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INHIBITION ACTIVITY ASSESSMENT OF BIOSYNTHESIZED OF CUNPS BY AN ECO-FRIENDLY TECHNIQUE USING PLANT EXTRACTS AGAINST *Staphylococcus aureus*

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ABSTRACT

The study investigated the Minimum Inhibition Concentration (MIC) of CuNPs biosynthesis using aqueous *Myrtus communis* and *Nerium oleander* leaves extracts against *Staphylococcus aureus* bacteria isolated from Al-Madaen Public Hospital in Baghdad-Iraq. Ten concentrations (50.0 to 0.09) mg/ml of CuNPs were prepared to determine MIC values for each extract concentration against *S. aureus* bacteria. Various techniques were used to characterize CuNPs, and UV-visible light was used to screen the surface plasmon resonance (SPR) for CuNPs in scope from 200 to 600 nm. The Cu Fourier transform infrared spectroscopy (FTIR) and atomic force microscopy (AFM) revealed the nanoparticle's size and surface feature. A scanning electron microscope (SEM) was used to determine nanoparticle crystalline morphology and size. The results revealed that CuNPs of *M. communis*, MIC values varied from 0.09 mg/ml in well 10 of samples 1 and 2 to 0.3 mg/ml in well 8 of sample 4, whereas in the case of CuNPs of *N. oleander*, the MIC values were found to range from 0.09 mg/ml in well 10 of sample 2 to 0.7 mg/ml in well 7 of sample 1. In the case of mixed CuNPs, prepared from extracts of both plants, the values varied from 0.3 mg/ml in well 8 of sample 4 to 3.1 mg/ml in well 5 of sample 1. However, regarding *M. communis* alcoholic extract, MIC values ranged from 0.09 mg/ml in well 10 of samples 2 and 4 to 0.1 mg/ml in well 9 of samples 1 and 3. For *N. oleander* alcoholic extract, MIC values ranged from a minimum rate of 0.09 mg/ml to a maximum rate of 3.0 mg/ml in well 10 of sample 4 and in well 5 of sample, respectively. Finally, the MIC values of the mixed alcoholic extract of *M. Commines* and *N. Oleander* varied from 0.3 mg/ml in well 8 of sample 2 to 0.7 mg/ml in well 7 for samples 1, 3, and 4.

Key words: MIC, CuNPs, *S. aureus, M. communis, N. oleander.*

[*]**This article is taken from the doctoral dissertation of the first researcher.**

تقييم النشاط التثبيطي لجسيمات النحاس النانوية المحضرة حيويا بوساطة تقنيات صديقة للبيئة باستخدام المستخلصات النباتية ضد المكورات العنقودية الذهبية

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الخلاصة

بحثت الدراسة في تحديد اوطأ التراكيز المثبطة (MIC) لجسيمات النحاس النانوية CuNPs المحضرة حيويا باستخدام مستخلصات أوراق نبات الاس المائية وأوراق نبات الدفلة ضد بكتيريا المكورات العنقودية *Staphylococcus aureus* والتي عزلت من مستشفى المدائن العام في بغداد-العراق. إعدت عشرة تراكيز (50.0 إلى 0.09) ملغم/مل من CuNPs لتحديد قيم MIC لكل من التراكيز المستخلصة ضد بكتيريا *S. aureus*. استخدمت تقنيات مختلفة لتوصيف CuNPs، حيث استخدم الضوء المرئي فوق البنفسجي لفحص رنين البلازمون السطحي (SPR) لـ CuNPs، عند 200 إلى 600 نانومتر. كشف التحليل الطيفي للأشعة تحت الحمراء باستخدام تحويل فورييه (FTIR) ومجهر القوة الذرية (AFM) عن حجم الجسيمات النانوية وخصائص سطحها. تم تحديد التشكل البلوري وحجم الجسيمات النانوية بواسطة المجهر الالكتروني الماسح (SEM). كشفت النتائج أنه جسيمات النحاس النانوية CuNPs المحضرة من مستخلص نبات الاس المائي *M.communis*، ان قيم MIC قد تباينت من 0.09 ملغم/مل في االحفرة رقم 10 للعينة 1 و 2 إلى 0.3 ملغم/مل في الحفرة 8 للعينة 4، وعلى النقيض من ذلك، في حالة جسيمات النحاس النانوية CuNPs المحضرة من مستخلص الدفلة المائي *N. oleander*، تراوحت قيم MIC من 0.09 ملغم/مل في الحفرة 10 للعينة 2 إلى 0.7 ملغم/مل في الحفرة 7 للعينة 1. في حالة جسيمات النحاس النانوية CuNPs الممزوجة والمحضرة من مستخلصات كلا النباتين، تراوحت القيم من 0.3 ملغم/مل في الحفرة8 للعينة 4 إلى 3.1 ملغم/مل في لحفرة 5 للعينة 1. فيما يتعلق بالمستخلص الكحولي لنبات الاس *M.communis*، تراوحت قيم MIC من 0.09 ملغم/مل في لحفرة 10 للعينات 2 و 4 إلى 0.1 ملغم/مل في لحفرة 9 من للعينات 1 و3. اما بالنسبة للمستخلص الكحولي لنبات الدفلة *N. oleander*، فقد تراوحت قيم MIC من معدل أدنى 0.09 ملغم/مل. مل إلى معدل أقصى 3.0 ملغم/مل في لحفرة 10 للعينة 4 وفي لحفرة5 للعينة على التوالي. أخيرًا، تراوحت قيم MIC للمستخلص الكحولي المختلط لنبات الاس *M. Commines* والدفلة *N. Oleander* من 0.3 ملغم/مل في الحفرة8 للعينة 2 إلى 0.7 ملغم/مل في الحفرة 7 للعينات 1 و3 و4.

الكلمات المفتاحية: MIC، CuNPs، *S. aureus*، *M.ommunis*، *N. oleander*

INTRODUCTION

Nanotechnology has been used as a new trend in most biological, environmental, and medical studies worldwide and has received much attention **(Tawade & Wasewar, 2023)**. Nanoparticles of several heavy metal ions were eco-friendly and prepared from various plant materials to examine their possible impact on the activity of specific bacterial species **(Singh *et al.*, 2018; Ibrahim *et al.*, 2024)**. various works on metal nanoparticles have been recorded to severely impact bacterial activities regarding growth inhibition, biofilm formation, genetic expression, and other bacterial properties **(Kang *et al.*, 2019; Heine *et al.*, 2024)**. However, biosynthesized metal nanoparticles were prepared by various aqueous and alcoholic plant extracts and applied against different bacterial species where previous work has examined the installation of nanoparticles and found that extracts of various plant classes have been successfully to produce NPs **(Mittal *et al.*, 2013)**. AgNPs extracted from *Ferula asafetida*, *Phoenix dactylifera* and *Acacia nilotica* plants **(Mohammed *et al.*, 2018)**, ZnONPs extracted from *Albizia lebbeck* stem bark **(Umar *et al.*, 2019)**, CuNPs extracted by *Ailanthus altissima* leaves **(Awad & Amer, 2020)** and by A*loe vera* plant **(Abed et *al.*, 2021)**, Fe_2O_3NPs extracted by orange peels **(Bashir et *al*, 2020)**, MgONPs extracted by *N. incerta* **(Al Shammary, 2023)** and finally AuNPS extracted by *Lasiosiphon eriocephalus Decne* Plant **(Datkhile *et al.*, 2023)**. *Myrtus communis* and *Nerium oleander* plants are prevalent, widespread, and accessible species in the Iraqi environment and have been used in the biosynthesis of various metal nanoparticles where *Myrtus communis* plant extract was used with AgNPs **(Ajdari *et al.*, 2017; Alyousef *et al.*, 2019)** and with CuNPs **(Al-Jubouri *et al.*, 2022)**. CuNPs **(Sebeia *et al*, 2019)**, and SiO_2NPs **(El Messaoudi *et al*, 2022)**. This work used an aqueous extract of Myrtus communis and Nerium oleander to synthesize CuNPs and tested against *S. aureus* bacteria isolated from wastewater generated by Al-Madaen Public Hospital in Baghdad-Iraq.

MATERIAL AND METHOD

Collection of samples

Fifty-four S. *aureus* bacteria were isolated from wastewater collected from Al-Madaen Public Hospital in Baghdad-Iraq in closed glass bottles after being washed with distilled water and sterile by placing them in the oven at 200°C for four hours. After that, the specimen was saved in the cool box and taken to the lab for examination. The conventional detection of *S. aureus* depended on the cultural properties of Blood agar and Mannitol salt agar, followed by microbiological tests, including gram stain morphology, coagulase reaction, catalase reaction, and other standard routine tests for detection. Finally, the confirmed diagnosis for *S. aureus* isolates was performed using the Vitek2 system compact.

Plant collection

M. communis and Nerium oleander fresh leaves were harvested from the general garden of the town of Al-Madaen. The leaves were cleaned and washed thoroughly with deionized distilled water. The clean leaves stayed to dry at room temperature, and afterward, they were grinding into tiny pieces before being kept in a dark and dry location for later use.

Preparation extract

The methanolic extract of *Myrtus Communis and Nerium Oleander* was ready by using a known weight of plant leaves, and the leaves were left to dry in the oven at a temperature of 40 °C. 100 g of each plant were placed in separated glass beakers of 1000 ml and 300 ml of methanolic alcohol solvent with a concentration of 80% and left at a stirrer for 48 hrs **(Kim *et al.*, 2009)**.

Synthesis of CuNPs

According to **(Abboud *et al*., 2014; Daylee, 2022)** slight modification. CuNPs were prepared by taking 20 g of copper salts ($CuSO_4.5H_2O$) and dissolved in an aqueous extract of each of *M. communis* and *N. oleander* at a volume of 200 ml, and then the pH was calibrated to 8 by adding 0.1 from (NaOH) with continuous stirring for 10 min within room temperature until the color was convert. The extract was incubated at room temperature, placed in a shaker, and left overnight. Then, it was centrifuged at 8000 rpm for 30 minutes to obtain CuNPs. The NPs were refined and washed 3 times with deionized water with recurrence centrifugation. Lastly, the grain of CuNPs was placed in the oven to dry and collected for further use.

Characterization of CuNPs nanoparticles

The generation of CuNPs was proven by UV-light Visible spectroscopy. UV analyzed the CuNPs between 200-600 nm. FTIR pattern was conducted to discover the biomolecules responsible for reducing copper ions. Atomic force microscopy (AFM) proved and labeled synthesized nanoparticles' size, form, and external region. A scanning electron microscope (SEM) will also be used to determine the surface morphological characteristics of the biosynthesized CuNPs.

Minimum Inhibition concentration

A Stock solution of CuNPs, *M. communis,* and *N. oleander* leave alcoholic extract with varying concentrations of 6.25 to 50 mg/ml was prepared. The antibacterial activity was defined against *S. aureus*. This assay used the agar well diffusion method, in which wells (8 mm) were used. The prepared CuNPs and *M. communis* and *N. oleander* leave alcoholic extract were placed into the wells Petri dishes and incubated overnight at 37°C. The antibacterial capacities of CuNPs, M. communis, and N. oleander leaves extracted from alcohol were measured using a standard Zone of Inhibition assay **(Subhankari & Nayak, 2013)**.

100 µl of the stock solution was transferred sterilely into A1 of a microtiter plate containing 100 µl aliquots of sterile MHB. This results in a 50 % dilution of the stock solution to 50 mg /ml. After mixing the contents adequately of each well, 100 µl aliquots of A1 were transported to the subsequent hole in B1 (also including 100 µl aliquots of MHB), followed by blend and reach in yet another 50% dilution of the antimicrobial (to 25 mg/ml). The process was repeated for every line to get ten concentrations of 50.0 to 0.09 mg/ml as recommended by an early work **(Sabee *et al*., 2020)**. The MIC test of CuNPs biosynthesized by both plant's aqueous extract was carried out against the isolated *S. aureus* bacteria according to guidelines given by the Clinical and Laboratory Standards Institute (CLSI) as used by various workers **(Humphries *et al.*, 2021).**

RESULTS AND DISCUSSION

Synthesis of Cu NPs

Copper nanoparticles were successfully synthesized by utilizing *M. Comminus* and *N. Oleander* plant leaves aqueous extract. (Figure 1) shows the color changing to brownish-black. The crude extract of *M. Comminus* and *N. Oleander* is reducing a reducing factor for CuNPs. The formation of CuNPs was proven primarily based on converting the color of the reaction mixture within 24 hours. Shift the color from dark yellow to brownish black owing to the reduction of copper ion, which indicated the formation of CuNPs; the color alteration gradually was followed via measuring UV-visible absorption with time. The exposure time of the reducing factor to metal ions is managed for nanoparticle synthesis, so the synthesis of CuNPs develops as the reaction incubation time of copper increases with the reductant **(Rocchetti *et al*.,2017)**. This result agrees with **(Awad Amer, 2020)**, where Ailanthus altissima leaves were extracted to produce CuNPs.

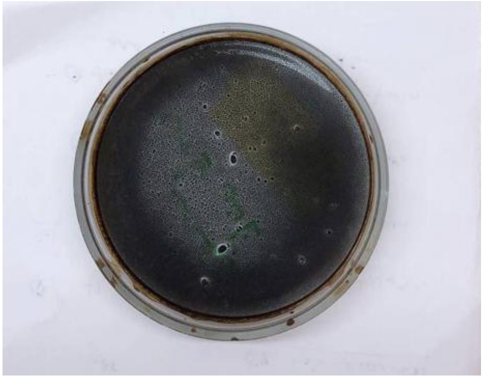
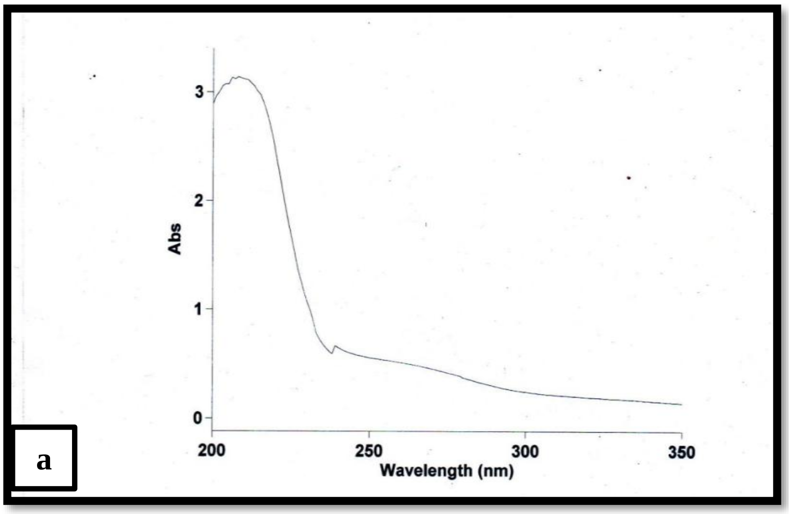


Figure (1): CuNPs biosynthesized using *M. Comminus* and *N. Oleander* plant leaves aqueous extracts.

Characterization of Copper nanoparticles

Ultra Violet-Visible (UV-Vis) Spectrophotometer was used to discover the chemical characteristics of *M. communis N. oleander* ,where the CuNPs biosynthesized by these two plants detected at wavelengths of 206 and 207 nm for CuNPs synthesized by *M. communis,* CuNPs synthesized by *N. oleander*, respectively. The results showed that the sample had formed CuNPs using *M. communis* extracts, as evidenced by the significant peak of characteristic emission found at 206 nm in the spectra of the Surface Plasmon Resonance (SPR) absorption band. A previous study **(Kalpana *et al*., 2016)** showed similar results, with a broad peak obtained at 219 nm, Figure. 2 (a, b). Regarding CuNPs synthesized *N. oleander* extract, this technique showed the wavelengths at 207 nm where a significant characteristic emission peak, Figure. 2b. Cu-NPs were reported by other studies at several wavelengths between 200 and 1000 nm at a scan average of 200nm/min **(ALBERT& Gonsago,2023)**.



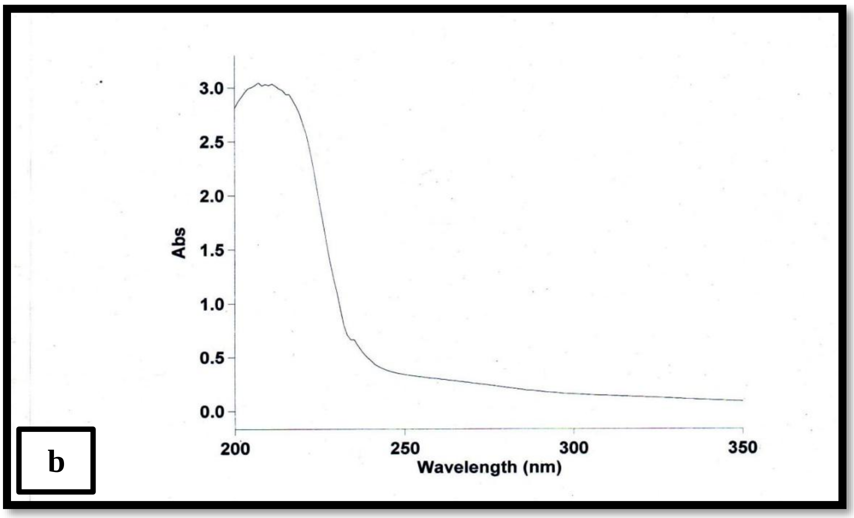


Figure (2): a) UV-Vis Spectroscopy of CuNPs synthesized by M. communis, b) Spectroscopy of CuNPs synthesized by N. oleander.

Atomic Force Microscopy (AFM)

The average diameter of CuNPs with *M. communis* and *N. oleander* extract by AFM examination is presented in (Table 1).

Table (1): the average diameter CuNPs synthesis by *M. communis* and *N. oleander* by AFM.

Extract	Dimeter	
CuNPs synthesis using *M. communis aqueous extract*	Avg. Diameter:52.37 nm	<=10% Diameter:15.00 nm
	<=50% Diameter:40.00 nm	<=90% Diameter:90.00 nm
CuNPs synthesis by *N. oleander extract*	Avg. Diameter:48.42 nm	<=10% Diameter:20.00 nm
	<=50% Diameter:40.00 nm	<=90% Diameter:80.00 nm

The surface shape formation of the CuNPs from *M. communis* was examined by atomic force microscopy (AFM) and found to have an average diameter of 52.37 nm. AFM images have shown that the biosynthesized CuNPs from *M. communis* NPs were spherical (Figure. 3a). In the case of the CuNPs from *N. oleander*, AFM analysis examination revealed that the surface shape formation of CuNPs from *N. oleander* had an average of 48.42 nm. The AFM images of the biosynthesized CuNPs from *N. oleander* were spherical (Figure. 3b).

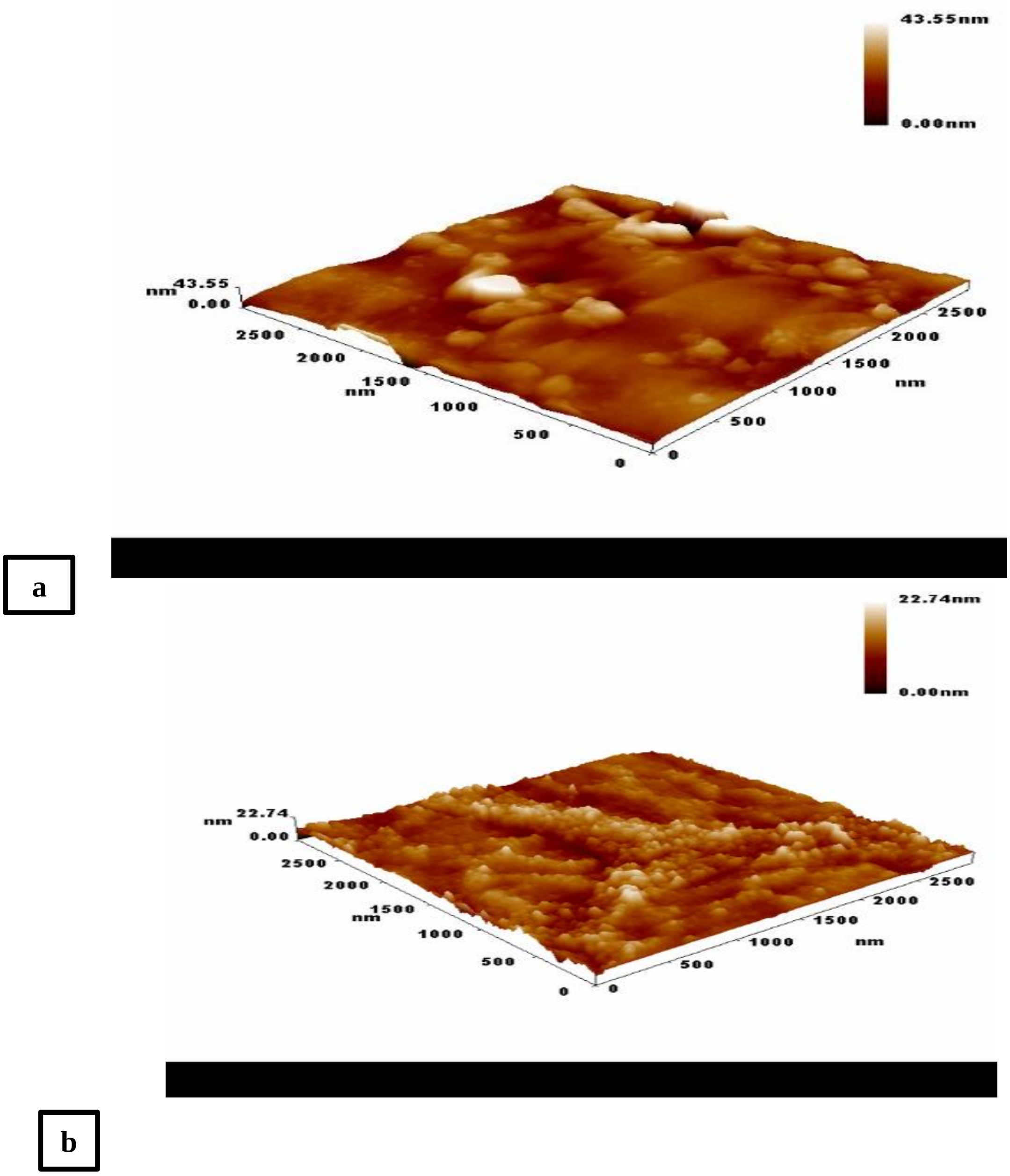


Figure (3): AFM appears as the average diameter of CuNPs synthesis by a) *M. communis* and b) *N. oleander*.

A study examined other extracts; the average CuNPs size produced from the plant extracts *Ziziphus mauritiana L*. in a different investigation was identical to the average size of the particles **(Memon *et al.*, 2020)**.

FT-IR Analysis

The FT-IR analysis was applied to examine the capping and stabilizing capacity of the extracting leaf. The FTIR bands of green synthesized CuNPs using leave extract of *M. communis* indicated the O-H stretch or N-H (alcohol or aliphatic primary amine), they were varied from 3431.13 - 3398.34 cm^{-1}, and for C=C stretch (alkene) was 1629.74 cm^{-1} while for C-H bending (alkene) was 1382.87, finally for C=C stretch (alkene), they were found to range from 819.69 to 794.62. (Table, 2).; (Figure. 4). A study examining CuNPs extracted from citrus peels found FTIR measurements 500-4000 cm^{-1} **(Tshireletso *et al.*, 2021)**. In the case of CuNPs synthesized by *N. oleander*, it was recorded that the absorption frequencies (cm^{-1}) of O-H stretch (alcohol) were varied from 3460.06 to 3431.13 cm^{-1} whereas for C=C stretch (alkene), it was 1629.74 cm^{-1}. For C-H (alkene) was 1384.79 cm^{-1}, while for C-N stretch (amin), the absorption frequencies (cm^{-1}) varied from 1083.92 to 1041.49 cm^{-1} and for C-Br stretch (halo compound), Table, 2 and Figure.5. Another study that examined CuNPs extracted from citrus peels has found FTIR measurement to be 500- 4000 cm^{-1} **(Kalpana *et al*., 2016)**.

Table (2): FT-IR spectroscopy measurement of CuNPs biosynthesized using *M. communis* aqueous and *N. oleander* aqueous extract.

Examined samples	Frequency of Absorption (cm^{-1})	Bonds	Compound class Of Functional Groups
CuNPs biosynthesized Using *M. communis* aqueous extract	3431.13-3398.34	O-H stretch or N-H	Alcohol or aliphatic primary amine
	1629.74	C=C stretch	alkene
	1382.87	C-H bending	alkane
	1064.63	C-N stretch	amine
	819.69-794.62	C=C stretch	alkene
CuNPs biosynthesized using *N. oleander* aqueous extract	3460.06-3431.13	O-H stretch	Alcohol
	1629.74	C=C stretch	Conjugated alkane
	1384.79	C-H	alkane
	1083.92-1041.49	C=N stretch	amine
	615.25-507.24	C-Br	Halo compound

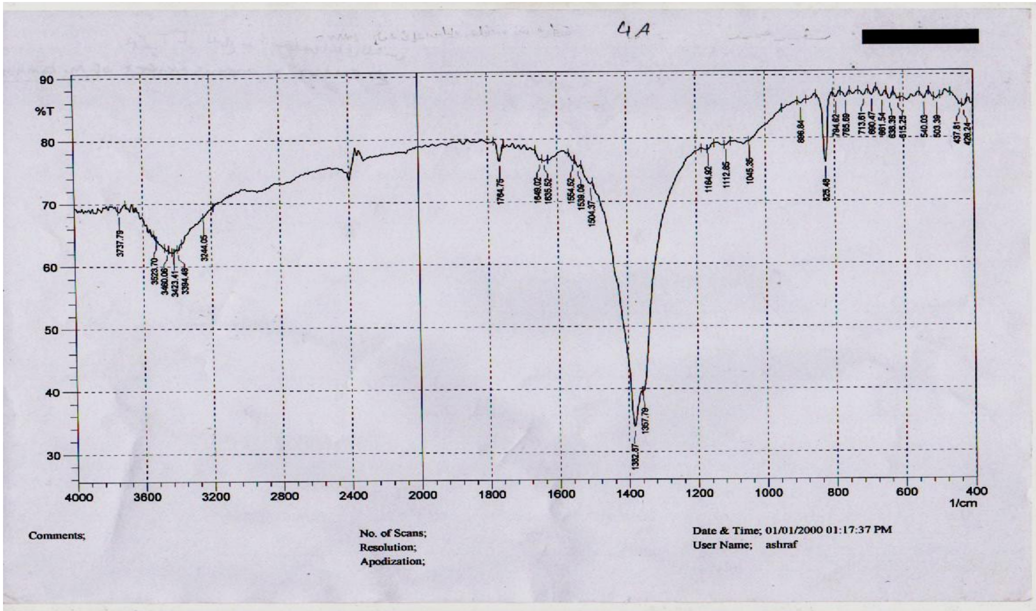


Figure (4): FITR of CuNPs synthesized using *M. communis* aqueous extract.

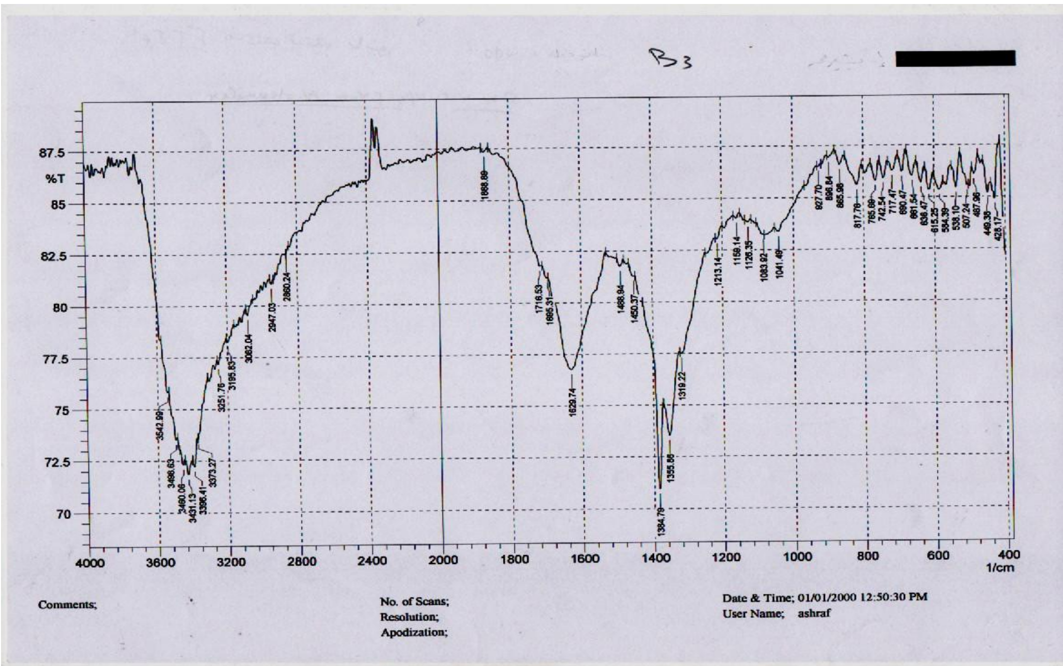


Figure (5): FITR of CuNPs synthesized using *N. oleander* aqueous extract.

Scanning Electron Microscope (SEM)

Scanning electron microscope is one of the most common methods for characterizing produced nanoparticles, and it was used to evaluate the green CuNPs biosynthesized using *M. communis and N. oleander aqueous* extract, shape, and morphology. The findings show that nanoparticles come in all sizes and forms. Particles of a spherical shape were seen in these green synthesized CuNPs using *M. communis and N. oleander;* they are mostly spherical, have a smooth surface, and well dispersed with a close compact arrangement. The average particle diameter was 27.15, 28.32, 35.02, and 38.85nm, to CuNPs biosynthesis using *M. communis* (Figure. 6 a). The average particle diameter of CuNPs using N. oleander was also found to be 31.65, 37.06, 48.86, 49.81, and 60.89 nm (Figure. 6 b). Recent study has reported similar SEM results of CuNPs of *M. communis* where CuNPs biosynthesized in this study were examined as antioxidant and catalytic substances (**Al-Saadi, 2022)**.

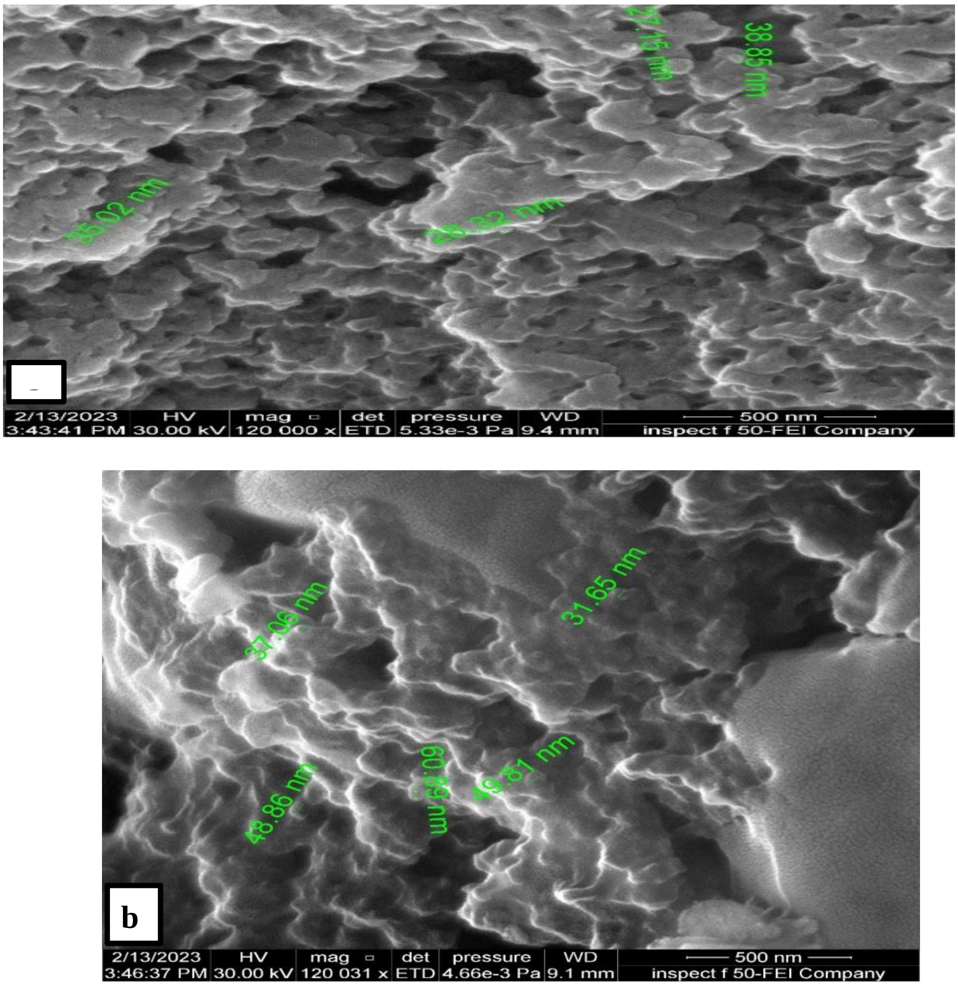


Figure (6): The SEM image shows the size and structure of CuNPs that biosynthesized from a) *M. communis, b) N. oleander.*

Minimum Inhibition concentration

According to the results of this study, (Table 3) shows MIC values that affect all *S.aureus* bacteria samples of different culturing wells in all examined NPs. This study recorded various MIC values related to the *S.aureus* bacteria samples, culturing wells, and different examined CuNPs. Regarding CuNPs of *M. communis*, a 0.09 mg/ml MIC value was recorded in well number 10 of both 1 and 2 bacterial samples, but it was found to be 0.1 mg/ml in well 9 of sample 3, whereas the value of 0.3 mg/ml was detected in well 8 of a bacterial sample of 4 (Table, 3). A study found the best MIC of *M. communis* extract at 25.0 mg/ml for *Candida Albicans* **(Issazadeh *et al.*, 2012)**, whereas recent work **(Mir *et al.*, 2020)** has found MIC of *M. communis* extract tested against Gram-positive bacteria varying from 4.87 to 78.0 µg/ml (0.00487 – 0.078 mg/ml). Regarding CuNPs synthesized using *N. oleander*, the MIC value was reported as 0.7 mg/l in well number 7 of bacterial sample 1, and 0.09 mg/ml was found in well number 10 of sample 2. Meanwhile, 0.1 mg/ml was detected in well number 9 for each bacterial sample of 3 and 4 (Table 3). In a previous study, the extract of *N. oleander* was investigated against Gram-negative and positive bacteria, including *S. aureus*, and reported the lowest (25 µg/ml, which equals 0.025 mg/ml) MIC of plant extract as an effective concentration **(El Sawi *et al.*, 2010)**.

Table (3): MIC values of all samples of various wells for examined Nanoparticles**.**

CuNPs synthesized using *M. communis*	1	10	0.09
	2	10	0.09
	3	9	0.1
	4	8	0.3
CuNPs synthesized using *N. oleander*	1	7	0.7
	2	10	0.09
	3	9	0.1
	4	9	0.1
Mixed of CuNPs synthesized using *M. communis* and *N. oleander*	1	5	3.1
	2	6	1.5
	3	6	1.5
	4	8	0.3
M. communis alcoholic Extract	1	9	0.1
	2	10	0.09
	3	9	0.1
	4	10	0.09
N. oleander alcoholic Extract	1	5	3
	2	9	0.1
	3	6	1.5
	4	10	0.09
Mixed of *M. Commines* and *N. Oleander* alcoholic extract	1	7	0.7
	2	8	0.3
	3	7	0.7
	4	7	0.7

For the mix of CuNPs produced by *M. communis* and *N. oleander,* the minimum MIC value at concentration 0.3 mg/ml was found in well number 8 of sample 4, whereas the maximum MIC value at concentration 3.1 mg/ml was recorded in well number 5 of sample 1. Also, a 1.5 mg/ml MIC was detected in well number 6 of 2 and 3 samples.

An early study investigated the MIC of CuNPs and ZnONPs against positive and negative Gram bacteria and found a concentration varying from 0.002 to 0.128 mg/ml as an effective MIC concentration **(Chavan *et al.*, 2016)**. Also, similar work has examined MIC but by AgNPs against *S. aureus* bacteria and reported a concentration of 0.625 mg/ml **(Parvekar *et al.*, 2020)**. Also, a recent study **(Hamk, 2023)** examined MIC values of ZnONPs against similar bacteria (*S. aureus*), which reported a MIC of 1.0 mg/ml as the most effective concentration.

MIC of CuNPs against *S. aureus* may be affected by factors such as contact time, CUNPs concentrations, bacterial species, strain **(Shiravand & Azarbani, 2017)**, and pH and temperature **(Hamk, 2023)**. Regarding *M. communis* alcoholic extract, a MIC value of 0.1 mg/ml was found in well number 9 of both 1 and 3 samples, while 0.09 mg/ml was recorded in well number 10 of samples 2 and 4. An early study has examined the MIC of alcoholic extract of *M. communis* against *S. aureus* bacteria and reported a value of 0.25 mg/ml as an effective inhibitory concentration **(Mohammed, 2020)**. Also, similar other work tested the MIC of alcoholic *M. communis* extract against *S. aureus* bacteria and found MIC of about 0.2 mg/ml **(Taheri *et al.*, 2013)**. A study investigated the MIC of methanolic leaf extract *M. communis* against (+ve) bacteria and recorded MIC values varied from 0.0048 to 0.078 mg/ml **(Mir *et al.*, 2020)**.

In the *N. oleander* methanolic extract, the highest MIC value of 3.0 mg/l was recorded in well number 5 of bacterial sample 1, while the lowest MIC value of 0.09 mg/ml was found in well number 10. The test that found MIC values of 0.1 mg/ml and 1.5 mg/ml were detected in well numbers 9 and 6 of 2 and 3 samples, respectively (Table 3). A study examined MIC by *R. officinalis* and *N. oleander* methanol leaf extracts on *E. coli, P. aeruginosa, S. aureus, and B. cereus* and found that *S. aureus* was most resistant to plant extract **(Abderrahim *et al.*, 2019)**. For mixed *M. Commines* and *N. Oleander* alcoholic extracts, the maximum MIC value of 0.7 mg/ml was found in well number 7 of the bacterial samples 1, 3, and 4, whereas the minimum MIC value of 0.3 mg/ml was recorded in well number 8 of sample 2.

The current study seems to be the first to test the combination effects of mixed *M. Commines* and *N. Oleander* alcoholic extract, whereas similar studies have not yet been found. However, such combination effects due to MIC values varied from 0.3 to 0.7 mg/ml were higher than those of *M. Commines* (0.09 - 0.3 mg/ml) and for *N. oleander* extract that ranged from 0.09 to 0.1 mg/ml, and these findings may confirm that the effects of mixed both *M. Commines* and *N. Oleander* were better than those of each plant examined separately.

CONCLUSION

This work describes a low-cost, low-impact, straightforward technique for producing copper nanoparticles using *M. communes* and *N. oleander* leaf extract. This method does not require chemical reagents, surfactant templates, or even high temperatures. Copper acetate was utilized as a significant part of the synthesis of Cu NPs. FTIR, AFM, and UV-VIS spectroscopy have all been used to characterize the biosynthesized nanoparticles. CuNPs that were biosynthesized exhibited outstanding antibacterial activity.

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