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PREPARATION OF GREEN COPPER OXIDE NANOPARTICLES USING ROSEMARY PLANT EXTRACT

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Abstract: The use of nanoparticles, represents one of the most significant advancements in modern science and technology, capturing the interest of researchers and engineers alike. Plant is an intriguing natural source for producing nanoparticles, as some species contain substances that facilitate the formation of these particles naturally. For instance, certain plants exhibit numerous biologically active compounds such as flavonoids and tannins, which can be utilized as nanomaterials. The process of extracting plant materials and converting them into nanoparticles is carried out using advanced preparation techniques biotechnological method. The advantage of this approach is that it provides an environmentally friendly means to produce nanoparticles effectively and reliably. In this work, the aqueous solution of Rosemary leaves extract has been used as a reducing and capping agent to prepare CuO- NPs. After preparation and purification, CuO-NPs have characterized using UV-VIS spectroscopy, FTIR and XRD. Anti- bacterial activity for these nanoparticles have been investigated.

1.1 General Introduction

The word "nano" in the English language refers to everything that is small in size and delicate in body. The word "nano" is derived from the word "nanos" and from the Greek word "dwaref" which means "dwarf" or billionth of a whole[(1)]. A "nanometer" is a measure of one thousandth of a millionth of a meter, that is, one billionth of a meter, that is, it represents one eighty-thousandth of the diameter of a single human hair! It is therefore the smallest and most accurate metric unit known to date, and nothing smaller can be built. It is the scale that scientists use when measuring an atom and the electrons that orbit its nucleus[(2)]. The word nanotechnology is used in the sense that it is nanotechnology, microtechnology, or microtechnology. **Fig 1**

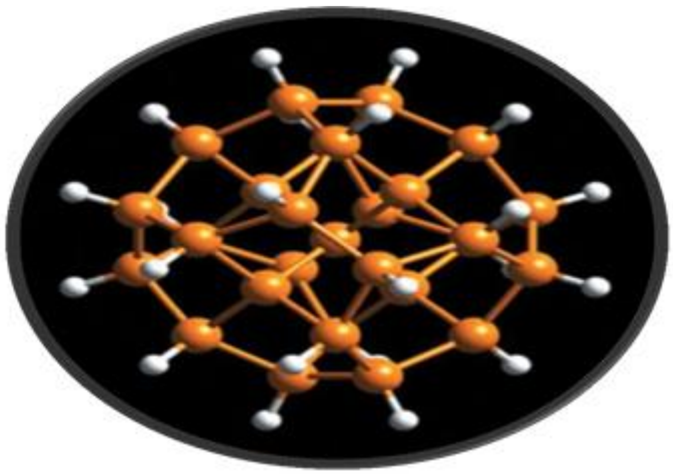


Figure (1-1) : Nanoparticles Exampl

By controlling and dealing with the molecular level and arranging it atom after atom in the way we desire to produce a specific substance[3].Nanotechnology is a vital area of modern research that deals with the design, synthesis, and manipulation of particle structures ranging in size from 1 to 100 nm[4].Nanoparticle Ultrafine unit with dimensions measured in nanometres (nm; 1 nm = 10−9 metre). Nanopa rticles exist in the natural world and are also created as a result of human activities. Because of their submicroscopic size, they have unique material characteristics, and manufactured nanoparticles may find practical applications in a variety of areas, including medicine engineering catalysis, and environmental remediation[5].

The prefix nano is derived from the Greek word Nanos, which means "dwarf," and refers to one billionth (10-9m) in size [6]. Nanoparticles have been synthesized from a variety of animal, plant, and microorganism sources. Copper has been widely used among metallic nanoparticles due to its stable and catalytic properties. Copper nanoparticles have sparked a lot of interest due to their excellent physical and chemical properties, as well as their low preparation cost [7]. Top-down and bottom- up approaches are commonly used to synthesize nanoparticles. In the top-down approach, bulk materials are gradually broken down into nanosized materials. Atoms or molecules are assembled into nanometer-scale molecular structures using the bottom-up approach. For nanoparticle chemical and biological synthesis, a bottom-up approach is commonly used.[7]

1.2 Previous study

Nanoparticles and structures have been used by humans in fourth century AD, by the Roman, which demonstrated one of the most interesting examples of nanotechnology in the ancient world [8]. The Lycurgus cup, from the British Museum collection, represents one of the most outstanding achievements in ancient glass industry. It is the oldest famous example of dichroic glass. Dichroic glass describes two different types of glass, which change color in certain lighting conditions. This means that the Cup have two different colors: the glass appears green in direct light, and red-purple when light shines through the glass [9]. In 1990, the scientists analyzed the cup using a transmission electron microscopy (TEM) to explain the phenomenon of dichroism .

The observed dichroism (two colors) is due to the presence of nanoparticles with 50–100 nm in diameter. X-ray analysis showed that these nanoparticles are silver-gold (Ag-Au) alloy, with a ratio of Ag:Au of about 7:3, containing in addition about 10% copper (Cu) dispersed in a glass matrix.[10]. The Au nanoparticles produce a red color as result of light absorption (~520 nm). The red-purple color is due to the absorption by the bigger particles while the green color is attributed to the light scattering by colloidal dispersions of Ag nanoparticles with a size > 40 nm. The Lycurgus cup is recognized as one of the oldest synthetic nanomaterials. A similar effect is seen in late medieval church windows, shining a luminous red and yellow colors due to the fusion of Au and Ag nanoparticles into the glass(11).In 1857, Michael Faraday studied the preparation and properties of colloidal suspensions of ―Ruby‖ gold. Their unique optical and electronic properties make them some of the most interesting nanoparticles. Faraday demonstrated how gold nanoparticles produce different-colored solutions under certain lighting conditions(12).

2. Materials and Methods

2.1 Materials

2.1.1 Apparatuses

Table (2-1) Tools and Instruments used in this stady

Tools and Instruments	Company
UV-Vis spectrophotometer	UV-1800/ Kyoto, Japan
FT-IR spectrometer	Shimadzu (8400S)/Japan
X-ray diffraction (XRD)	Philips Xpert /Holland
Centrifuge	D-78532 Germany
Oven	D-91126 Schwabach FRG/ Germany
Sensitive balance	Sartotius / Germany
Beaker	National / Japan
Funnel	DragoLAB/China
Stirring Rod	National / Japan
Watch glass	National / Japan
Filter paper	DragoLAB/China
Cylinder	National / Japan
Conical flask	945307 /THE.U.S.A

2.1.2 Chemicals

Table (2-2) Chemicals used in this stady

Chemicals	Company
Copper chloride	Chemos GmbH & Co. KG Sonnenring 7 84032 Altdorf Germany
Ethanol	Chemos GmbH & Co. KG Sonnenring 7 84032 Altdorf Germany
Rosemary plant extract	A shop selling medical herbs in Karbala
Distilled water	
Sodium hydroxide	Fisher Scientific 1 Reagent Lane Fair Lawn, NJ 07410

2.2 Methods

2.2.1 Preparing the extract

- A quantity of rosemary plants was collected and washed with distilled water and Ethanol to get rid of contaminants.

Figure(2-1): Rosemary leaves

- 500 grams of Rosemary leaves were grounded.

Figure(2-2): Powder Rosemary leaves

It was placed in a beaker, 1 liter of distilled water was added, and it was placed on a heater to boil for two hours at a temperature of 70 C.

- The solution was left to cool and then filtered.

A green-olive extract was obtained Fig (2-3)**.**

Figure (2-3) : Rosemary plant extract.

2.2.2 Synthesis of Nano Oxide (CuO-NPs)

A weight of 0.55 grams of hydrated Copper Chloride was taken.

It was dissolved in 800 ml of distilled water, stirring to complete the dissolution process.

Then 25 ml of Rosemary extract was added to the solution.

It was heated to a temperature of 80°C and Sodium hydroxide was added to neutralize the solution.

During the heating process, an unstable precipitate was observed to form, and after heating, a stable brown precipitate was formed at the bottom of the beaker.

The sediment was filtered and washed several times with distilled water and absolute ethanol to get rid of impurities.

Finally the precipitate was placed in the oven for half an hour. Then kept for later use.

2.2.3 UV-Vis Spectroscopy

The synthesis of CuO-NPs was confirmed by and UVvisible absorption spectra. and the maximum absorption was determined with the scanning at the range from 190 to

800.

2.2.4 Fourier Transform-Infrared (FT-IR) Spectroscopy

FT-IR analysis was done for both extract and synthesized CuO-NPs.

FT-IR spectroscopy is commonly used to examine interactions between NPs and capping agents. Using this method, the interactions of the functional group and the metal NPs can be checked when two or more functional groups are present in the capping agent.

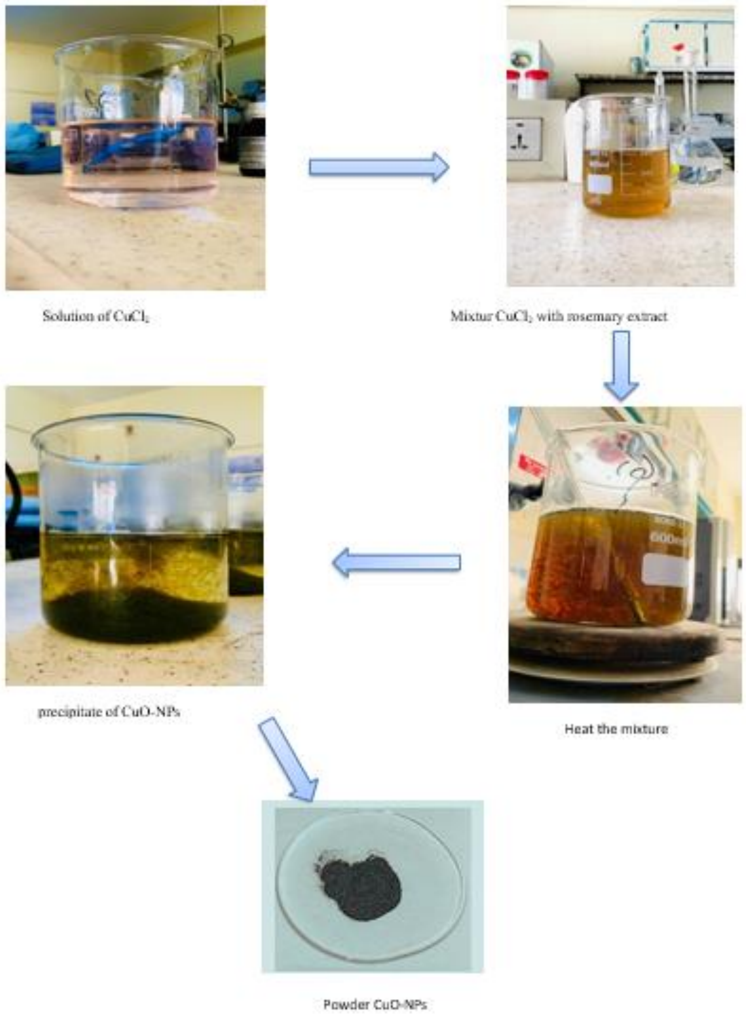
Sample Preparation for FT-IR Spectroscopy

Both plant extract and CuO-NPs were measured with the KBr disk in the wavelength ranging from 400 to 4000 cm^{-1}.

2.2.4 X-ray Diffraction Analysis

X-ray diffraction (XRD) is an effective non-destructive method for the characterization of crystalline materials. This provides information on structures, phases, desired crystal orientation (texture), and other structural parameters, such as average crystal size, crystallinity, strain, and crystal defects.

The synthesized CuO-NPs were characterized using (XRD) in Baghdad



Figure(2-4): Method of Preparation CuO-NPs

2.2.3 Preparation of Anti-bacterial

The antibacterial activity of CuO-NPs was determined using the agar well diffusion method . The antibacterial activity was assessed against Gram-negative bacteria (Klebsiella pneumonia and Pseudomonia aeruginosa) and Gram-positive bacteria (Staphylococcus aureus and lactobacillus salivarins) .

➢ **Procedure:**

1. Muller-Hinton agar plates were inoculated with tested bacteria.
2. Agar wells of 6 mm diameter were made using cork borer.
3. A dilute solution of CuO-NPs powder was prepared.
4. All wells were filled with (1 ml) of test sample (CuO-NPs).
5. The plates were incubated at 37 C for 24 hours.

3. Results

3.1 Rosemary leaves extract

In this work Rosemary has been chosed because it contains many compounds that have biological properties such as antibacterial, antioxidant, anti-inflammatory, etc.

compounds is polyphenols, terpenoids, alkaloids, sugars, proteins, and phenolic acids.

It was classified according to the Biology Laboratory at the College of Science at the University of Babylon as :

Table(3-1): Plant classification

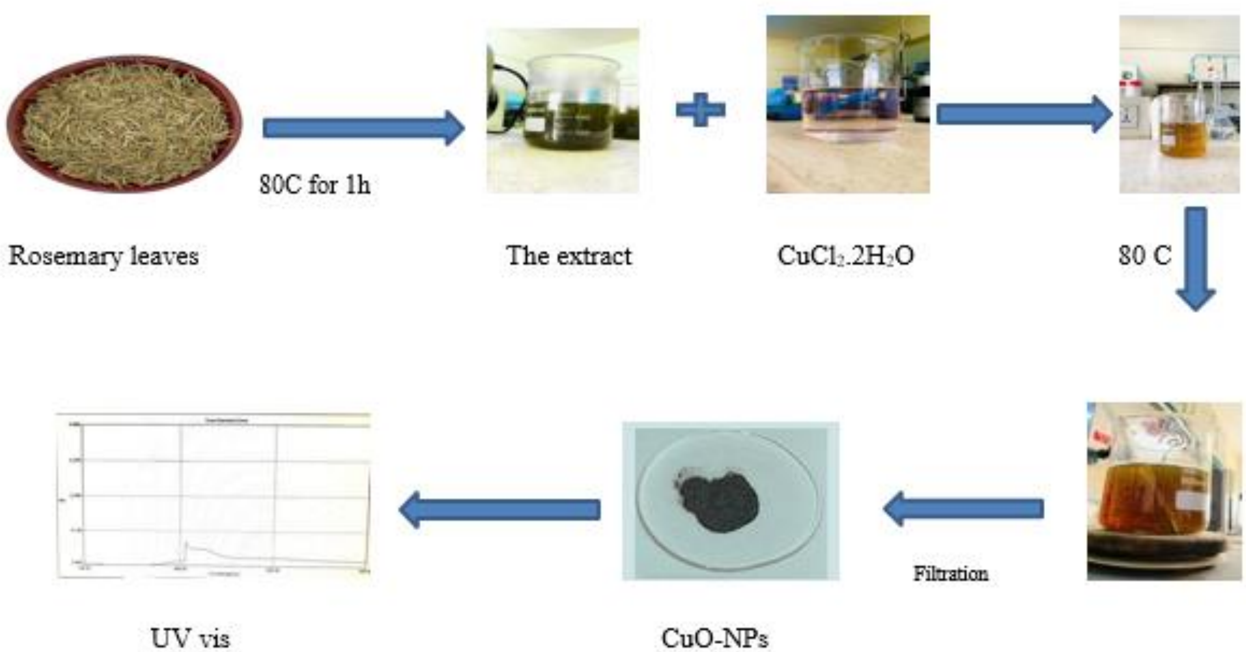
Partition unit	Rosemary plant
Kingdom	Plant
Division	Tracheophyt
Class	Angiosperm
Subclass	Dicotyledons
Order	Lamiales
Family	Lamiaceae
Subfamily	Nepetoideae
Tribe	Mentheae
Genus	Thymus L
Species	Thymus vulgaris L

3.2 Synthesis of Copper Oxide Nanoparticles

The color change in the aqueous extract with copper chloride solution may be due to the presence of bioactive compounds in the aqueous extract. Various phytochemicals are responsible for the reduction of copper ions Probably, the flavonoids or phenolic compounds in Rosemary leaves act as the reductions of metal ions, whereas saponins and tannins may act as the capping agents. Moreover, polyhydroxy groups may be responsible for the reduction of Cu ions into metal NPs.

The basic medium was used because the basic medium produces nanoparticles of small sizes that are more active.

When the solution was heated, it was observed that nanoprecipitate formed at the bottom of the beaker.



Figure(3-2): Synthesis CuO-NPs

3.3 UV visible spectroscopy

The synthesis of CuO-NPs from rosemary leaf extract was characterized using UV– vis spectroscopy, which showed an absorption band at 420 nm Fig(3-3). Broad, single and strong absorption peaks showed the formation of CuONPs in the sample.

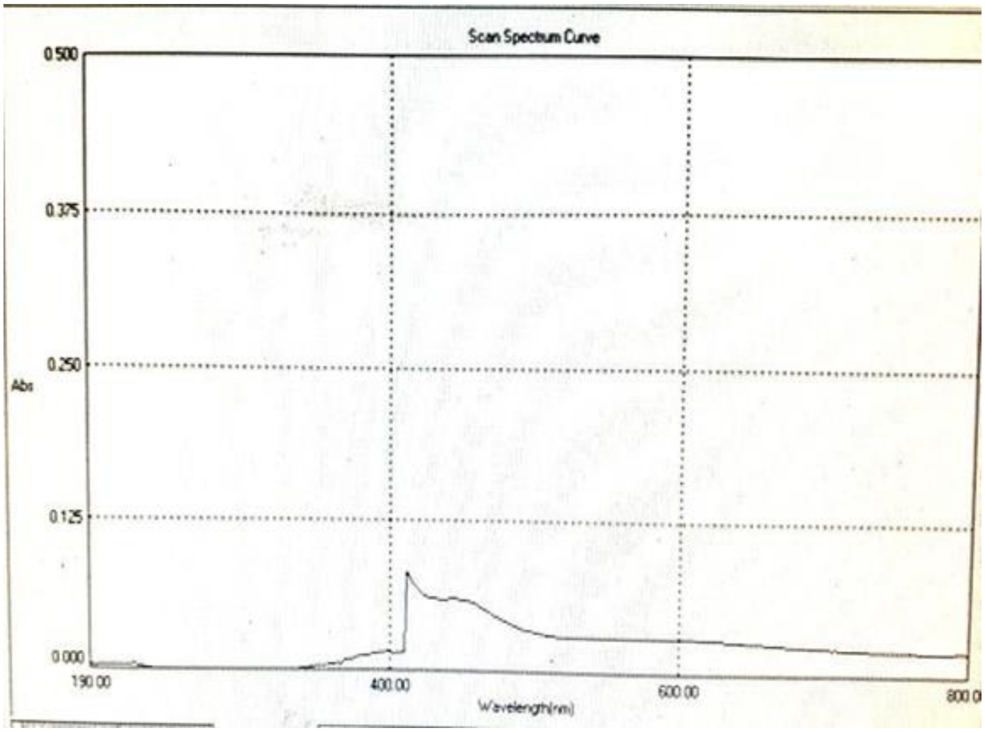


Figure (3-3): UV-Visible spectra of CuO-NPs at 420 nm.

3.4 Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR characterization is used to identify the functional groups and observe the interaction between biomolecules of Rosemary extract **(Fig3-4**) and CuO-NPs (**Fig 3- 5**). The FT-IR spectrum of Rosemary leaves extract showed peaks at 3306.10cm^{-1} (O–H stretching vibrations), 2931.80 cm^{-1} (C–H) and CH_2 vibration of aliphatic hydrocarbons, , 1608.69 cm^{-1} (C=C stretching vibrations), 1423.51 cm^{-1} (O–H bending vibrations), 1257.63 cm^{-1} (C–O asymmetric stretching in cyclic polyphenolic compounds) and 1072.46 cm^{-1} (O–H deformation), pheromone(O-H groups), also some signals emerged in 1587.47, and 1072.46 cm^{-1} are related to C=C stretching and C-OH bending, respectively. The Rosemary extract peak locations and absorption intensities were compared to those produced from CuO-NPs, and results showed that some band positions and absorption intensities from the plant extract peak were replicated in the CuO-NPs FT-IR spectrum with a slight shift in peak position. Moreover, peaks at 3306.10 cm^{-1} in the FT-IR spectrum of the leaves extract are shifted to 3446.91cm^{-1} in the FT-IR spectrum of CuO-NPs. Moreover, a band at 1423.51 cm^{-1} is present in the plant extract which has disappeared in the

FT-IR spectrum of CuO-NPs.

Another band at 705.97 cm^{-1},690.54 cm^{-1},663.53 cm^{-1},559.38 cm^{-1},524.66 cm^{-1} **(Fig3- 7**) was observed in the FT-IR spectrum of CuO-NPs due to the presence of CuO- NPs, as it is not present in the FT-IR spectrum of leaves extract. Therefore, the absence of the carbonyl band of leave extract and appearance of a new peak at 705.97 cm^{-1},690.54 cm^{-1},663.53 cm^{-1},559.38 cm^{-1},524.66 cm^{-1} in the FT-IR spectrum of CuO-NPs indicated that interaction of biomolecules of leaves extracts occurred the through carbonyl band with CuO-NPs.

The peaks around 705.97 cm^{-1},690.54 cm^{-1},663.53 cm^{-1} corresponds to the Cu–O stretching vibration of copper oxide nanoparticles in the monoclinic structure. The absorption peaks at 3446.91 cm^{-1} and 1639.55 cm^{-1} corresponds to the OH stretching vibration and HOH bending mode of adsorbed water molecules, since the nano crystalline materials possess high surface area to volume ratio leading to the absorption of moisture in the lattice. The absorption band at 1031.95cm^{-1} corresponds to the C–O stretching of phenol and alcoholic compounds.

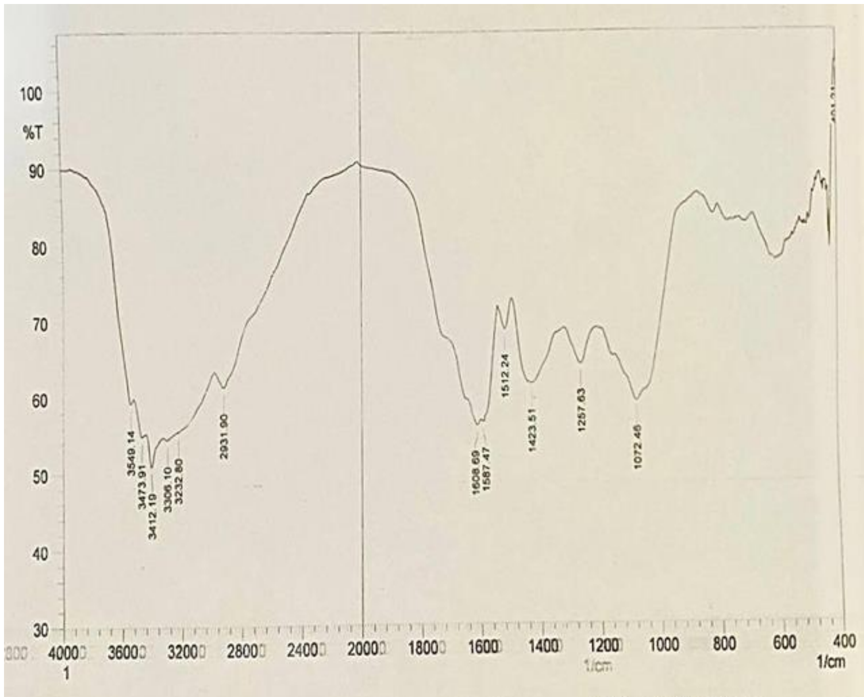


Figure (3-4): FT-IR spectrum of Rosemary leaves extract

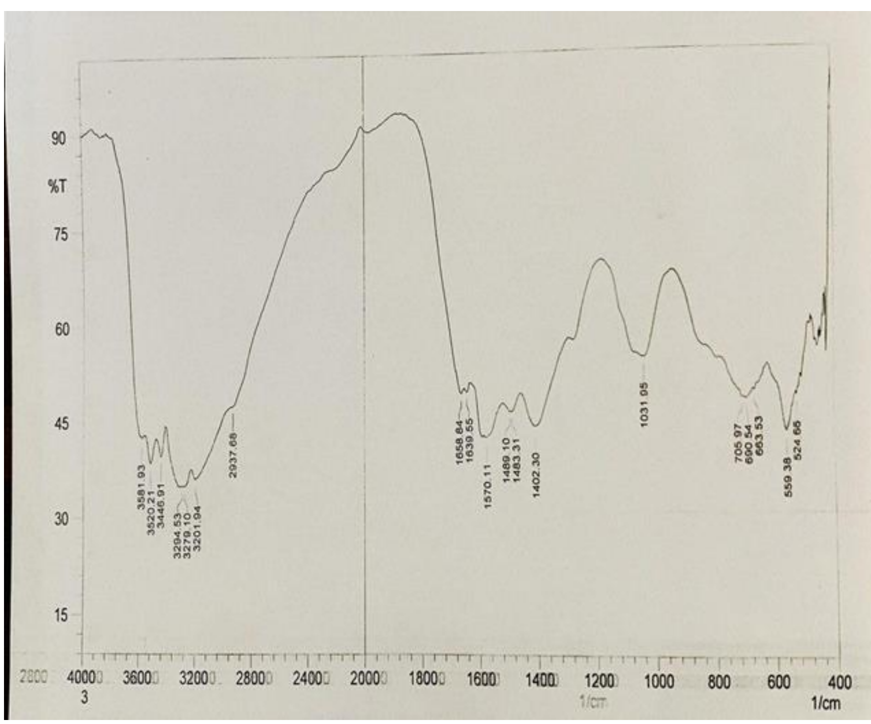


Figure (3-5): FT-IR spectrum of synthesized CuO-NPs

3.5 X-ray Difraction (XRD)

The structure has been identified a chemical formula of CuO-NPs according to Automated analysis laboratory in Baghdad . The XRD diffraction analysis revealed three featured peaks at 2θ values of 40, 47 and 67 , corresponding to (111), (200), and

(220) planes of copper oxide **Fig(3-6)**. The structure of CuO-NPs suggested by these observation is hexagonal wurtzite. The obtained data was matched with the previous stady [(41)], **Fig(3-7)**.

Comparison of the X-ray measurement results with previous results showed that they are similar.

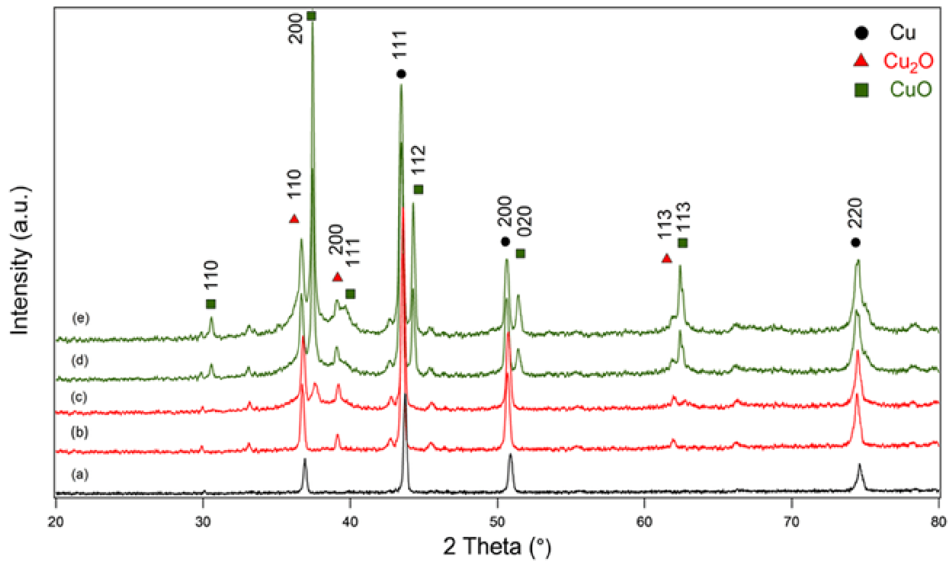


Figure (3-6) : X-ray to Previous study (standard)

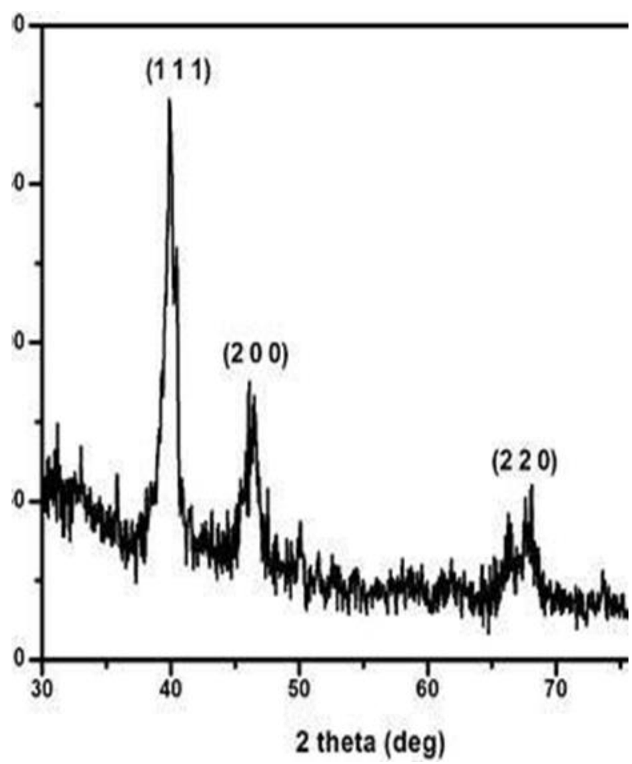


Figure (3-7): X-Ray diffraction (XRD) of synthesized CuONPs from Rosemary leaves extract

3.6 Anti-bacterial activity

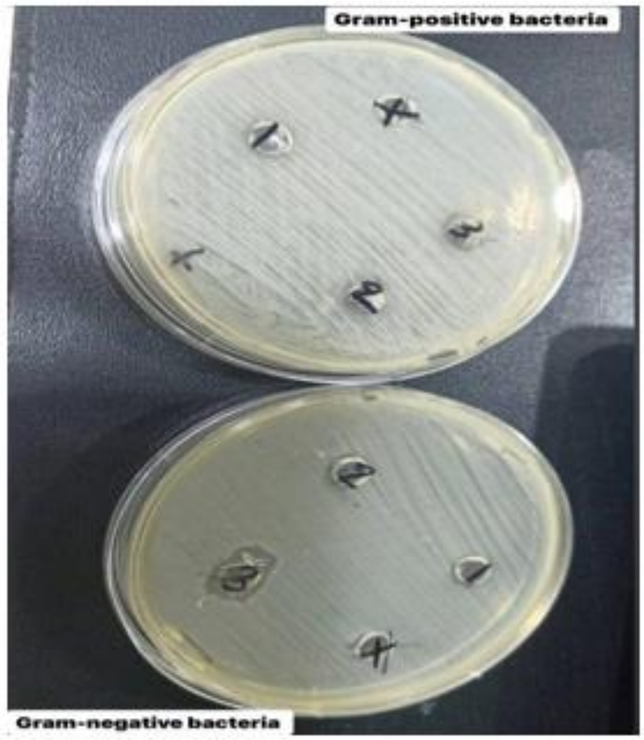
The antibacterial activity for each CuO-NPs and Rosemary leaves extract were tested against Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (E-Coli). As shown in **(Fig 3-8)**, Rosemary leaves extract showed a pronounced antimicrobial activity against all the tested bacteria compared to CuO-NPs When conducting an antibacterial test, it was confirmed that CuO-NPs work to inhibit bacteria .

These results of antibacterial activity of Rosemary leaves extract were compared with the Antibiotics(ZnO-NPs),(AgO-NPs),(Ampicillin) and synthesized CuO-NPs **(Fig 3-9)**. Nano copper oxide has the greatest degree of inhibition of bacterial growth.

Figure(3-9): The antibacterial inhibition

(A) Ampicillin , **(B)** Rosemary leaves extract

(C) ZnO-NPs,**(D)** AgO-NPs ,**(E)** CuO-NPs



Figure(3-8): Gram-positive bacteria and

Gram-nagative bacteria

3.6.1 Mechanism of NPs action in bacteria cells

NPs can attack bacteria cell through multiple mechanisms: the formation of ROS leading to membrane, protein, and DNA damage; direct interaction occurs with cell membrane because some metal-based NPs can generate metal ion via dissolving, for example, inhibition of electron transport chain; and the regulation of bacterial metabolic processes [42] . **Fig (3-10)**

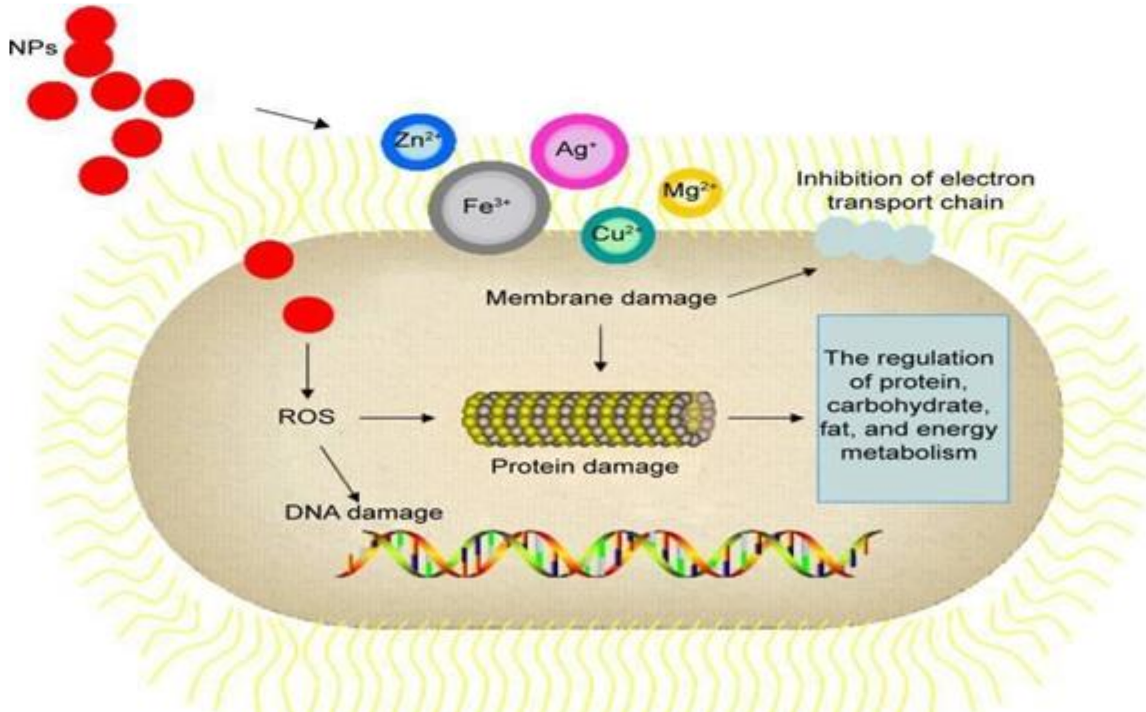


Figure (3-10): Mechanisms of NP action in bacteria cells

Conclusions

1. Copper Nanoparticles were successfully synthesized Using aqueous Rosemary leaves extract.
2. CuO-NPs have slaw significant inhibiton agent (E-Coli) grow negative bacteria in comparation to Ampicillin.

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