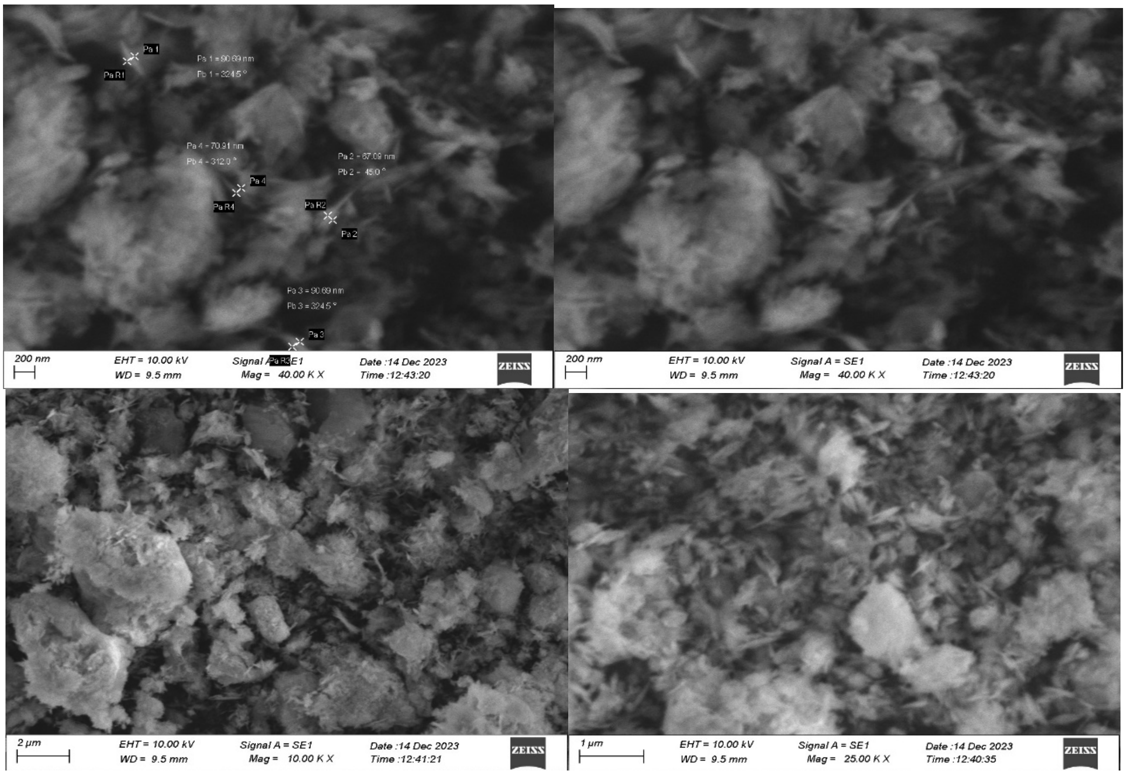


Figure10. SEM image of MgO nanoparticle

ANTIMICROBIAL ACTIVITY

We evaluated the antimicrobial activity of both copper oxide and magnesium oxide nanoparticles. Compared to the magnesium oxide nanoparticle, the copper oxide nanoparticle has the maximum zone of clearance. The magnesium oxide nanoparticles do not disperse in the agar medium, so the activity was minimal. The nanocomposite (1:1 ratio of copper and magnesium oxide), copper oxide, and magnesium oxide were tested on Staphylococcus aureus and E. coli.

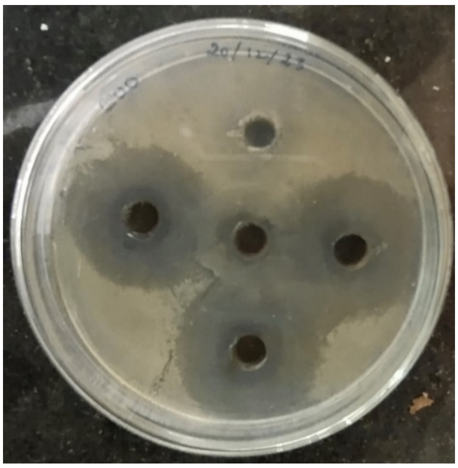


Figure 11. Of Copper oxide nanoparticle on bacillus subtilis

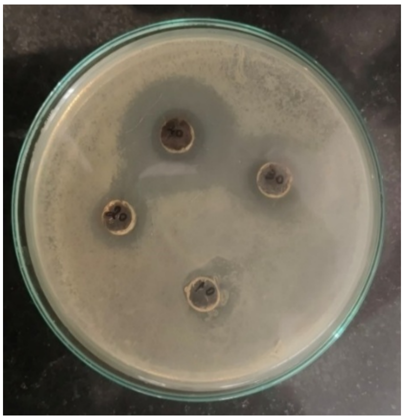
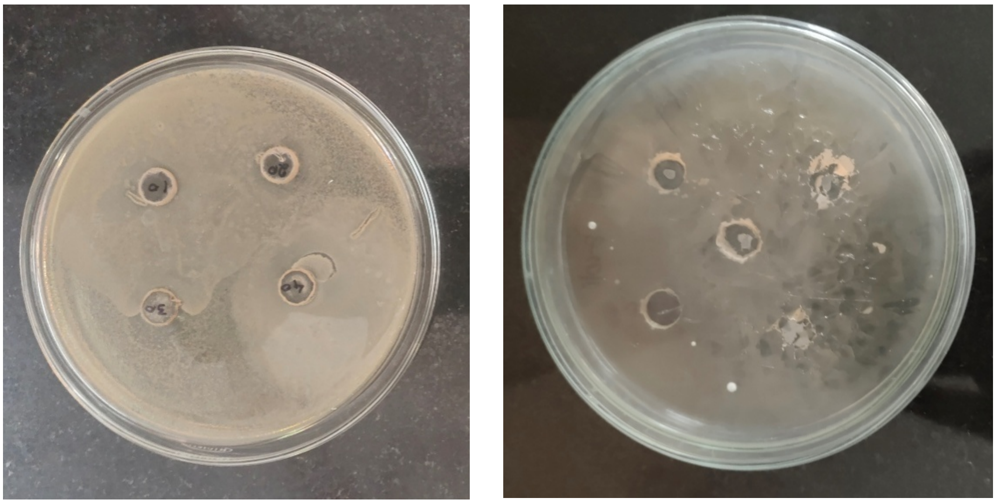


Figure 12. Copper oxide activity on nanoparticle on E. coli



B.Subtilis *E. coli*

Figure 13. MgO nanoparticles on Bacillus subtilis and E. coli

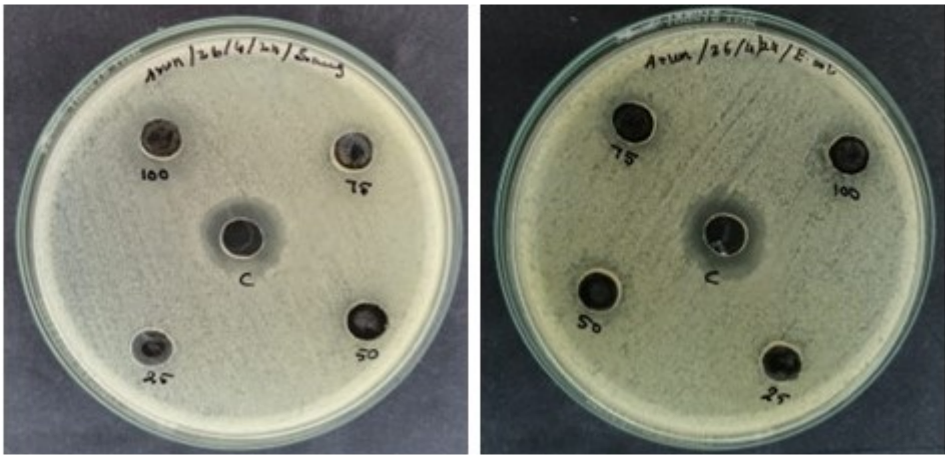


Figure 14. antimicrobial activity of nanocomposite in S. aureusandE. Coli

CYTOTOXICITY STUDY (MTT Method)

Using the MCF-7 breast cancer cell line, a copper and magnesium oxide nanoparticle nanocomposite was investigated. The MCF-7 cell count in Table 1 steadily decreases as copper and magnesium oxide nanoparticle concentrations increase. The graph showing nanocomposite concentration vs cell viability demonstrates that the nanocomposite increases as the number of cells decreases.The above figures are microscopic images of MCF-7 cells treated with different concentrations. Each figure has a different cell count according to the concentration of the nanocomposite. In the control image, there are a lot of cells. Because it is not treated with any samples. The other three figures (Figs. 4.9.3,4,5) show decreasing

cell counts. The concentration of 100 µg/mL shows the highest activity.
The half maximal inhibitory concentration (IC50) can be calculated from the formula **% inhibition = 100 – (OD of sample/OD of control) x 100.** The half-maximum inhibitory concentration of nanocomposite in the MCF-7 cell line is 53.06±0.9 µg.

Table 1. MTT absorption value

Cells	**CuOand MgO nanocomposite (1;1) on MCF-7 breast cancer cell line**										
Blank	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
0.068	0.978	0.911	0.838	0.782	0.658	0.564	0.424	0.318	0.227	0.121	0.039
0.055	0.964	0.915	0.833	0.782	0.654	0.535	0.429	0.333	0.226	0.117	0.047
0.044	0.979	0.916	0.827	0.748	0.654	0.532	0.415	0.337	0.229	0.123	0.045
	0.974	0.914	0.833	0.771	0.655	0.544	0.423	0.329333	0.227	0.120	0.044
Mean	**0**	**6**	**14**	**21**	**33**	**44**	**57**	**66**	**77**	**88**	**96**
Viability	**100**	**94**	**86**	**79**	**67**	**56**	**43**	**34**	**23**	**12**	**4**
	IC50 = 53.06±0.9 µg										

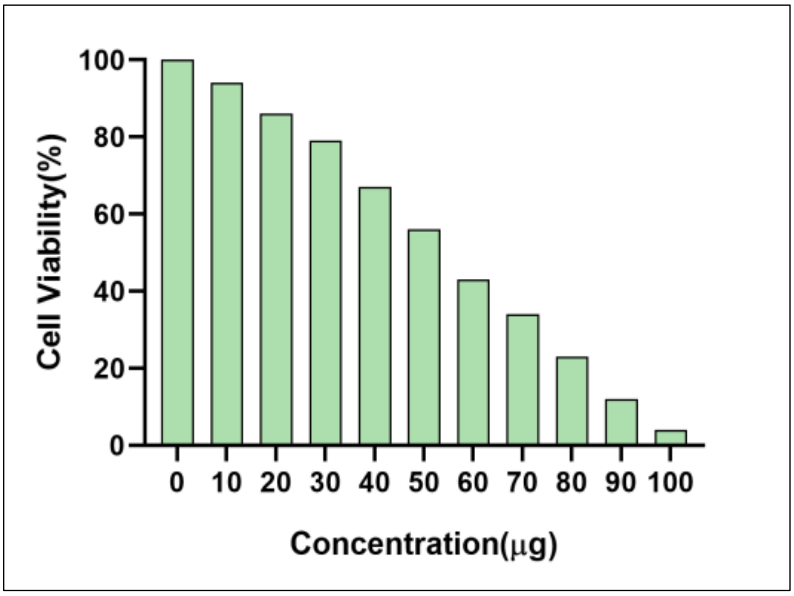


Figure 15. Cell viability vs concentration graph

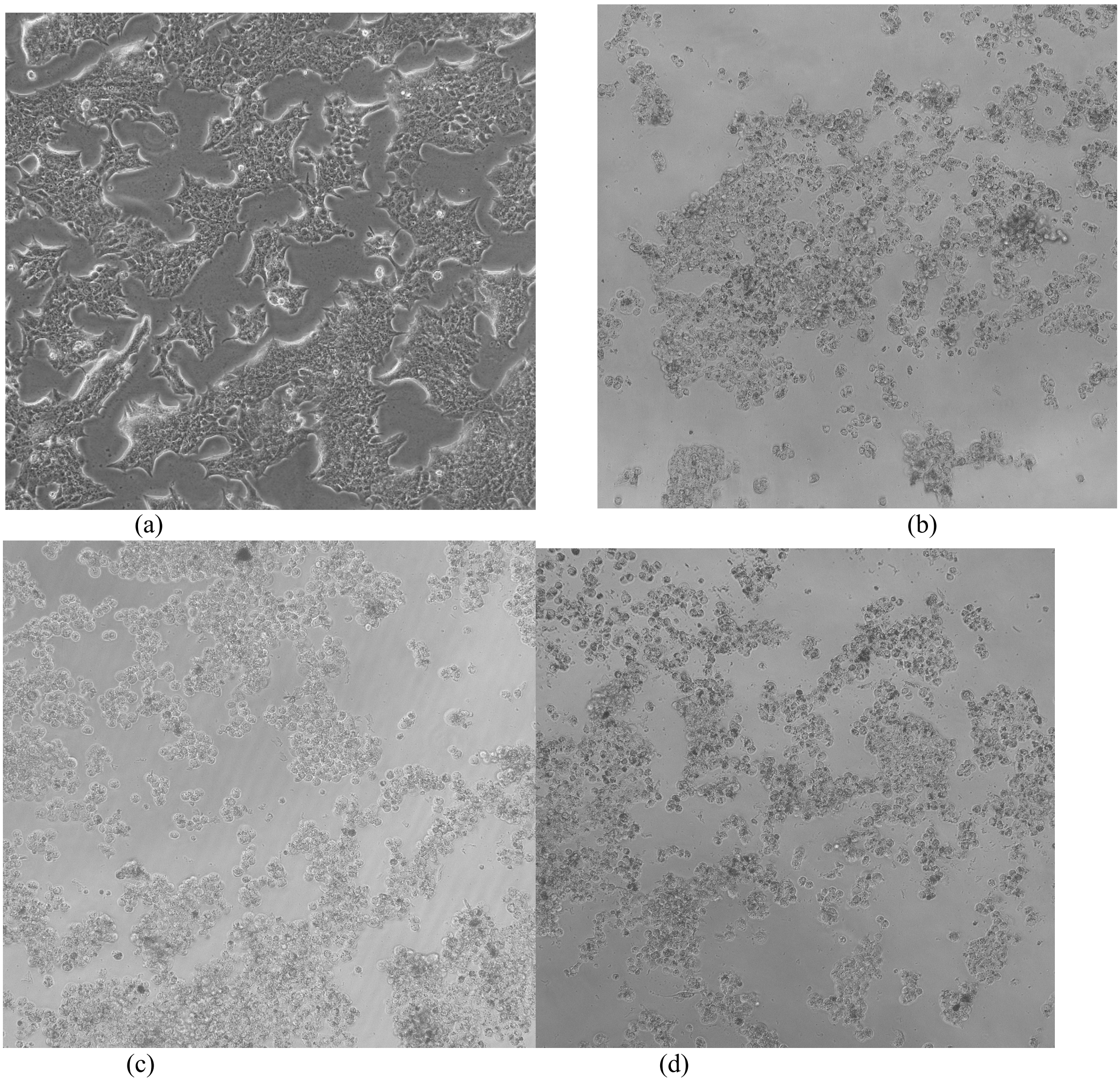


Figure 16. MCF-7 cell line a-control, b-30, c-50, d-100µg/ml

CONCLUSION

In this work, copper sulphate pentahydrate and magnesium sulphate heptahydrate were used as starting materials to synthesise copper oxide and magnesium oxide nanoparticles utilising the sol-gel method. One of the reducing and capping agents is sodium hydroxide. In a muffle furnace, calcination is used to turn copper hydroxide and magnesium hydroxide into copper oxide and magnesium oxide, respectively, at 400 °C. The synthesised nanoparticles are about 80 nm in size on average. The nanoparticles' zeta potential value fell between -11 and -13 mV. This demonstrates how the hydrophobicity of the nanoparticle contributes to the stability of the dispersion. The functional group of the nanoparticle was determined via FTIR analysis.

The spread plate method was used to evaluate the nanoparticle's antibacterial activity. Maximum activity is shown by copper oxide nanoparticles, but no antibacterial activity was seen in magnesium oxide nanoparticles because they were unable to disperse in the well. To examine the nanoparticle's surface morphology, SEM examination is carried out. The MCF-7 cell was used to investigate the metal nanocomposite's anticancer properties. The MCF-7 cell line demonstrated superior cytotoxicity activity towards the nanocomposite. Next, the pills' ability to dissolve was examined at both blood and gut pH levels.

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