The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89 (2), Page 6923- 6927

Green Synthesis of Cu/CuO Nanoparticles and Their Antibacterial Effects against *Streptococcus Mutans*

Nagwan Mohammed Saeed [1], Luma Musa Ibrahim [*2], Suha Ali Abdulameer [3], Sahar Hamdan Alani [4]
[1,2,4]Collage of Dentistry, Al-Farahidi University, Baghdad, Iraq
[3] Department of Orthodontics, Al Rafidin University Collage, Baghdad, Iraq
*Corresponding author: **Luma Musa Ibrahim**,email : lumamusa@uoalfarahidi.edu.iq, Mobile: +9647901431473

ABSTRACT

Background: Antibiotic resistance is a serious public health concern that is becoming worse. Although several new antibiotics have been created in recent years, none have demonstrated enhanced efficacy against bacteria that are multidrug resistant.

Objective: Long recognization for the all-encompassing antibacterial properties of Cu/CuO NPs.

Subjects and Methods: FT-IR spectroscopy, UV-visible spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy were used to analyze the produced nanoparticles (SEM).

Results: The UV-visible spectroscopy revealed a Surface Plasmon Resonance (SPR) at 355 nm which is characteristic of silver nanoparticles. FT-IR spectral analysis showed the functional groups which were responsible for the reduction of AgNPs and capping and stabilization. The antimicrobial assay confirmed good inhibitory activity against *Streptococcus mutans*. **Conclusion:** The outcomes demonstrated that the addition of Cu/CuO NPs to medications had considerable antibacterial effects.

Keywords: Biosynthesis, Cu/CuO NPs, Antimicrobial**,** *Streptococcus mutans.*

INTRODUCTION

Pinpoint accuracy nanomaterials have caused quite a stir lately as researchers work to create novel medicinal and analytical procedures for use on people. Cu/CuO nanoparticles are being used more often as antibacterial agents in clothing, bandages, medical equipment, and household items like refrigerators and washing machines. Cu/CuO NPs have attracted a lot of attention in many research fields, including optics, electronics, magnetism, mechanics, catalysis, energy science, nanobiotechnology, and nanomedicine, particularly as an antimicrobial. This is due to their distinctive physical and chemical properties as well as their significant applications [(1)].

An essential component of nanotechnology is the creation of a straightforward, affordable, dependable, and ecologically friendly chemical method for the production of nanomaterials [(2)]. The demand to create an ecologically acceptable nanoparticle production method is growing. Standard processing techniques often yield small quantities of Cu/CuO NPs, while chemical techniques are hazardous and time-consuming [(3)].

One of the essential procedures in nanoscience is the design of a nontoxic technique for the fabrication of nanostructured materials, Covered nanoparticles have been shown to be more effective against bacteria, and encapsulating chemicals are essential to the long-term stability of nanomaterials [(4)]. Furthermore, biologically produced nanostructures offer enormous promise since nps are readily enclosed with a sterol shell which thus provides a physiologic dissolution rate, as these are crucial for the clinical field as well as the limitation of many other production techniques [(5)]. Cu/CuO NPs have significant antiseptic properties that are effective towards highly pathogenic variants as well as Gram-positive and Gram-negative bacteria [(6)]. Cu/CuO NPs have antimicrobial and anti-properties, and they work in concert with several types of antibiotics including b-lactams, sulfonamides, and Cephalosporins [(7)].

Both Gram-negative and Gram-positive bacteria are significant contributors to a variety of illnesses, particularly in hospitals, and are often probiotics. With all main classes of antibiotics used to treat a range of pulmonary illnesses, dermatitis, and sexually associated infections, an increase in the prevalence of resistance to antibiotics has been noted. Numerous scholars are interested in discovering innovative, efficient, resistance-free, and affordable antimicrobial reagents due to the ubiquity and rising number of microbes that are resistant to many antibiotics as well as the rising expenses of health care [(8)]. Due to such difficulties and demands, metal cleaning agents have been created and are now widely used. Compared to probiotics, these agents have a far reduced propensity to cause the emergence of antimicrobial sensitivity. Among the most prevalent illnesses in the world are dental caries and dental plaque, which are brought on by a concoction of microbes and dietary residues. In the presence of fermentable carbohydrates like sucrose and fructose, certain forms of acid-producing bacteria, in particular Streptococcus mutans, colonize the tooth surface and harm the hard tooth structure. This study examines the creation of green nanoparticles that block S. mutans [(9)].

MATERIALS AND METHODS

Preparation of plant extract

copper nitrate (Cu_2O, Alfa Aesar, 99.9%), 0.1 M, was created and dissolved in 100 mL deionized water. The Accol plant extract (Alhagi graecorum) weighs 1 g, and it is dissolved in 100 ml of deionized water before being placed on a magnetic jacket for 10 minutes at 60

Received: 6/7/2022
Accepted: 11/9/2022

m, centrifuged for 15 minutes at 4000 RPM, filtered using filter paper, and kept in a cool area, as depicted in **Fig. 1**.

Synthesis of Cu/CuO NPs by using Alhagi graecorum

While the extract is set on a magnetic stirrer and heated to 80°C, the copper nitrate solution is progressively distilled into the Accol plant extract. As can be seen in **Figure 1**, the solution's color changes after 60 minutes, signaling the creation of the molecule Cu/CuO NPs.

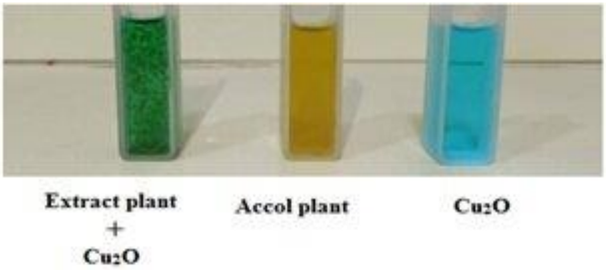

Fig.1: Stages of composition Cu/CuO NPs

Antibacterial assay

Clinical isolates

Streptococcus mutans, the bacterium that causes dental caries, was identified by the College of Dentistry at Al Farahidi University in Baghdad, Iraq. On nutritional agar plates kept at 4 °C, all bacterial clinical isolates were routinely maintained.

Bacterial sensitivity test

In bacterium trap cells, the bacteria used by the specimen on the supplied culture medium were captured, and the diffusion method was used to evaluate the amount of bacterial growth inhibition. After that, the tablets were made and soaked for a few minutes in the nanoparticle solution before being placed in all of the bacterial plates. Cu/CuO NPs were studied to see how they affected bacterial inhibition. The findings were recorded the following day by measuring the zones of inhibition surrounding each disc using a ruler after that the plates had been incubated at 37°C for 24 hours.

Ethical considerations:

College of Dentistry, Al-Farahidi University, and the Baghdad Hospital's Ethics Committee gave their approval for the study . Informed written consent was obtained from each participant in the study after assuring confidentiality under code 1106/8. All research involving human beings complied fully with the World Medical Association's Declaration of Helsinki.

RESULTS

X-ray diffraction analysis

Cu/CuO NPs made into a greenway are shown in X-ray diffraction data as shown in (Table **1**). The XRD pattern for produced Cu/CuO NPs using an accol plant extract is shown in **Fig.2.**

Table 1: Cu/CuO NPs made into a greenway are shown in X-ray diffraction data.

Sample	2θ (Deg.)	hkl	FWHM (Deg.)	K.λ	$\theta = rad$	Cos θ	C.S	Average
Cu	43.84	(111)	0.5904	0.1386	0.0174	0.928141	14.536229	11.011263
	50.94	(200)	1.1808	0.1386	0.0174	0.903393	7.46725	
	74.44	(220)	0.9055	0.1386	0.0174	0.797517	11.03025	
CuO	18.3	(020)	1.1808	0.1386	0.0174	0.987353	6.832274	19.47941
	24.4	(021)	1.1808	0.1386	0.0174	0.977553	6.900766	
	33.6	(002)	0.1968	0.1386	0.0174	0.957578	42.2684	
	35.2	(042)	0.3444	0.1386	0.0174	0.953474	24.25728	
	42.7	(131)	0.5904	0.1386	0.0174	0.931788	14.47941	

By using x-ray diffraction assessment, the density, phase recognition, and crystallinity of the Cu/CuO NPs were evaluated. Cu signals were observed in the diffraction pattern of the XRD investigation ,Cu is responsible for the planes (111), (200), and (220), as well as the peaks at 2 angles of 43.8°, 50.9°, and 74.4°. XRD measurements verified the production of CuO nanoparticles. $D = \frac{0.9\,\lambda}{\beta\,\cos\theta}$ ... (1)

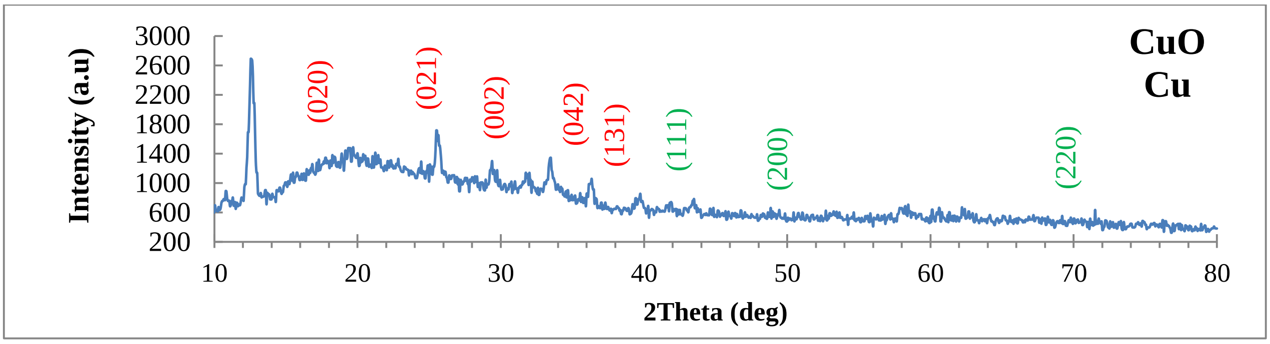

Fig. 2: XRD spectrum of Cu\CuO NPs

The band-gap energies of Cu/CuO nanostructures are growing; it might be a sign of the quantum entrapment impact owing to the shrinking size of the structure, as shown in **Table (2).**

Table.2 shows the band gaps in the samples' UV-Vis absorption spectra.

Manufacturing method	Absorption wavelength(nm)	Band gap (eV)
Cu/CuO NPs chemical method	355	3.49

Field-Emission scanning electron microscope analysis

The morphological investigation was carried out using field emission scanning electron microscopy. The Cu/CuO nanoparticles are shown in **Fig. 3 (a, b).** 2 µm and 200 nm FESEM pictures. These photos depict the spherical shape of nanoparticles. The spherical shape indicates a particle size of 30–74 nm. Surprisingly, the crystallite size determined by XRD is virtually identical to the particle size determined by FESEM.

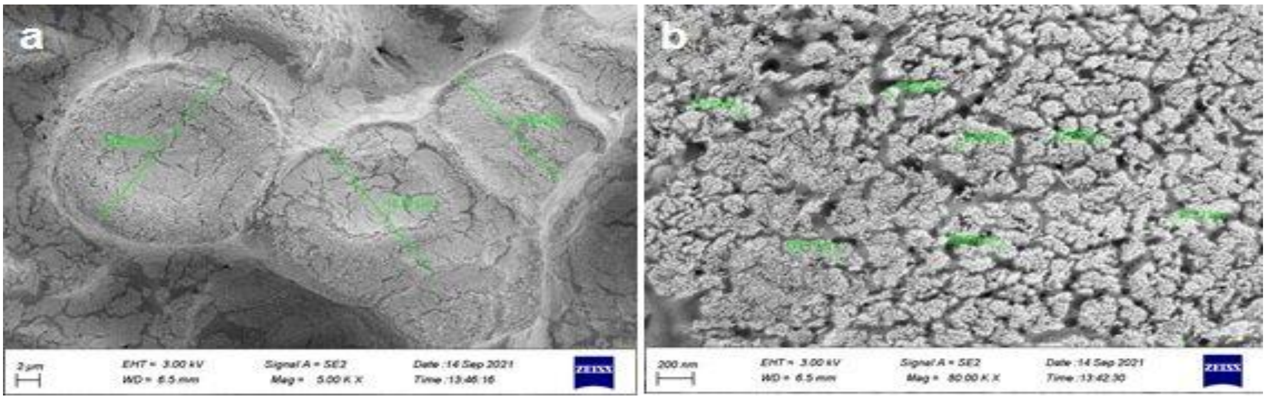

Fig 3: FESEM image of Cu/CuO NPs (a) 2 µm, (b) 200 nm.

Transmission electron microscope (TEM) analysis

Using the transmission electron microscope (TEM), to examine the size, shape, moreover, nanoparticles Cu/CuO morphology, as seen in Fig 4. The nanoparticles overlap, allowing for the creation of a flower-like nanostructure with a spherical and oval shape and no large agglomerations.

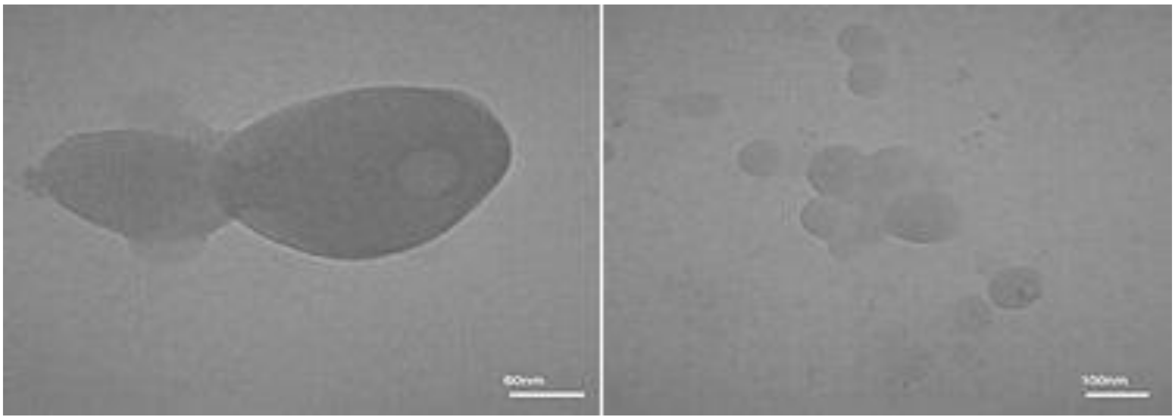

Fig 4: 100 nm and an 80 nm TEM picture of Cu/CuO nanoparticles.

Optical Properties

Optical Energy Band Gap (E_g)

Cu/CuO NPs' UV uptake spectra were obtained to figure out their band gap, as shown in **Fig 5.**

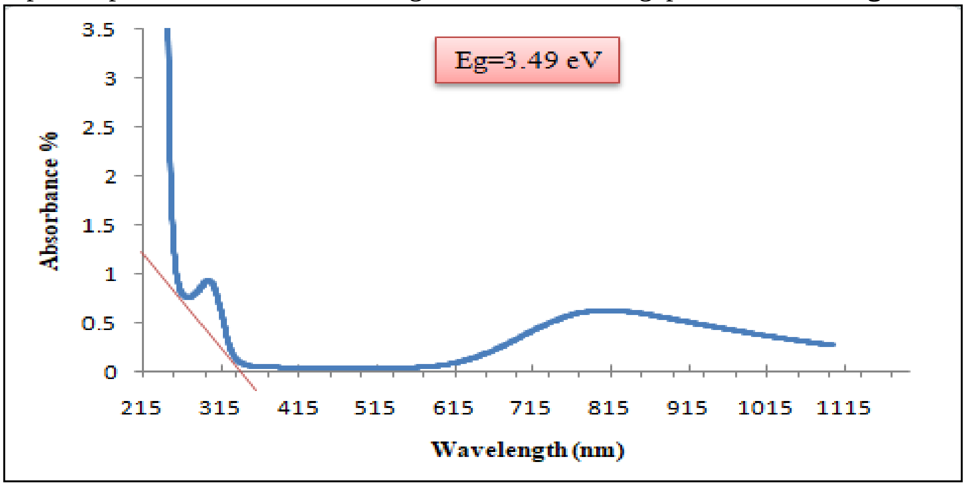

Fig.5: Cu/CuO NPs absorbance spectra as a function of wavelength.

Biological properties:

Table 3 illustrates the effect of Cu/CuO NPs at 100% concentration on G-positive *S. mutans*. The greatest suppression proportion for G-positive microbes reached 27 mm in NPs at a concentration of 100%.

Table.3 The inhibitory efficacy of Cu/CuO NPs

Type bacterial	Sample	Inhibition zone / mm
		100%
S. mutans	Cu/CuO	27
Control	Cu/CuO	0

DISCUSSION

The planes (020), (021), (002), (042), and (131) in XRD analysis suggest monoclinic crystallite formation at two angles of 18.3, 24.4, 33.6, 35.2, and 42.7, respectively. These appear to be extremely similar to those in JCPDS File No. 5-0661. The outcome is consistent with the information provided. Table 1 displays the diffract angles, half-width, and particle size [10, 11]. The widening of the nanoparticles' diffraction peaks may be seen in the x-ray patterns, and the crystallite size (D) can be determined [12] using the Scherer formula . Band gap energy may be calculated using the maximum absorption in nanomaterials. The surface's significant barrier confines the holes and electrons of the valence band, as predicted by quantum confinement theory. The band gap (Eg) is increased by the minimum photonic transfer of energy by the valence to the conduction band [13].

The optical energy bandgap for Cu/CuO Nanoparticles has been determined using the absorption edge, which correlates towards the primary specimen solubility boundaries. The band-gap energies of Cu/CuO nanostructures are growing; it might be a sign of the quantum entrapment impact owing to the shrinking size of the structure. As the number of nanomillions grows; the surface-to-volume index rises, as well as changing structural and optical characteristics generally [14].

The majority of bacterial cell membranes feature stomata or holes at the nanoscale. To influence bacterial development through their impact on cell function, the nanomaterials must first enter or pass through such barriers, then settle down sufficiently [15].

There are many reasons why nanostructures restrict bacterial growth because their mass, durability, and additional density in the growth environment are comparable to those of microbial mortality. This gives nanoparticles the extra ability to engage with bacteria , the theory is the same, regardless of how researchers have exploited nanoparticles to limit bacterial growth: such particles have a strong charge that may link to the surface of the bacterial cell, which has an adverse charge, leading particles to gather on the cell membrane's surface. Bacteria lose membrane functions including breathing, permeability, and electron transfer as a consequence of damage to the membrane caused by a change in its physical and chemical characteristics [16, 17]. In one study, Nanoparticles lead to down-Regulation of responsible pathogenic gene expression in Proteus *spp* isolated from UTI [18]. The resistance of certain pathogenic bacteria that produced a deadly illness may be lowered, however, by using magnetic fields and loud sounds as a physical force treatment. The reason for this is that both magnetic fields and sounds are examples of physical forces [19], in a general sense, nanoparticles have the potential to be used either as a replacement for or in addition to the traditional therapies for a variety of illnesses.

Because of the information that has been presented up to this point, it was essential to evaluate Cu/CuO Nanoparticles in terms of their efficacy against a diverse collection of pathogenic bacteria,viruses, and parasites. Some of these bacteria include *Clostridium perfringens* [20], *Brucella melitensis* [21], *Proteus vulgaris* [22,23] , *Staphylococcus aureus* [24], *Pseudomonas aeruginosa* [25], and *Toxoplasma spp* [26,27], SARS-Cov-2 [28].

CONCLUSIONS

In this work, a method is developed to clarify the reinforcing impacts on Cu / CuO NPs regardless of the use of medications. To do this, researchers chemically created Cu/CuO NPs.

Then, employing various methodological approaches, Cu/CuO Nanoparticles were determined. These results indicate that the biosynthesized Cu/CuO NPs can be used as a cofactor for the inhibition of Streptococcus mutans. Our results, therefore, lend credence to the idea that Cu/CuO NPs have potent antiseptic properties that must be exploited to boost the effectiveness of current medications over bacteria.

Conflict of interest: no conflict.
Sources of funding: by co-authors.
Author contribution: Authors are contributed equally in the study.

REFERENCES

1. **Reddy K (2017):** Green synthesis, morphological and optical studies of CuO nanoparticles. J Mol Struct., 1150: 553-557.
2. **Gebremedhn K, Kahsay M, Aklilu M (2019):** Green synthesis of CuO nanoparticles using leaf extract of Catha edulis and its antibacterial activity. J Pharm Pharmacol., 7(6): 327-342.
3. **Fuku X, Modibedi M, Mathe M (2020):** Green synthesis of Cu/Cu2O/CuO nanostructures and the analysis of their electrochemical properties. SN Appl Sci., 2(5): 1-15.
4. **Qasim M, Udomluck N, Chang J *et al*. (2018):** Antimicrobial activity of silver nanoparticles encapsulated in poly-N-isopropylacrylamide-based polymeric nanoparticles. Int J Nanomedicine , 13: 235–249.
5. **Carrion C, Nasrollahzadeh M, Sajjadi M *et al*. (2021):** Lignin, lipid, protein, hyaluronic acid, starch, cellulose, gum, pectin, alginate and chitosan-based nanomaterials for cancer nanotherapy: Challenges and opportunities. Int J Biol Macromol., 178 (1): 193-228.
6. **Rajendaran K, Muthuramalingam R, Ayyadurai S (2019):** Green synthesis of Ag-Mo/CuO nanoparticles using Azadirachta indica leaf extracts to study its solar photocatalytic and antimicrobial activities. Mater Sci Semicond., 91: 230-238.
7. **Tharchanaa S, Priyanka K, Preethi K *et al*. (2021):** Facile synthesis of Cu and CuO nanoparticles from copper scrap using plasma arc discharge method and evaluation of the antibacterial activity. Mater Technol., 36(2): 97-104.
8. **Chakraborty N, Banerjee J, Chakraborty P *et al*. (2022):** Green synthesis of copper/copper oxide nanoparticles and their applications: a review. Green Chem Lett Rev., 15(1):187-215.
9. **Forssten S, Björklund M, Ouwehand A (2010):** *Streptococcus mutans*, caries and simulation models. Nutrients, 2(3): 290-298.
10. **Suramwar N, Thakare S, Khaty N (2012):** Synthesis and catalytic properties of nano CuO prepared by soft chemical method. Int J Nano Dimens., 3(1): 75-80.
11. **Kalita C, Karmakar S (2018):** Analysis of Structural and Optical Features of CuO Nanoparticles Synthesized at Different Molarities. Int j sci res phys appl sci., 6: 2348-3423.
12. **Abdalameer N, Mazhir S, Aadim K (2020):** The effect of ZnSe Core/shell on the properties of the window layer of the solar cell and its applications in solar energy. Energy Rep., 6:447-458.
13. **Ahamed A, Ramar K, Kumar P (2016):** Synthesis and characterization of ZnSe nanoparticles by co-precipitation method. J Nanosci Nanotechnol., (1):148-150.
14. **Morones J, Elechiguerra J, Camacho A *et al*. (2005):** The bactericidal effect of silver nanoparticles. Nanotechnology, 16(10):2346.
15. **Ahmadi F, Kordestany A (2011):** Investigation on silver retention in different organs and oxidative stress enzymes in male broiler fed diet supplemented with powder of nanosilver. Am Eurasian J Toxicol Sci ., 3(1): 28-35.
16. **Asharani P, Wu Y, Gong Z *et al*. (2008):** Toxicity of silver nanoparticles in zebrafish models. Nanotechnology, 19(25): 255102.
17. **Abdalameer N, Khalaph K, Ali E (2021):** Ag/AgO nanoparticles: Green synthesis and investigation of their bacterial inhibition effects. Mater Today, 45: 5788-5792.
18. **Saleh T, Hashim S, Malik S *et al*. (2019):** Down-Regulation of flil Gene Expression by Ag Nanoparticles and TiO2 Nanoparticles in Pragmatic Clinical Isolates of *Proteus mirabilis* and *Proteus vulgaris* from Urinary Tract Infection. Nano Biomed. Eng.,11(4): 321-332.
19. **Ali M, Al-Rubaii B (2021):** Study of the Effects of Audible Sounds and Magnetic Fields on *Staphylococcus aureus* Methicillin Resistance and *mec*A Gene Expression. Trop J Nat Prod Res., 5(5):825-830.
20. **Hashim S, Fakhry S, Rasoul L *et al.* (2021):** Genotyping toxins of *Clostridium perfringens* strains of rabbit and other animal origins. Trop J Nat Prod Res., 5(4):613–616.
21. **Awadh H, Hammed Z, Hamzah S *et al*. (2022):** Molecular identification of intracellular survival related *Brucella melitensis* virulence factors. Biomedicine (India) , 42(4):761–765**.**
22. **Abdul-Gani M, Laftaah B (2017):** Purification and characterization of chondroitinase ABC from *Proteus vulgaris*, an Iraqi clinically isolate. Curr Sci., 113(11):2134-2140.
23. **AL-Imam M, AL-Rubaii B (2016):** The influence of some amino acids,vitamins and anti-inflammatory drugs on activity of chondroitinase produced by *Proteus vulgaris* caused urinary tract infection. Iraqi J Sci., 57 (4A):2412-2421.
24. **Fakhry S, Hammed Z, Bakir W *et al*. (2022):** Identification of methicillin-resistant strains of *Staphylococcus aureus* isolated from humans and food sources by use mecA 1 and mecA 2 genes in Pulsed-field gel electrophoresis technique. Bionatura, 7(2), 44. http://dx.doi.org/10.21931/RB/2022.07.02.44.
25. **Shehab Z, AL-Rubaii B (2019):** Effect of D-mannose on gene expression of neuraminidase produced from different clinical isolates of *Pseudomonas aeruginosa*. Baghdad Sci J.,16(2):291–298.
26. **Jiad A, Ismael M, Salih T *et al*. (2022):** Genotyping and evaluation of interleukin-10 and soluble HLA-G in abortion due to toxoplasmosis and HSV-2 infections. Ann Parasitol., 68(2):385–390.
27. **Jiad A, Ismael M, Muhsin S *et al*. (2022):** ND2 Gene Sequencing of Sub fertile Patients Recovered from COVID-19 in Association with Toxoplasmosis. Bionatura, 7(3), 45. http://dx.doi.org/10.21931/RB/2022.07.03.45.
28. **Rasoul L, Nsaif M, Al-Tameemi M *et al*. (2022):** Estimation of primer efficiency in multiplex PCR for detecting SARS-Cov-2 variants. Bionatura, 2022, 7(3), 48. http://dx.doi.org/10.21931/RB/2022.07.03.49.