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Antibacterial Activity and Cytotoxicity of Spinel Copper Ferrite Nanoparticles Synthesized by using Sol Gel Technique and Lemon Juice as Substrate

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ABSTRACT

The objective of the present study was to prepare $CuFe_2O_4$ ferrite nanoparticles using the sol-gel combustion method, employing lemon juice as a surfactant and energy agent. This method is located within the green chemistry, representing an environmentally friendly and less expensive approach compared to other methods. The nanoparticles were subsequently evaluated as antibacterial agents against different pathogenic bacteria. Before the antibacterial assays, a cytotoxicity test was conducted to evaluate their safety when applied to organisms. The structural, morphological, elemental composition, and magnetic properties of the samples were analyzed using Fourier-Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), Field Emission-Scanning Electron Microscopy (FE-SEM), and Energy Dispersive X-Ray Detection (EDX). The X-ray diffraction patterns confirmed both the phase purity and the particle size to be 24.27 nm. The results demonstrated that the $CuFe_2O_4$ nanoparticles exhibited substantial antibacterial activity against both Gram-negative bacteria (*Sphingomonas paucimobilis*) and Gram-positive bacteria (*Staphylococcus lentus* and *Bacillus subtilis*). The antibacterial efficacy was more pronounced against Gram-negative bacteria, with inhibition diameter 5.46mm and 10.64mm at concentrations of 5000 ppm and 10000 ppm, respectively. When making a comparison, the effectiveness against Gram-positive bacteria displayed a slight reduction. Inhibition zones measured 2.76 mm and 8.33 mm for *Staphylococcus lentus*, while they were 3.58 mm and 5.35 mm for *Bacillus subtilis*. These measurements were observed at nanoparticle concentrations of 5000 ppm and 10000 ppm, respectively. Furthermore, the study confirmed the safety of the $CuFe_2O_4$ nanoparticles by assessing their toxicity on human red blood cell at different concentrations (50, 100,250,500,1000,5000, and 10000 ppm).

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INTRODUCTION

Over time and with the advancement of pharmaceutical antibiotics, the world witnessed the emergence of bacterial strains exhibiting both virulence and resistance to diverse antibiotics, even in cases where they previously lacked such traits.(Terreni et al., 2021). This circumstance calls for the development of innovative strategies to generate and improve antibacterial agents, or to reinvigorate the advancement of antibiotics that have already been established and proven successful in the market. (Miethke et al., 2021), (Nas et al., 2018). Therefore the best and environmentally strategy involves the use of metallic and metal-oxide nanoparticles (1–100 nm), as zinc, silver, and copper, which display antibacterial machines in their bulk form, other resources, such as iron oxide, are not antibacterial in their bulk form but may show antibacterial properties in nanoparticulate form is begin used(Mendes et al., 2022).The mechanisms by

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which these metals nanoparticles act as antibacterial agents not fully understood,however, there are some explanations for this mechanism, which are related to the physical structure of nanoparticles(destruction of bacterial membranes by nanoparticles), and the other relates to stimulating the release of antibacterial metal ions from their surfaces(Nas et al., 2018).

The twenty-first century can be considered the golden decade in the field of nano-applications in various scientific fields, whether applied or biological, and their importance is increasing day by day(Ansari et al., 2018). Nano-metal oxides are often prepared chemically or physically. The physical methods includes: energy ball milling and ultrasonic shot peening(Li&Zhang, 2006),while the chemical methods, includes:pulse electro-deposition(Li & Zhang, 2006) reduction of chemical salts of metals (Tan et al., 2003).Chemical and physical methods usually involve many drawbacks, such as: high economic cost, long time for production, as well as some times include toxic substances in the synthesis route. That is why science has turned to the biological production of nanoparticles, because it is safe and environmentally friendly (Pandit et al., 2022).Working within green chemistry involves the use of many organisms to produce metals nanoparticle; plants, bacteria and fungi, however, previous studies confirmed that plant extracts are highly effective in producing metallic nanoparticles, as they are characterized by the following: the abundance of their types, their low cost and ease of handling, as well as they work at low temperatures and do not require the use of harmful substances during the reaction(Jadoun et al., 2021; Ali et al., 2021; Angeline et al. 2019). Ferrite nanoparticles have great interest compared to their counterparts of metal and metals nanoparticles, due to their superior nano-properties and the high ratio of surface to volume (Bilal et al., 2022). Ferrite can takes various shapes; garnet, hexaferrite or orthoferrite and spinel(Ansari et al., 2018). Among the different forms of ferrite, the greatest interest has emerged in the spiral one, for the following reasons: their renowned magnetic, catalytic, high adsorption, optical , electronic properties and easy to form with control on the size of their nanoparticles (Singh & Thirupathi, 2017).Various methods can be used to prepare ferrite nanoparticles, which for example and are not limited to: combustion (Kadyrzhanov et al., 2019),sol–gel(Ashour et al., 2018), solvothermal (Zhang et al., 2016). and biogenic method using bacteria *Geobacter sulfurreducens* (Céspedes et al., 2014). Although there is a lot of research on the antibacterial activity of many metals and metal oxide nanoparticles, such as, silver (Ali et al., 2018),gold (Payne et al., 2016), ZnO , Al_2O_3 , Fe_3O_4 , and magnetic iron oxide α-Fe_2O_3(Ansari et al., 2018), however, the available information about the antibacterial properties of nano-ferrite needs further updating(Ansari et al., 2018). Recently different studies investigated the antibacterial activity of different metals nanoparticles: Harikumar (2016)studied the antibacterial activity of copper nanoparticles and copper nanocomposites against *Escherichia Coli* bacteria, Ansari et al. (2018); studied the antibacterial activity of spinel chromium-substituted copper ferrite nanoparticles for biomedical application, Angeline et al. (2019) studied the synthesis and characterization copper oxide nanoparticles, and their antibacterial activity, Ashour et al. (2018), investigated the antimicrobial activity of metal-substituted cobalt ferrite nanoparticles. Vergis et al.(2018) evaluated the antimicrobial activity and cytotoxic effect on MCF-7 Cell Line of combustion derived $CuFe_2O_4$ nanomaterial using aloe-vera extract.

For the importance of the spinel ferrite in the biological application particularly as the antibacterial agent, present study focuses on the prepare the spinel ferrite of copper using the lemon juice as row material and tested their antibacterial activity against different pathogenic bacteria.

MATERIALS AND METHODS

Combination of $CuFe_2O_4$ nanoparticles

The copper oxide nanocomposite was prepared as follow:

14.54g of $Fe(NO_3)_3.9H_2O$ (M.W 404) was mixed with 8.669g of $Cu(NO_3)_2.6H_2O$ (M.W 241.60) the mixture then dissolved in the extract of lemon juice(45mL), to maintain neutral medium, pure 99% ammonium solution (NH_4OH) was added and continuous moving using a magnetic stirrer for 30 min at 80°C, till the entire solution turned into a gel. Dried the gel using an oven at 80°C to obtain a constant weight and crushed it to get a soft powder. Finally, the powder calcination using a furnace device at 600 °C for 5 hours.

Characterization techniques

For characterization of nanoparticles, many advanced techniques were used: including FTIR spectra, X-ray diffraction (XRD), scanning electron microscopy with energy dispersive X-ray spectroscopy (FESEM -EDX).

Fourier-transform infrared spectroscopy FT-IR

For functional group identification that associated with the $CuFe_2O_4$ NPs synthesis process, FT-IR technique was used. The infrared spectrum of the sample was obtained in the wavelength range from 400-4000 cm^{-1} using Perkin Elmer FTIR spectrophotometer (SHIMADZU 6100, Japan).

X-ray diffraction (XRD)

The X-ray diffraction measurement is nondestructive analytical technique gives information about the crystal structure and properties of synthetic materials and this was achieved by PANalytical device

. The practical application of the Debye-Scherrer equation was used to measure the size of $CuFe_2O_4$ nanoparticles: The Debye-Scherrer equation: $\tau_{hkl} = (K*\lambda)/(\beta_{hkl}*cos(\theta_{hkl})$ (1)

where τ represents the perpendicularity of the particle's size to the natural line of (hkl) plane, β_{hkl}

It is full width at the middle of maximum, θ_{hkl} is the Bragg angle of (hkl) peak, K is constant equals to 0.9 and λ is the wavelength of the X-ray (Vinila et al., 2014).

Field Emission Scanning Electron Microscopy (FESEM)

The Field *Emission Scanning Electron Microscopy* device was used for getting information about the morphology of $CuFe_2O_4$ NPs.

Energy Dispersive X-Ray Detection (EDX)

This technique used in order to know the type of chemical elements present in the sample, even if their percentage is very small, as the principle of work depends on the exchange between the electron beam emitted from the device and the sample material.

Cytotoxicity Assay

The cytotoxicity assay was accomplish according to(Nair et al., 1989) and as the following :-

The human blood solution was prepared by mixing 1 ml from fresh human blood with 20 ml of normal saline. Different concentrations from prepared nanoparticle was made using DMSO as solving agent. (50, 100,250,500,1000,5000, and 10000 ppm). The prepared blood solution was distributed into separate tubes, each containing 2ml of blood solution, and 100µl from each concentration was added, and left them on room temperature. The turbidity of solution was taken in 10,30, and 60min.The toxicity of the nanocompound was indicated according to the concentration that showed a clear solution, as an indication to non-lysis of the red blood cells. The DMSO was used and control compound against the prepared studied nanoparticles concentrations.

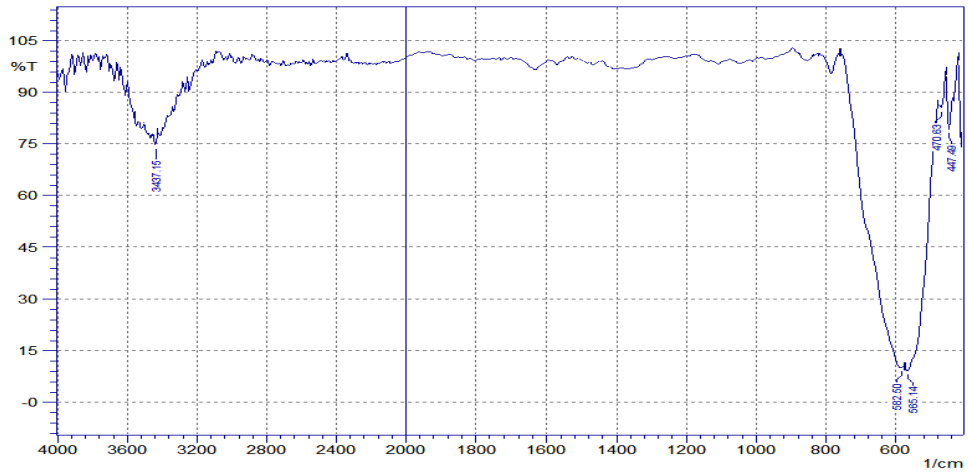

Fig. 1. FT-IR spectra of $CuFe_2O_4$

Antibacterial activity

Different concentrations of $CuFe_2O_4$ NPs were prepared (2000, 5000, 10000 ppm), using DMSO as solvent agent. The antibacterial activity of $CuFe_2O_4$ NPs was confirmed using the disc diffusion method against *Staphylococcus lentus*, *Sphingomonas paucimobilis* and *Bacillus subtilis*; where bacteria was cultured on the surface of Mueller Hinton agar plate (Himedia). Disk with the diameter(15mm)was saturated with the suspension of $CuFe_2O_4$ NPs with different concentrations putted on the surface of plate and incubated for 24 hr. at 37°C(Elango et al., 2017).The DMSO was used as a control agent.

RESULTS AND DISCUSSION

FT-IR spectroscopy

Fig.1 showed the spectra of the calcified sample at 600°C, which indicated presence many absorption peaks in the range 400cm^{-1} – 4000 cm^{-1} The broad absorption band centered at 3437.15 $cm^{-1}$is assigned to O–H stretching vibrations and the band at 1630 cm^{-1} is attributable to H–O–H bending vibration mode. These indicate the presence of traces of moisture from the air in the sample(Anandan & Rajendran, 2011). The located in the 400–600cm^{-1} region by (Cu-O) and (Fe-O) is stretching vibration(Kareem, 2018). FTIR plays a crucial role in determining the chemical composition, phase, surface properties, and reactivity of nanoparticles, aiding in their characterization and the development of tailored nanomaterials(Eid, 2021)

X-ray diffraction (XRD)

Fig.2 showed the result of identification $CuFe_2O_4$ using X-ray diffraction technique. for comparison the present result, the reference standard data(JCPDS file No: 34-0425) has been used (Lv et al., 2008). The comparison data indicated that the prepared sample included within the spinel cubic. The practical application of the Debye-Scherrer equation showed that the size of $CuFe_2O_4$ nanoparticles was 24.27nm

Metal components and surface morphology

To determine the surface morphology of the prepared nanoparticles, the Field Emission Scanning Electron Microscopy (FESEM) was utilize. Fig.3 reveled the results of the microscopic survey, which displayed that the prepared particles showed a polyhedral shape with little porosity and few agglomerations. It is also indicated that the prepared compound has crystal sizes within

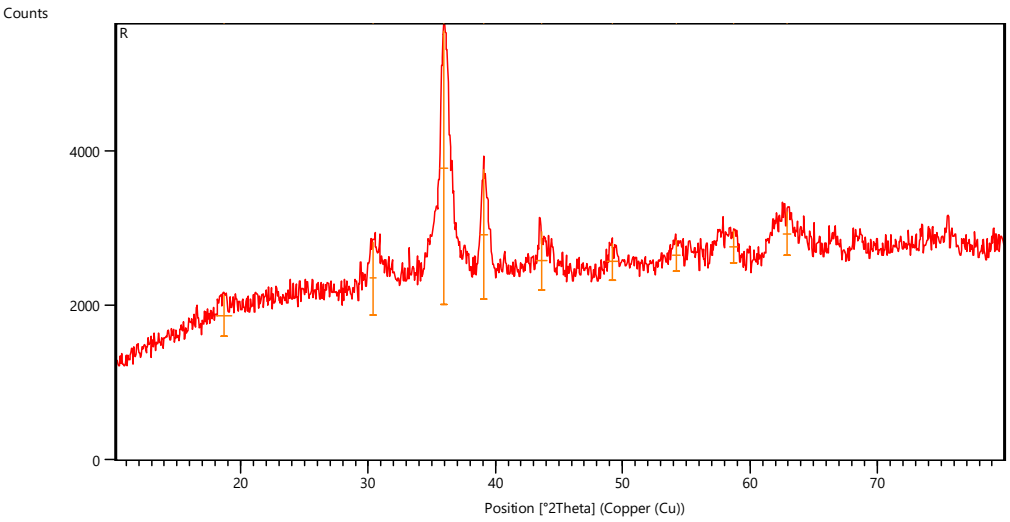

Fig. 2. The XRD spectrum of $CuFe_2O_4$

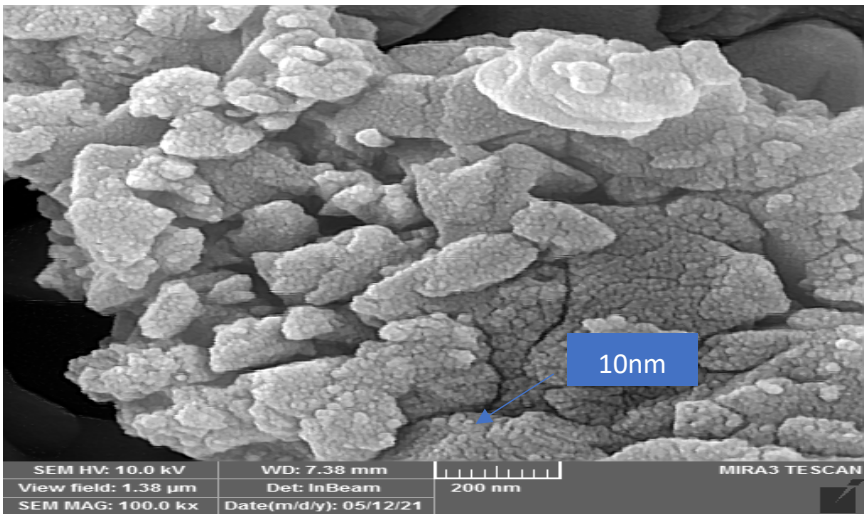

Fig. 3. The morphology of $CuFe_2O_4$ under FESEM microscope

the nanoscale range, as the (Image-J) program was adopted, which was used to identify the grains and calculate their sizes through image analysis. After conducting the process of determining the shape of the granules through the summary obtained from the program, the average particle size was calculated, as the average particle size was (14.32nm). On the other hand, to confirm the structural purity, the EDX analysis of different areas was adopted for each sample. The EDX technic also provided conceive about the sample components and elements'mapping. Figure 4 and Table 1 show the results of examining the prepared nanoparticle by EDX, which showed the presence of copper, oxygen and iron in different proportions with a small percentage of impurities, these which is consistent with theoretical calculations.

Cellular Cytotoxicity

Table 2 and Fig. 5 showed the results of the cytotoxicity, results indicated that the NPs don't has any toxicity against the human blood solution, where all concentrations used don't showed any turbidity as a result of non lysing of the red blood cell by the prepared nanocopound, and according to the result can advance their application in both medical and pharmaceutical.

Table 1. The percentage of metals measured by EDX analysis

metals	Weight %
Fe	45.13
Cu	27.35
O	27.52
Total %	100

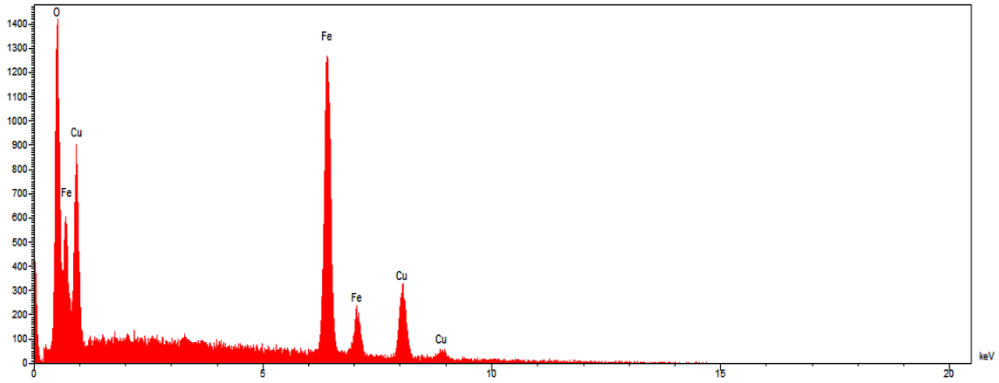

Fig. 4. Elements percentage calculated by EDX analysis

Table 2. The cytotoxicity of $CuFe_2O_4$ nanoparticle against human blood solution

Nanoparticle	**Concentrations(ppm)**						
$CuFe_2O_4$NPs	50	100	250	500	1000	5000	10000
toxicity	NT	NT	NT	NT	NT	NT	NT

NT: non toxics

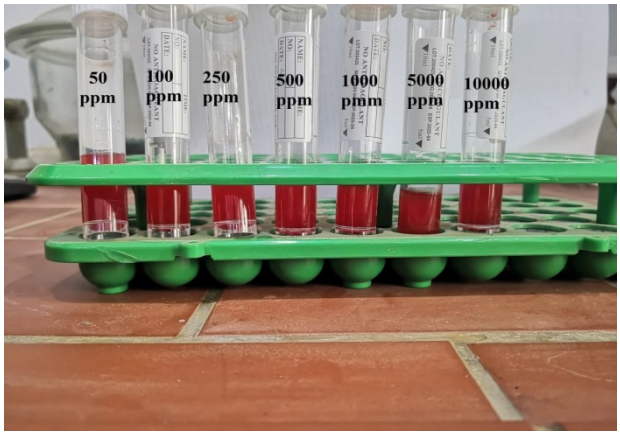

Fig. 5. Cytotoxicity of the prepared $CuFe_2O_4$ nanoparticle against human blood

Antibacterial activity

To evaluated the antibacterial activity of $CuFe_2O_4$ NPs, against pathogenic bacteria, it was tested against three types of bacteria, two of which were Gram-positive (*Staphylococcus lentus, and Bacillus subtilis)*, and the last one was negative *(Sphingomonas paucimobilis).* The both concentrations from $CuFe_2O_4$NPs compound used (5000 and 10000 ppm), showed good anti-bacterial activity, but the most effective was the highest concentration (10000 ppm), as indicated by the measurements of the diameter of inhibition zone (table 3), and (Figure 6). Different

Table 3. The diameter of inhibition zone(mm), resulted from antibacterial activity of $CuFe_2O_4$ NPs in the different concentration(ppm)

Bacteria	Nanoparticle concentration(ppm)	Inhibition zone (mm)
Sphingomonas paucimobilis	5000	5.46
Staphylococcus lentus	5000	2.76
Bacillus subtilis	5000	3.58
Sphingomonas paucimobilis	10000	10.64
Staphylococcus lentus	10000	8.33
Bacillus subtilis	10000	5.35

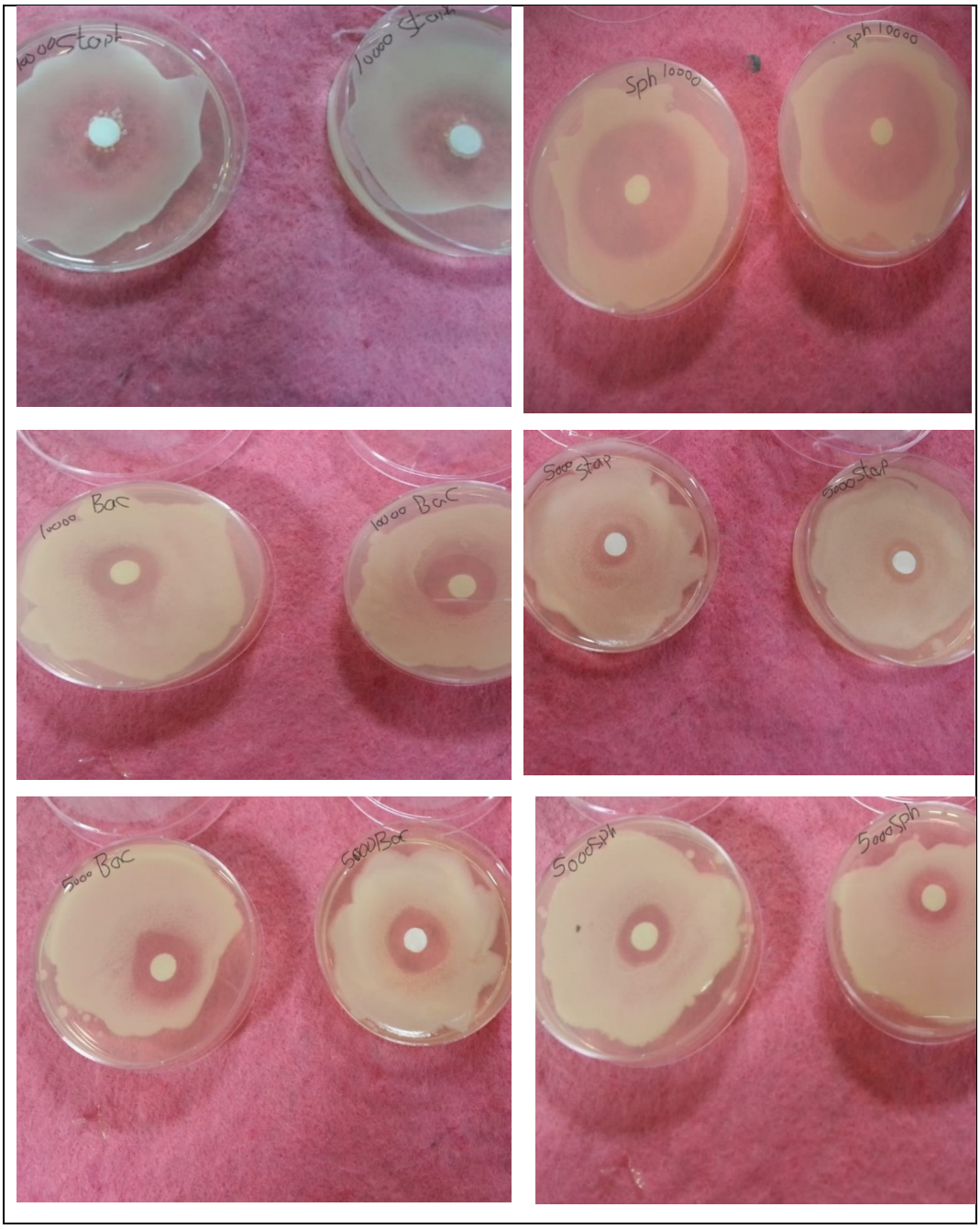

Fig. 6. the antibacterial activity of $CuFe_2O_4$ NPs in the different concentration(ppm) against different types of bacteria

approach has been usually projected by the scientists for the activity of nanoparticles against bacteria:- The small size of nanoparticle ,which can penetrate the bacterial cell walls and their ability to bind with the nucleic acids, this is which can considered the most causes of $CuFe_2O_4$ NPs antibacterial effect (Angeline et al. 2019),(Elango et al., 2017). As well as their causing of oxidation and ROS generation, which lead to death of bacterial cell(Hsueh et al., 2017; Ali et al., 2021). Results of the diameter size of the inhibition zone indicated, that the nanoparticle compound showed stronger inhibition against negative bacteria(*Sphingomonas paucimobilis)* (5.46 and 10.64 mm) in the concentrations (5000 and 10000 ppm) respectively, compared to the positive one (2.76 and 8.33 mm) for *Staphelococcus lentus and (*3.58 and 5.35mm*) for Bacillus subtilis* in the concentration(5000 and 10000 ppm) respectively.(Emami-Karvani, 2012; Ansari et al., 2018) indicated more inhibition activity of ZnO nanoparticles against Gram negative bacteria than the positive one. The explanation of the above results depending on the difference in the structure of the cell wall for both types of bacteria as well as the physiological and metabolic differences of the cell.

CONCLUSION

The primary focus of this study was to employ an environmentally friendly and sustainable method for synthesizing $CuFe_2O_4$ nanoparticles using lemon juice as a stabilizing agent. Highly developed techniques like FTIR and XRD were employed to validate the formation of $CuFe_2O_4$ nanoparticles and analyze their crystal structure. Visual confirmation of particle formation at the nanoscale was obtained through FSEM imaging. The antibacterial investigation conducted using these environmentally conscious synthesized $CuFe_2O_4$ nanoparticles clearly demonstrated their significant antibacterial efficacy. The copper nanoparticles exhibited inhibitory effects on the growth of both Gram-negative and Gram-positive bacteria, which was evident from the substantial diameter of the inhibition zones. This indicates the potential of copper nanoparticles to act as effective antibacterial agents. Furthermore, the assessment of cytotoxicity revealed encouraging results, as there were no observed toxic effects on human blood solutions across all concentrations tested.

GRANTSUPPORT DETAILS

The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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