

ANTI- INFLAMMATORY AND ANTI- BACTERIAL ACTIVITY OF COPPER NANOPARTICLES SYNTHESIZED FROM *MYRTUS COMMUNIS* LEAVES EXTRACT

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ABSTRACT

A variety of organisms, including plants and bacteria, fungi, seaweeds, and microalgae, are involved in the biological synthesis of nanoparticles. Copper nanoparticles (CuNPs) can be synthesized using plant extracts, which is considered to be one of the safest methods in green chemistry. Copper-NPs were synthesized from the leaves of *M. communis*, which were extracted with water. The first indication that CuNPs have been synthesized is the change in color of the solution from light yellow to dark brown. Several different techniques were used to characterize CuNPs. The Surface Plasmon Resonance (SPR) of the nanoparticles in the range of 300 to 700 nm was investigated using ultraviolet-visible absorption spectroscopy (UV-Vis), and the Fourier Transforming Infrared analysis (FT-IR) was used to identify functional groups in biomolecules that act as a reducing and capping agent for NPs. The X-ray diffraction (XRD) analysis of CuNPs revealed that they are crystalline. In this study, the size and surface properties of biosynthesized nanoparticles were determined using atomic force microscopy (AFM). Copper-NPs had an average size of 53.55 nm, according to the results. In this study, the antibacterial and anti-inflammatory activity of CuNPs and extract were investigated. The antibacterial activity of CuNPs and *M. communis* extract was evaluated against Gram-negative bacteria (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus*, and *Lactobacillus salivarinis*). Zone inhibition of up to 25 mm was observed in *Staphylococcus aureus* when the extract concentration was 100000 µg/mL. At various concentrations, the anti-inflammatory activity of both the extract and the CuNPs was assessed in vitro using the assays (albumin denaturation assay, membrane stabilization assay, and proteinase inhibitory activity). According to the findings, CuNPs demonstrated a significant anti-inflammatory activity when compared to a standard drug.

Keywords: Copper nanoparticles, Plant extracts, Antibacterial, Anti-inflammatory activity

الجبوري وآخرون

مجلة العلوم الزراعية العراقية - 2022: 53(3): 698-711

الفعالية المضادة للالتهاب والمضادة للبكتيريا لدقائق النحاس النانوية المحضرة من مستخلص اوراق نبات الياس

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باحث

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المستخلص

هناك كائنات حية مختلفة بما في ذلك النباتات والبكتيريا والفطريات والأعشاب البحرية والطحالب الدقيقة ، تساهم في التخليق الحيوي للدقائق النانوية. ان جسيمات النحاس النانوية (CuNPs) يمكن تخليقها باستخدام المستخلصات النباتية ، والتي تعتبر من أكثر الطرق أماناً في الكيمياء الخضراء. تم تصنيع دقائق النحاس النانوي المستخلص المائي لاوراق نبات الياس. أول مؤشر على تخليق النحاس النانوي هو تغير لون المحلول من الأصفر الفاتح إلى البني الداكن. تم استخدام العديد من التقنيات لتصنيف دقائق النحاس النانوية. تم التحري عن رنين البلازمون السطحي (SPR) للجسيمات النانوية في النطاق من 300 إلى 700 نانومتر باستخدام مطيافية الامتصاص المرئي - فوق البنفسجي (UV-Vis) ، وتم استخدام اطياف الأشعة تحت الحمراء (FT-IR) لتحديد المجموعات الوظيفية في الجزيئات الحيوية التي تعمل كعامل مختزل وسد لـ NPs. كشف تحليل حيود الأشعة السينية (XRD) الشكل البلوري لـ CuNPs. في هذه الدراسة ، تم تحديد حجم وخصائص سطح الدقائق النانوية المُصنَّعة حيويًا باستخدام مجهر القوة الذرية (AFM) إذ بلغ متوسط حجم النحاس النانوي 53.55 نانومتر. في هذه الدراسة تم فحص النشاط المضاد للبكتيريا والمضاد للالتهابات لكل من CuNPs والمستخلص. تم تقييم النشاط المضاد للبكتيريا لكل من CuNPs ومستخلص نبات الياس ضد البكتيريا السالبة لصبغة كرام (*Klebsiella pneumoniae* و *Pseudomonas aeruginosa*) والبكتيريا الموجبة لصبغة كرام (*Staphylococcus aureus* و *Lactobacillus salivarinis*). إذ لوحظ ان قطر التثبيط كان 25 مم بالنسبة لبكتريا *Staphylococcus aureus* عندما كان تركيز المستخلص 100000 ميكروغرام / مل. تم تقييم النشاط المضاد للالتهابات لكل من المستخلص و CuNPs في المختبر بتركيز مختلفة وباستخدام فحوصات (فحص مسخ الألبومين ، وفحص الاستقرار للغشاء ، وفحص الفعالي المثبطة للبروتينيز). وفقًا للنتائج ، أظهرت CuNPs نشاطاً مهماً مضاداً للالتهابات عند مقارنتها بالعقار القياسي.

الكلمات المفتاحية: جزيئات النحاس النانوية ، مستخلصات نباتية ، نشاط مضاد للبكتيريا ، مضاد للالتهابات

Received:10/1/2021, Accepted:15/4/2021

INTRODUCTION

Nanotechnology is primarily concerned with the synthesis and application of nanomaterials, which can be accomplished through the use of various systems. The large surface area to volume ratio of nanoscale materials causes them to have a wide range of chemical, physical, optical, magnetic, and electrical properties at the nanoscale level. One of the most important aspects of nanotechnology is the synthesis of nanoparticles (NPs) with one dimension less than 100 nm (6). Nanoparticle preparation requires precise control over the shape and size of nanoparticles. Copper is widely used because of its unique physical and chemical properties which make it an excellent material for nanomaterial preparation (25). Among the many remarkable properties of copper nanoparticles (CuNPs) are their high surface-to-volume ratio, high yield strength, ductility, hardness, flexibility, and rigidity. Copper nanoparticles (CuNPs) are used in a variety of applications. When used in a variety of applications, CuNPs have been shown to have anti-inflammatory, antibacterial, antioxidant, and antifungal properties, as well as cytotoxicity and anticancer properties (10). To achieve the green synthesis of CuNPs, many plant parts or whole plants have been used. This is due to the presence of a large number of bioactive compounds in plants that act as reducing and capping agents. As a result, plant extracts are effective for this purpose (2). There are many different types of phytochemicals present in plants, such as, (alkaloids, phenolic acid, flavonoids, terpenoids, and coumarins) that have different bioactivities. These include antioxidant, anti-inflammatory, and antibiotic activities. Plants also contain essential oils and fixed oils that have various bioactivities. The demonstration of antimicrobial activity against both Gram-negative and Gram-positive bacteria indicates that the plant may be a source of bioactive substances with a broad spectrum of activity which could be a source of bioactive substances (23). This plant, *Myrtus communis* Linn., also known as common Myrtle, is a member of the Myrtaceae family that is widely used in traditional herbal medicine as an antiseptic in rural areas, where it is consumed as a decoction (5); (29). Inflammation is a

component of the complex biological response of vascular tissues to harmful stimuli, and it is frequently associated with pain. It involves a variety of biological events, including an increase in vascular permeability, an increase in protein denaturation, and membrane alteration all of which contribute to pain (8). The present study aimed to synthesize and characterize CuNPs and then, evaluate their anti-inflammatory and antibacterial activity .

MATERIALS AND METHODS Plant Collection

The green, fresh leaves of *M. communis* were obtained from the garden of the Department of Chemistry, College of Sciences, University of Kerbala. First, the leaves were washed out of contamination with tap water and then with distilled water. After that, the plant leaves were dried at room temperature then crashed into small slices, and then kept in a dry, dark place for further use .

Preparation of *M. communis* Leaves Extract

For this experiment, twenty grams of small pieces of dry *M. communis* leaves were added to 200 mL of deionized water and allowed to boil for 10 minutes on a shaker water bath at 50 °C. Following that, the crude extract was filtered through filter paper Whatman No.1 to remove any impurities. The filtrate was used to create (CuNPs), which was then used in other applications.

Qualitative Phytochemical Analysis of *M. communis* Leaves Extract

The crude solution of *M. communis* leaves extract was submitted to phytochemical analysis. These phytochemicals were included alkaloids, coumarins, flavonoids, resins, saponins, sugars, tannins, phenolic compounds, terpenes, and steroids (9).

Synthesis of CuNPs

An aqueous solution of (1mM) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was prepared for the synthesis of (CuNPs), 50 mL of *M. communis* leaves extract (the pH of the extract was adjusted to pH 10 by the addition of 0.1 mM NaOH) was mixed with 50 mL of copper sulfate and stirred continuously for 30 minutes at room temperature (27). The color was gradually changed from light yellow to dark brown, indicating copper nanoparticles' formation. The mixture was centrifuged at 8000 xg for 15 minutes to get the pellets of CuNPs. The nanoparticles were washed three

times by deionized water and dried using a desiccator containing calcium carbonate .

Characterization of CuNPs

Ultraviolet-Visible Spectroscopy

To characterize the CuNPs, an ultraviolet-visible spectrometer (UV-1800/ Kyoto, Japan) was used, which is widely used for the characterization of nanoparticles and the detection of the Surface Plasmon Resonance property (SPR) of CuNPs, among other applications. The maximum absorption was measured by scanning the spectrum between 300 and 700 nm.

Fourier Transform-Infrared (FT-IR) Spectroscopy:

To better understand the interaction of nanoparticles and to identify the potential biomolecules responsible for capping and efficient stabilization of metal nanoparticles synthesized using *M. communis* leaves extract, FT-IR measurements were performed. The pellets of CuNPs were measured with the KBr disk in the wavenumber range of 400 to 4000 cm^{-1} using an FT-IR spectrophotometer (Shimadzu (8400S)/Japan) in the wavenumber range of 400 to 4000 cm^{-1} .

X-ray diffraction Analysis

The x-ray diffraction (XRD) (Philips Xpert/Holland XRD) was used to determine the average crystal size, crystallinity, strain, and crystal defect. It was decided to use Cu-k radiation ($k=1.50456$) in the range of (10° to 80°). The crystal size of all prepared samples was calculated using Scherer's equation, which was written as follows:

$$D = 0.9 \lambda / \beta \cos \theta$$

D is the average size of crystallite, λ is the X-ray wavelength, θ is the angle of Bragg in radians, and β is the full width of the peak in radians at half its maximum .

Atomic Force Microscopy (AFM)

The surface morphology, aggregation, shape, size, and distance of biosynthesized CuNPs were revealed through atomic force microscopy (AFM Scanning Probe Microscope). The highest measurement was obtained using AFM image analysis software .

In vitro anti-inflammatory activity

Inhibition of albumin denaturation: Minor modifications were made to the Mizushima *et al* (22) method, which was then followed. It was necessary to prepare the reaction mixture

(0.5 mL) by adding 0.45 mL bovine serum albumin (1 percent aqueous solution) to varying concentrations (5, 10, 20, 25, 50, 100 $\mu\text{g}/\text{mL}$) of aqueous extract, CuNPs, and aspirin. Using a small amount of HCl, the pH of the reaction mixture was brought down to 6.3. After that, samples were incubated at 37°C for 20 minutes before being heated at 51°C for 20 minutes to complete the process. After cooling, the turbidity of the samples was determined spectrophotometrically at 660 nm using a spectrophotometric instrument. The following formula was used to calculate the percentage of protein denaturation that was prevented :

$$\text{inhibition\%} = \frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \times 100$$

where A control is the absorbance of solution without test sample, A sample is the absorbance of solution with sample extract or CuNPs or standard.

Protein inhibitory action

The experiment was carried out by the modified method developed by Gunathilake *et al* (15). The reaction mixture (2 mL) was containing 0.06 mg trypsin, 1 mL Tris HCl buffer (20 mM) (pH 7.4) and 1 mL test sample at different concentrations. The mixture was incubated at 37 degrees Celsius for 5 minutes before 1 mL of casein (0.8 percent by weight to volume) was added. For an additional 20 minutes, the mixture was incubated at room temperature. Two milliliters of 70% perchloric acid were added to conclude the reaction. The cloudy suspension was centrifuged, and the absorbance of the supernatant at 210 nm was measured in comparison to a buffer used as a blank. In this case, the percentage inhibition of proteinase activity was calculated using the above-mentioned formula.

Membrane Stabilization test

Preparation of Red Blood Cells (RBCs)

Suspension: It was necessary to collect fresh whole human blood (10 mL) and transfer it to the heparinized tubes. Afterward, the tubes were centrifuged for 10 minutes at 1800 xg, and they were washed three times with an equal volume of normal saline to remove any remaining debris. After measuring the volume of blood in the sample, it was reconstituted as a 10 percent volume-to-volume suspension with normal saline.

Heat-Induced Hemolytic Assay: To prepare the reaction mixture (2 mL), 1 mL of test sample solution and 1 mL of a suspension of 10 percent RBCs were mixed. In the control test tube, no test sample was added; instead, only saline was added to the tube. Aspirin was taken as a standard. Thirty minutes after being placed in the water bath, all of the centrifuge tubes containing the reaction mixture were removed. The tubes were cooled at the end of the incubation period by running water from a faucet. The reaction mixture was centrifuged at 2500 xg for 5 minutes, and the absorbance of the supernatants was measured at 560 nm. As previously stated, the percentage of membrane stabilization activity was calculated using the formula mentioned above (18).

Hemolytic Activity: This assay was carried out by the method Bouma (7) to determine the effect of CuNPs on hemolysis; therefore, the following blood specimens from healthy nonsmokers' donors were used in this study. As a general rule, 30 µL of CuNPs or extract solution was added to 0.02mL of blood at the following concentrations: 5, 10, 20, 25, 50, and 100 µg/mL. and mixed well for 5 seconds. A total of 20 mL of normal saline was added to avoid any unnecessary hemolysis. The mixture was centrifuged at 1800 xg for 10 minutes, resulting in a clear separation. After that, the mixture's optical density (O.D.) was measured at a wavelength of 540 nm. This test's primary goal is to determine the percentage of hemolysis caused by CuNPs compared to a control group with 100 percent hemolysis. Consequently, diluting blood with 100 times the amount of distilled water was performed to complete hemolysis 100 percent). The positive control, Triton X-100, was used, and after measuring the absorption, the hemolysis percentage was calculated using the following equation:

$$\text{Hemolysis\%} = (\text{AT}-\text{AS}) / (\text{A } 100\%-\text{AS}) \times 100$$

Where, AT is the absorbance of the test solution, AS is the absorbance of the normal saline, and A 100% is the absorbance of 100% hemolysis.

Antibacterial Assay: The antibacterial activity of *M. communis* leaves extract and CuNPs was determined using the agar well diffusion method (4). Serial dilution of each extract and CuNPs was prepared at various

concentrations (25000,50000,75000,100000 µg/mL). The antibacterial activity was assessed against Gram-negative bacteria (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus* and *Lactobacillus salivarius*). Muller-Hinton agar plates were inoculated with tested bacteria (10^5 - 10^6 CFU/mL). Agar wells of 10 mm diameter were made using a sterile cork borer. All wells were filled with 100 µL of each CuNPs and extracted and then incubated at 37°C for 24 hours. The inhibition zones diameter of each sample was measured in millimeters.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis of *M. communis* leaves extract: The chemical constituents of *M. communis* leave extract were investigated. These constituents were flavonoids, resins, saponins, sugar, tannins, phenolic compound, and terpenes (Table 1).

Table 1. Phytochemical tests of *M. communis*

Chemical constituents	Detection indicator	Results
Alkaloids	Orange spots	-
Flavonoids	Yellow color	+
Resins	Turbidity	+
Saponins	Foaming remaining for a long time	+
Sugars	Red precipitate	+
Tannins	White mucilage precipitate	+
Phenolic compound	Green color	+
Terpenes	Brown color	+
Steroids	Blue color	-

Synthesis of Cu nanoparticles

After mixing the solution of copper sulfate with aqueous leaves extract, the reaction mixture changes gradually from light yellow to dark brown color within 30 minutes. The new color was ascribed to the excitation of surface plasmon vibrations in the CuNPs, which indicates the formation of copper nanoparticles directly as shown in (Figure 1).

UV-Vis Spectroscopy Analysis

The absorption band of CuNPs has been detected in the range from 400 to 600 nm and the optimal absorption band was to be at 481 nm (Figure 2a). The intensity of the absorption increased with increasing incubation time and became constant after 24 hours, indicating the completion of the reaction (Figure 2b).

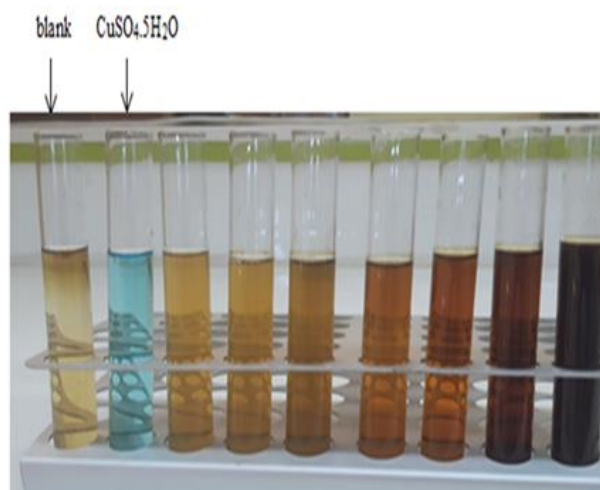


Figure. 1. Biosynthesis of (CuNPs) and the color change with time

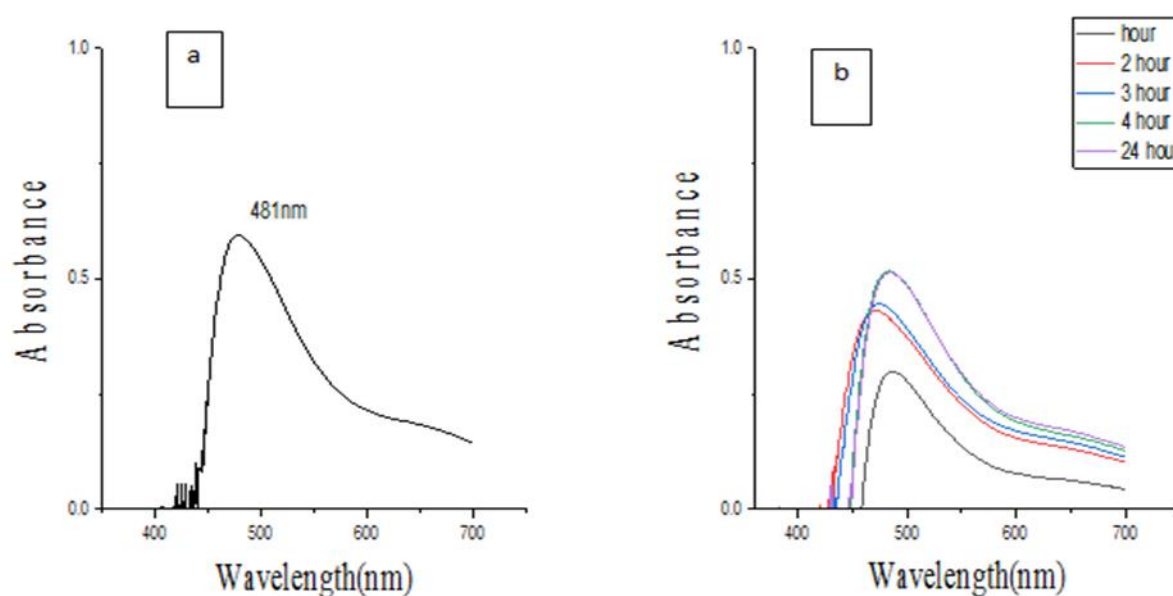


Figure. 2. UV-visible spectra of synthesized CuNPs. (a) CuNPs peak at 481 nm (b) UV-visible spectra of synthesized CuNPs over time

Fourier Transform-Infrared Spectroscopy (FT-IR): The analysis of FT-IR spectra provides information about the presence of biomolecules with a variety of functionalities in the underlying system. The (FT-IR) spectra of CuNPs and leaves extract are depicted in the diagram (Figure 3a and b). The comparison of the spectra of *M. communis* leaves extract and CuNPs reveals that there are only minor differences in the positions and magnitudes of the absorption bands between the two samples. In Figure 3a, the FT-IR spectrum of the leaves extract shows intense bands at 3335.03 cm^{-1} (O–H stretching vibrations), 2937.68 cm^{-1} (C–H) and CH_2 vibration of aliphatic hydrocarbons), 1726.35 cm^{-1} (C=O stretching vibration), 1616.40 cm^{-1} (C=C stretching vibrations), 1452.45 cm^{-1} (O–H bending

vibrations), 1367.58 cm^{-1} (C–O stretching of the ester group), 1240.27 cm^{-1} (C–O asymmetric stretching in cyclic polyphenolic compounds) and 1039.67 cm^{-1} (O–H deformation), whereas the FT-IR spectrum of synthesized CuNPs (Figure 3b) shows a broad peak in 3429.55 cm^{-1} due to O–H groups, also some signals emerged in 2922.25 , 1649.19 , 1554.68 and 1066.67 cm^{-1} are related to C–H asymmetric stretching C=O of aromatic rings, C=C stretching and C–OH bending, respectively. As a result, it appears more likely that many functional groups, such as alcohols, ketones, aldehydes, alkenes, and carboxylic acids, that are present as plant metabolites and reducing sugars of *M. communis* plants are responsible for the reduction of copper ions and stabilization of synthesized (CuNPs).

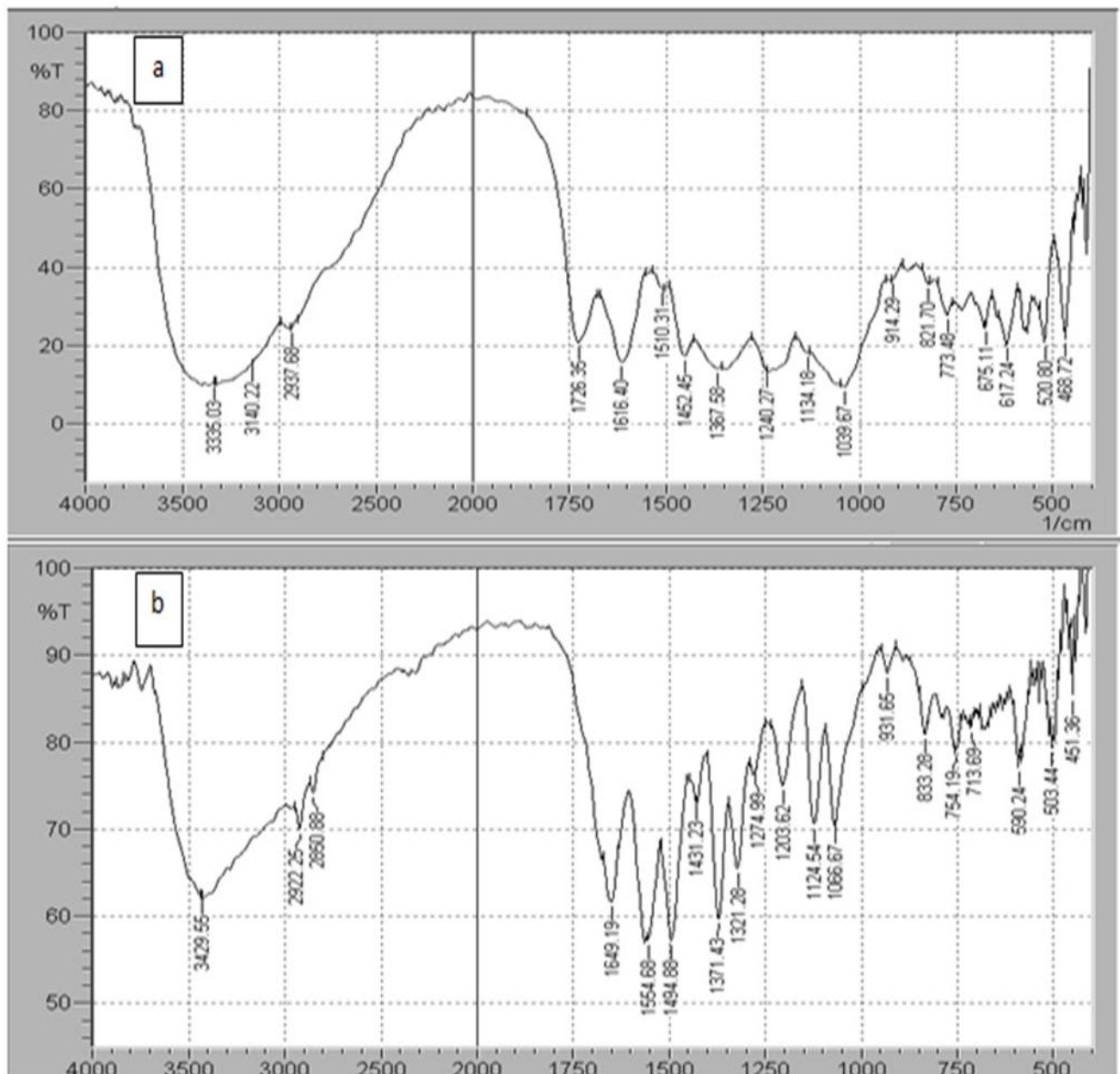


Figure 3. (a) FT-IR spectrum of *M. communis* leaves extract and (b) FT-IR spectrum of CuNPs

X-ray diffraction (XRD)

To determine the crystal structure and phase composition of the prepared sample, an XRD analysis was carried out. The XRD patterns of the CuNPs are depicted in Figure 4. Two-dimensional X-ray diffraction peaks at 43.43° (111) and 50.58° (200), as well as 74.23° (220), are well indexed to the JCPDS card numbers 96-901-3024, which represent the face-centered cubic structure of copper, and this confirmed that CuNPs were obtained as a single phase. Copper-NPs have an average crystal size of 39.16 nm, according to the research.

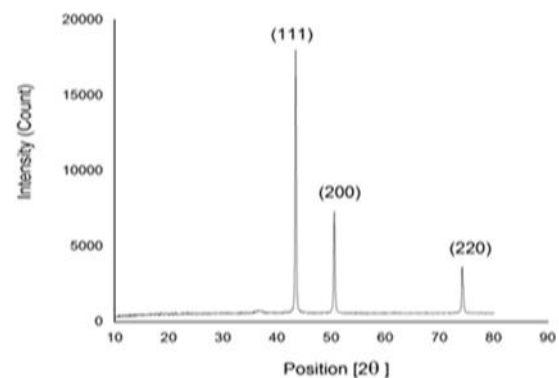


Figure 4. X-ray diffraction patterns for the synthesized CuNPs

Atomic Force Microscopy (AFM)

AFM analysis was used to determine the surface topology of the nanoparticles as well as the size of the nanoparticles. According to the experiment, the average particle size of the CuNPs measured in this study was 53.55 nm. The two-dimensional and three-dimensional images of all CuNPs revealed that they were all of uniform size and shape, as shown in the figure (Figures 5a and b).

In vitro Anti-inflammatory Activity

Inhibition of Albumin Denaturation: It is well documented that protein denaturation is a contributing factor to inflammation. Aspects of the investigation into the mechanism of anti-inflammatory activity included the ability of the extract and CuNPs to inhibit protein denaturation, which was carried out as part of the investigation. Copper-NPs, *M. communis* leaves extract, and aspirin as a standard anti-inflammatory drug were all effective in inhibiting heat-induced albumin denaturation at different concentrations, as shown in (Figure 6a), with the highest levels of inhibition (84.8%, 82.4 %, and 88%) achieved at 100 µg/mL for CuNPs, *M. communis* leaves extract, and aspirin as a standard anti-inflammatory drug, respectively .

Proteinase Inhibitory Activity

Proteinases have been implicated in the development of arthritic symptoms. Neutrophils are well-known for being a rich

source of proteinase, as evidenced by the fact that they contain numerous serine proteinases in their lysosomal granules. According to previous research, leukocyte proteinase is a key player in the development of tissue damage during inflammatory reactions, and proteinase inhibitors provide an extremely high level of protection against tissue damage during inflammatory reactions. CuNPs and *M. communis* extract demonstrated significant anti-proteinase activity at various concentrations, as demonstrated in Figure 1. (Figure 6b). CuNPs and the extract showed the greatest inhibition at 100g/mL (88.2 and 83.1 percent, respectively), whereas aspirin showed the greatest inhibition at 100g/mL (91.5 percent) .

Membrane stabilization test

The percentage of inhibition of heat-induced hemolysis of red blood cells at various CuNPs and extract concentrations is shown in (Figure 6c). The membrane stabilization of RBCs was investigated to determine the mechanism of anti-inflammatory action for each nanoparticle and extract. The effect may prevent neutrophils from releasing their lysosomal content at the site of inflammation. CuNPs showed the greatest inhibition (83.5 percent), followed by aqueous extract (76.6 percent), the standard drug, aspirin, showed the highest inhibition of 87.6 percent at 100 µg/mL .

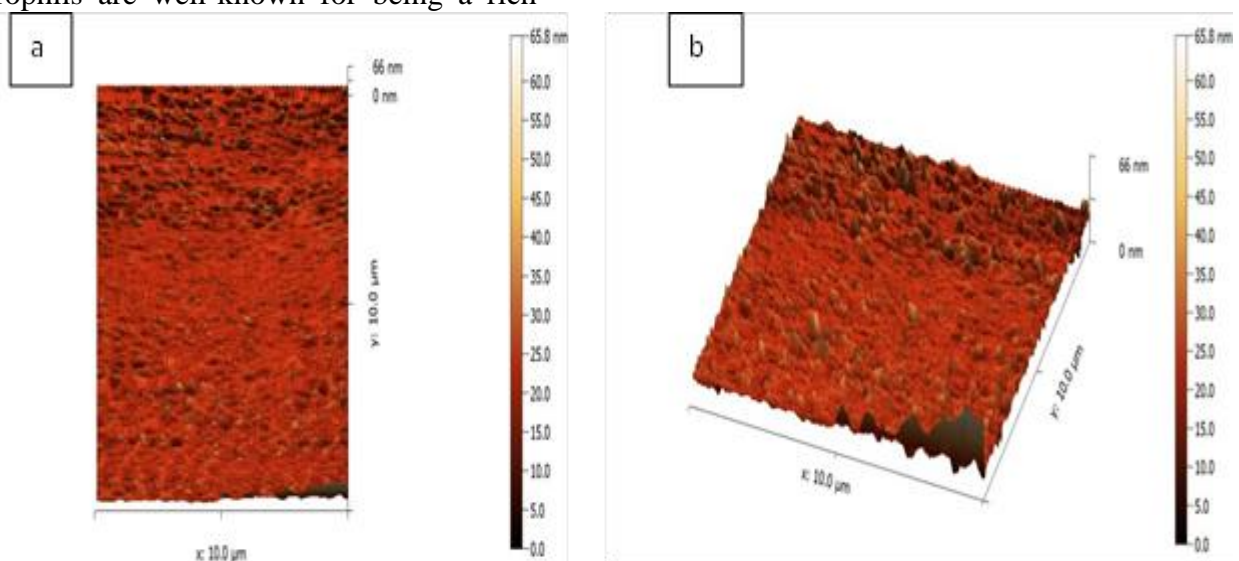


Figure 5. AFM images of CuNPs. (a) Two-dimensional image (2D) (b) three dimensional image (3D).

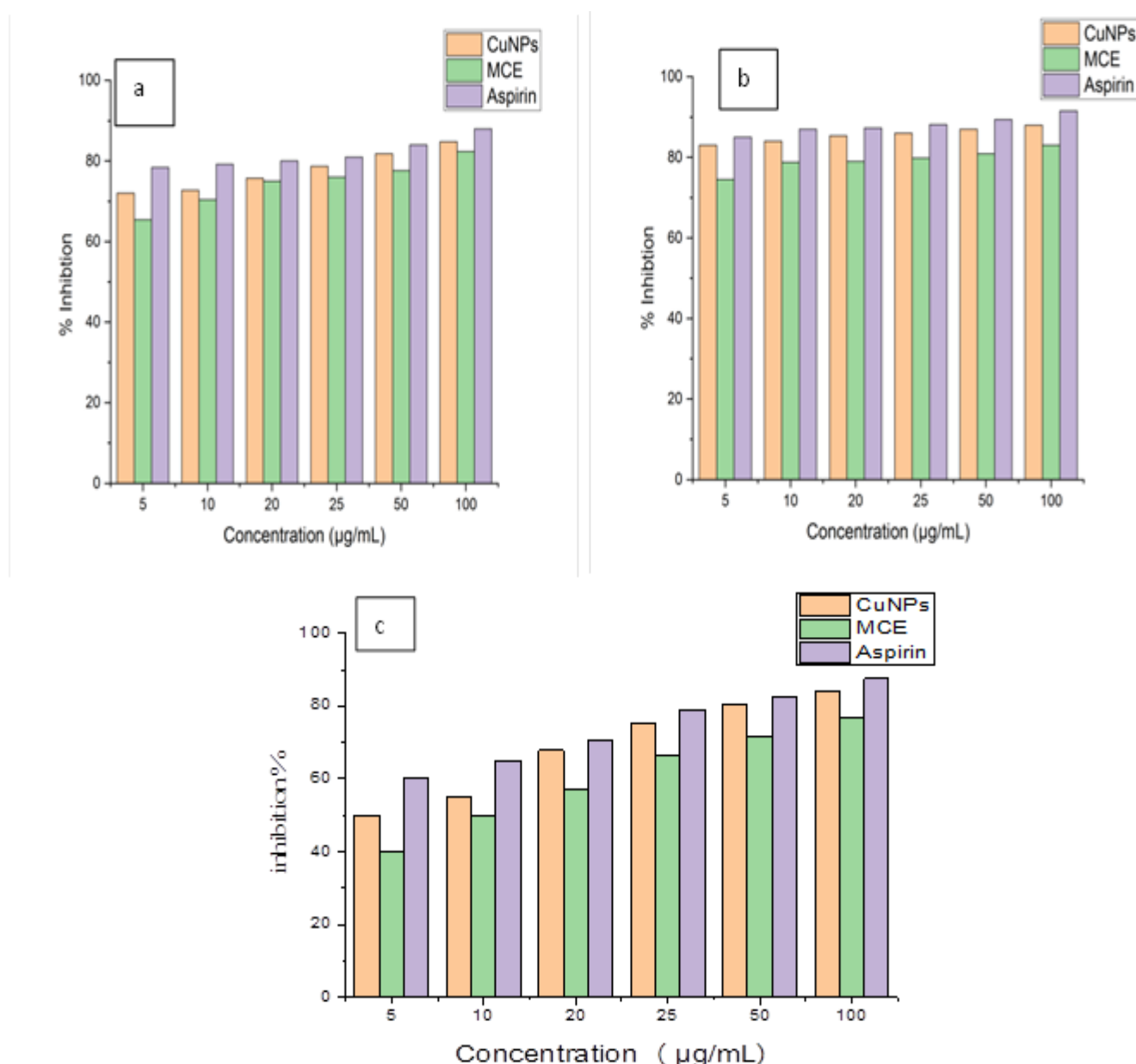


Figure 6. Effect of *M. communis* leaves extract and CuNPs on (a) albumin denaturation (b) protein inhibitory activity and (c) membrane stabilization

Hemolytic activity

The hemolytic activity of CuNPs from *M. communis* was tested against normal human erythrocytes. Both CuNPs and the extract showed little effect on blood hemolysis (Figure 7). The percentages of hemolysis induced by CuNPs were (7.35, 6.5, 3.40, 2.01, 1.5 %) /respectively (Figure 7a), whereas for the extract were (7.3, 4.3, 2.67, 1.5, 0.42, 0.34 %) (Figure 7b), comparing with negative and positive control .

Antibacterial assay

The antibacterial activity for each CuNPs and *M. communis* leaves extract were tested against Gram-positive bacteria (*Staphylococcus aureus* and *Lactobacillus*

salivarins) and Gram-negative bacteria (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*). As shown in (Table 2), *M. communis* leaves extract showed a pronounced antimicrobial activity against all the tested bacteria compared to CuNPs. *Staphylococcus aureus* and *Lactobacillus salivarins* were susceptible for *M. communis* at the concentrations (10000, 75000, and 50000 µg/mL) respectively, and they were intermediate at the concentration 25000 µg/mL compared with antibiotics whereas CuNPs didn't show antimicrobial activity against bacteria under study except for *lactobacillus* which showed intermediate resistant at the concentrations (10000, and 75000 µg/mL

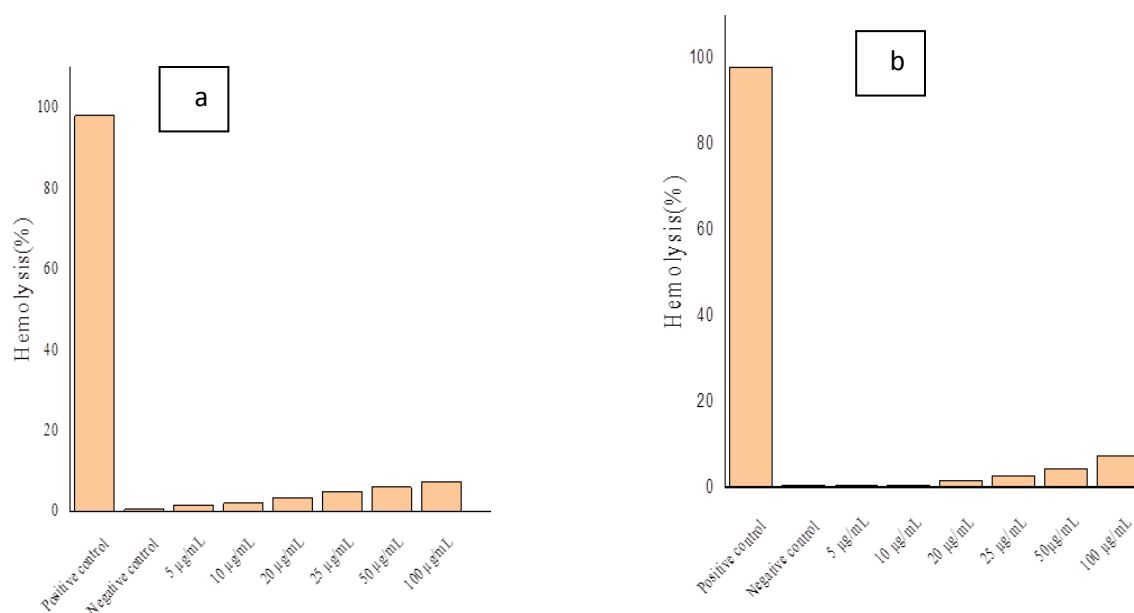


Figure 7. The percentage of hemolysis induced by (a) CuNPs and (b) *M. communis* extract. Triton X-100 was used as a positive control and normal saline as a negative control

Table 2. Anti-bacterial activity of CuNPs and *M. communis* extract against some pathogenic bacteria

Test sample	Concentration (µg/mL)	Inhibition zone diameter (mm)			
		Gram-negative		Gram-positive	
		<i>Klebsiella pneumonia</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Lactobacillus salivarins</i>
CuNPs	100000	10	-	-	14
	75000	-	-	-	14
	50000	-	-	-	7
	25000	-	-	-	5
Extract	100000	12	9	25	23
	75000	9	7	20	20
	50000	-	-	20	20
	25000	-	-	19	18
Antibiotic (Amoxicillin)	25	-	-	15	37
(Tetracycline)	30	-	-	-	30
(Trimethoprim)	5	-	-	25	35
(Gentamicin)	10	19	-	7	14
D.W)(-	-	-	-
(D.W+ ethanol)		-	-	-	-

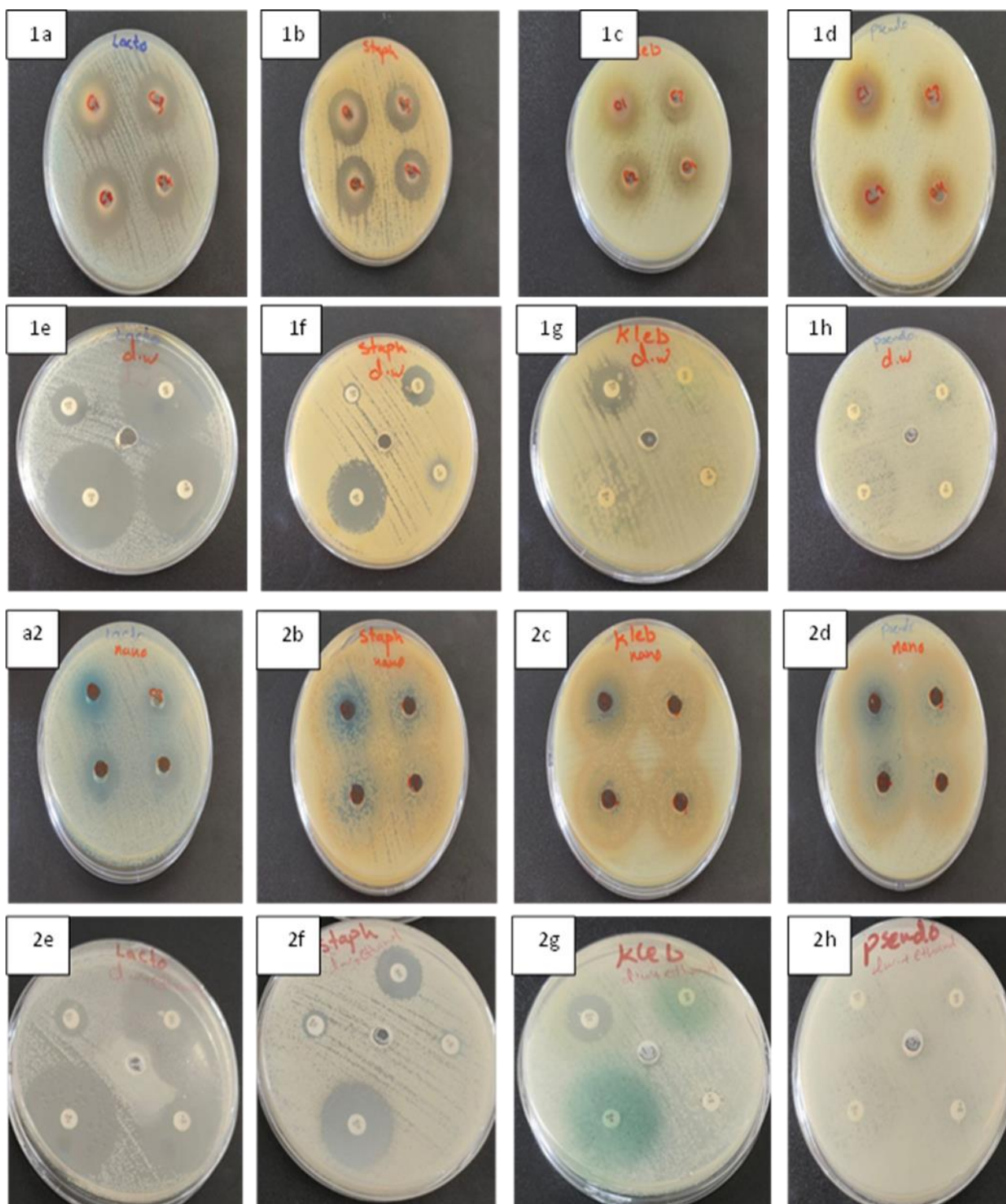


Figure 8. The antibacterial activity (1) *M. communis* leaves extract and (2) (CuNPs) (a) *Lactobacillus salivarius* (b) *Staphylococcus aureus* (c) *Klebsiella pneumoniae* and (d) *Pseudomonas aeruginosa*. while, 1(e, f, g, and h) and 2(e, f, g, and h) represent solvent (D.W and D.W+ ethanol) and Antibiotic (Amoxicillin), (Tetracycline), (Trimethoprim), and(Gentamicin) of *Lactobacillus salivarius*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and, *Pseudomonas aeruginosa* respectively.

Numerous studies have recently demonstrated that plant extracts can be used as a potential precursor for the synthesis of nanomaterials in a non-hazardous manner, according to the findings. Alkaloids, flavonoids, saponins, steroids, tannins, and other nutritional compounds are abundant in plants, as are flavonoids, saponins, and other natural compounds. For this reason, the plant extract contains a variety of secondary metabolites, which serve as both reducing and stabilizing agents in the bioreduction reaction that results in the formation of CuNPs (19). Following mixing of the aqueous extract of *M. communis* with $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, the color of the extract changes from light yellow to dark brown. This is due to the excitation of surface plasmon resonance (SPR) by the $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. The reduction of Cu^{2+} to Cu^0 is indicated by the change in color of the solution (3). The gradual color change was observed by measuring the UV-visible absorption over time, which was then plotted against time. The wavelength of 481 nm was found to be the most absorbent for CuNPs (Figure 2a). Copper-NPs were observed to form within the first few hours of the reaction, according to the UV-Vis analysis (Figure 2b). Nonetheless, the CuNPs solution was stable within 24 hours and remained stable thereafter. The presence of active phenolic compounds such as flavonoids in the *M. communis* extract before and after the bio-reduction process can be determined using FT-IR analysis. According to its complex nature, the *M. communis* extract exhibits numerous absorption peak patterns (16). The carbonyl and hydroxyl linkages in the components of the *M. communis* extract are responsible for the reduction of copper ions to copper nanoparticles (CuNPs) (29). FT-IR spectra of CuNPs revealed that some of the peaks observed in the plant extract were repeated in the CuNPs spectra, albeit with slight variations in band positions and intensities of the absorptions (Figure 3). The crystalline structure and purity of the NPs produced are demonstrated by the XRD results. The Bragg reflections at 43.43° , 50.58° , and 74.23° at 2θ values confirmed the crystallization structure of CuNPs at the three different temperatures. Bragg's law describes the action of X-ray diffraction as a rule of

thumb. According to standard X-ray diffraction powder patterns (28), the values of these angles were compared to the standard 2θ angle of CuNPs. The AFM was used to determine the surface morphology. The CuNPs had a spherical shape, and the average mean size of the CuNPs produced was 53.55 nm, indicating that they were of uniform size and shape, as shown in the figure (Figure 5). A possible explanation for this could be found in the fact that the leaves extract contained compounds that were responsible for the particle morphology (17). Copper-NPs synthesized from commercially available plant extracts such as *Nerium oleander*, *Punica tenuiflorum*, *Asparagus adscendens*, and *Eclipta prostrate* have irregular shapes, according to other studies (21), and their size was determined to be 40, 35, 56, and 42 nm, respectively, according to the researchers. Through the use of an in vitro method, the anti-inflammatory activity of plant extracts and synthesized CuNPs was evaluated. When these extracts and nanoparticles were tested in vitro as anti-inflammatory materials, their effects on protein denaturation, proteinase activity, and the HRBC (Human Red Blood Cells) membrane stabilization method were examined, it was discovered that the aqueous extract and nanoparticles showed the greatest amount of inhibitory activity (Figure 6). Because the extract contains biologically active compounds such as flavonoids and phenolic compounds, it is possible that the release of neutrophil lysosomal contents at sites of inflammation will be inhibited by these compounds. Some of the components of neutrophil lysosomes are bactericidal enzymes and proteases that, when released into the extracellular environment, cause additional tissue inflammation and damage (26),(14). The effect of CuNPs and the extract on blood hemolysis was investigated. When red blood cells come into contact with water, hemolysis occurs, and it is critical to thoroughly inspect the implant material before use (13). CuNPs have been tested in vitro on human blood erythrocytes to see if they have any effect on them (RBCs). CuNPs may be administered to the vascular system through injection or oral administration in medicine, and as a result, direct interaction between CuNPs and RBCs

may occur frequently. The earlier report on CuNPs demonstrated that small particles have higher hemolytic activity than large particles, and the contract that in silica nanoparticles increases hemolytic activity increases with large particles (20), (12) demonstrated that small particles have higher hemolytic activity than large particles. It was found that *M. communis* extracts had antibacterial activity against both Gram-positive (*Staphylococcus aureus* and *Lactobacillus salivarius* bacteria) and Gram-negative (*Klebsiella pneumonia*, *Pseudomonas aeruginosa* bacteria). These findings were compared to those obtained from the use of antibiotics (Amoxicillin), (Tetracycline), (Trimethoprim), and (Gentamicin), as well as those obtained from the use of synthesized CuNPs, which demonstrated antibacterial activity against (*Lactobacillus salivarius* bacteria), while other bacteria were resistant. The antibacterial activity of the *M. communis* plant was attributed to its unique phytoconstituents, which included tannins, saponins, steroids, cardiac glycosides, and other compounds. It was discovered that the difference in sensitivity between different microorganisms was primarily due to the phytochemical constituents present in the plant (24); (11). The antimicrobial activity of *M. communis* against seven pathogen bacteria was also investigated by Akin *et al.* It was found to have some activity against both Gram-positive and Gram-negative bacteria (1).

Conclusion

This study explained that CuNPs from *M. communis* leaves extract have anti-inflammatory activity and showed that *M. communis* extract has good activity against certain bacteria. These findings may be significant for using *M. communis* plant and CuNPs which are synthesized from it in the pharmaceutical fields after further study .

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