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Evaluation of Inhibition Effect of Nanohybrid Curcumin (Cur-ZnO) and Cur-free Against Some Types of Bacteria

N. Sh. Ahmed[1,]*, A.A.K. Al-Ghanimi[2], E.I. Al–Shamary[2]

Abstract

This study aimed to prepare an efficient nanohybrid food preservative by loading curcumin on zinc oxide. The prepared compound was characterized using microscopy and spectral methods. Fourier transform infrared spectrum of the nanohybrid curcumin showed a shift in the frequencies of some chemical groups towards high and low frequencies. X-ray diffraction (XRS) spectrum also revealed the emergence of new diffraction planes in the nanohybrid compound, Compared with the carrier spectrum of zinc oxide, suggesting that the preservative under study was in the nanoscale. Characterization with atomic force microscope (AFM) confirmed that the average dimeter of the nanohybrid preservative particles was 68.07 nm, while using scanning electron microscope revealed the appearance of zinc oxide in layers and well-defined hexagonal shapes superimposed on top of each other in irregular shape and sizes. Some of these shapes turn into shapes like scattered pieces of wood as well as the formation of compounds. with high porosity in the nanohybrid preservative. The inhibitory efficacy of the nanohybrid curcumin and free curcumin was evaluated against some gram-positive and gram-negative bacterial species isolated from food.

Keywords: Nanohybrid curcumin, characterization, XRD, AFM

INTRODUCTION

Nanotechnology is one of the modern technologies that recently brought about a global revolution through its entry into chemical, physical and biological studies, as it is no longer just science fiction in which the theoretical side surpasses the practical reality but has become a reality that receives the attention of many developed countries of the world [1]. Nanotechnology is attributed to nanometer because it is the most accurate metric unit of measurement with a length of 10^{-9} meters, the term "nano" refers to the Greek word "nanos" which means dwarf and which refers to objects of small sizes [2].

Nanocomposites are hybrid materials that have different phases, at least one of these phases is at the nanoscale, the layers of nanocomposites can be formed inserting the guest anion in the inner layers of the inorganic carrier such as zinc oxide, magnesium oxide and other metal oxides without changing the composition of these layers and this process is called 'interrelation'. On this basis, organic and inorganic composites can be used to synthesize an unspecified set of hybrids [3–5].

The mechanism of action of nanoparticles as inhibitors of microorganism varies according to

*__Author for Correspondence__
N. Sh. Ahmed
E-mail: nour.shokr1102a@coagri.uobaghdad.edu.iq

[1]Researcher, Department of Food Science, College of Agriculture University of Baghdad, Iraq
[2]Professor, Department of Biology, College of Science, University of Kerbala, Kerbala, Iraq
[3]Professor, Department of Food Science, College of Agriculture University of Baghdad, Iraq

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their types. These particles have a volume of less than 100 nm, which means increase in their area relative to their size and thus allow for increased binding with the cell wall, which in turn increases their inhibitory efficiency [6, 7].

The inhibitory action of nanoparticles against microorganism (bacteria and fungi) is summarized by several mechanisms that include inhibiting DNA synthesis, stopping the production and delivery of energy to the cell [8, 9], direct interference with and penetration of the cell membrane or wall, preventing the formation of biofilms, and the internal effects of damage to enzymes, proteins and nucleic acids inducing the host's immune response [10]. It also works to produce toxic reactive oxygen species (ROS) and their accumulation on the cell wall of bacteria that increase its permeability due to damage to the protein pumps responsible for regulating the process of nutrient influence to and from the cell and their loss of capacity [11].

Turmeric has been used as a type of food spice in food coloring and also in dyeing textiles, as it has been used in folk cosmetics and in the treatment of various types of diseases, and is commonly used in traditional Indian medicine to treat intestinal, gastrointestinal, pulmonary and hepatic disorders, loss of appetite, cough, sinusitis, in addition to the treatment of diabetic wounds, arthritis and rheumatism [12]. Many studies have also addressed the multiple functional biological activities of curcumin, as an anti-obesity [13], anti-inflammatory, anticancer, anti-diabetic and hepatic prevention [14], as an antioxidant, anticoagulant antifungal, antibacterial, antifibrosis, anti-ulcer and used to relieve postoperative inflammation, and is also used in some countries, including Thailand, in the form of capsules as dietary supplements to activate muscles and improve performance in exercise and possesses. Curcumin additionally has effects on lipids and lipid metabolism [15]. Teow et al. [16] described the biological effect of curcumin as an antidote to a wide range of genera and types of bacteria such as *S. aureus*, *Mycobacterium tuberculosis*, *Salmonella paratyphi*, and *Trichophyton gypseum*. Recent research has also indicated that curcumin is effective against many drug-resistant bacterial strains [17]. This study aimed to preparation of an efficient nanohybrid food preservative from curcumin and evaluation it’s inhibitory activity against some food spoilage microorganism.

MATERIALS AND METHODS

The nanohybrid preservatives were prepared according to the method described by Kolekar et al. [18], with some modification. Briefly, 50 mL of the free-curcumin were added to the solution of zinc oxide (ZnO), prepared by dissolving 1 g of ZnO in 50 mL of ethanol 50% and the mixture was magnetically stirred at room temperature for 2 hours. The mixture was put in a shaking incubator at 37°C for 18 hours, followed by incubation in an incubator at 37°C for 24 hours. The mixture was centrifuged at 5000 rpm for 20 minutes, washed several times with deionized water, and dried at 25°C. It should be noted that the prepared nanohybrid food preservative is given Cur-ZnO symbol.

Characterization of the Nanohybrid Curcumin

Several technics were used to characterize the nanohybrid curcumin under study, including Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), atomic force microscopy (AFM), scanning electronic microscopy (SEM), and precise analysis of C, H, and N elements.

FT-IR: The infrared spectrum for each of the nanohybrid curcumin and free-curcumin as well as the zinc oxide were carried out by making disk from each the compound with potassium bromide (KBr) after grinding well and measuring the infrared spectrum in a wave number range 4000 to 400 cm^{-1}.

XRD: X-ray diffraction spectrum was used to characterize the nanohybrid curcumin and free-curcumin. XRD shows the difference in the thickness of the layer before and after the insertion process for curcumin by using Bragg’s law ($n\lambda = 2d \sin\theta$).

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Atomic Force Microscopy (AFM): In order to measure the diameters, sizes and aggregation of the nanoparticles, the samples of the nanohybrid curcumin were characterized by AFM. Samples were assessed in the Chemical Department of Baghdad University's College of Science.

Accurate Analysis of C, H and N elements: C, H and N percentages in the nanohybrid curcumin and free curcumin were analyzed.

Evaluation of Inhibition Effect of Nanohybrid Curcumin Cur-ZnO Against Some Spoilage Bacterial Isolates

The diffusion method using drilling as described by Patil et al. [19] was used to detect the inhibitory efficacy of hybrid nano preservatives after being dissolved in dimethyl sulfoxide (DMSO).

The inhibitory efficacy of nanohybrid curcumin Cur-ZnO and Cur-free against *Klebsilla pneumonia, E. coli, E. coli O157, Staphylococcus aurous, Salmonella cholera, Bacillus cereus, Acintobacter boumannii*, *Proteus hauseri Comlex*, *Entero gergoviae*, and *Hevani alvei* was tested according to the well diffusion assay method described by Patil et al. [19]. We used concentrations of the Cur-ZnO and Cur-free of 0.02, 0.05, 0.07, 1, 5, 10, 15, and 20 mg/mL and zinc oxide at a concentration of 20 mg/mL as a control treatment after being dissolved in DMSO.

RESULTS AND DISCUSSION

Characterization of Nanohybrid Curcumin Cur-ZnO by FT-IR

FT-IR spectroscopy is used to identifying organic molecules by detecting the active groups and bonds present in the molecules and this is done by exposing the sample to infrared energy and measuring the vibration energy of the bonds that bind these groups, as these vibrations take several forms such as expansion, curvature, torsion and oscillation depending on the structure of the molecules and the composition of the active groups in them [20].

Infrared Spectrum for Zinc Oxide

It was noticed that the zinc oxide layers showed an inconspicuous band located at the frequency range of 400 to 500 cm^{-1} (Figure 1) attributable to the vibration of the (Zn-O) metal bond [21].

Infrared spectrum for free curcumin is shown in Figure 2. Several of the unique bands of free-curcumin appeared. The band at 3504cm^{-1} refers to a stretching vibration of the hydroxyl bond (O-H) stretch interfering with the stretch of the aromatic C-H bond. as reported Nasir et al. [22]. The frequency of the OH group ranges between 3200 and 3600 cm^{-1}. The appearance of the vibration frequency of the aromatic C-H group is at 3020 cm^{-1} according to Silverstein and Bassler [23]. While at the frequency 2939 cm^{-1}, the emergence of an absorption bands belonging to the C-H aliphatic group is observed, according to Nasir et al. [22] its appearance is limited to 2800 to 3000 cm^{-1}. The band at 1629 cm^{-1} is attributable to the vibration of the carbonyl group C=O. The band at a frequency of 1597 cm^{-1} is attributed to the vibration of olefinic C=C. The two bands observed at the frequencies 1460 and 1506 cm^{-1} are due to the structural extension of the benzene ring. The appearance of the band at the frequency 1427 cm^{-1} is attributed to the curvature of the CH3 group. The two bands at frequencies 1278 and 1028 cm^{-1} are for the asymmetric and symmetrical stretching of the C-O-C etheric group, respectively. The band shown at 1155 cm^{-1} indicates the phenolic C-O stretch. A band is observed at the frequency 813 cm^{-1} indicating the curvature of the C-H aromatic bonds beyond the plane [23].

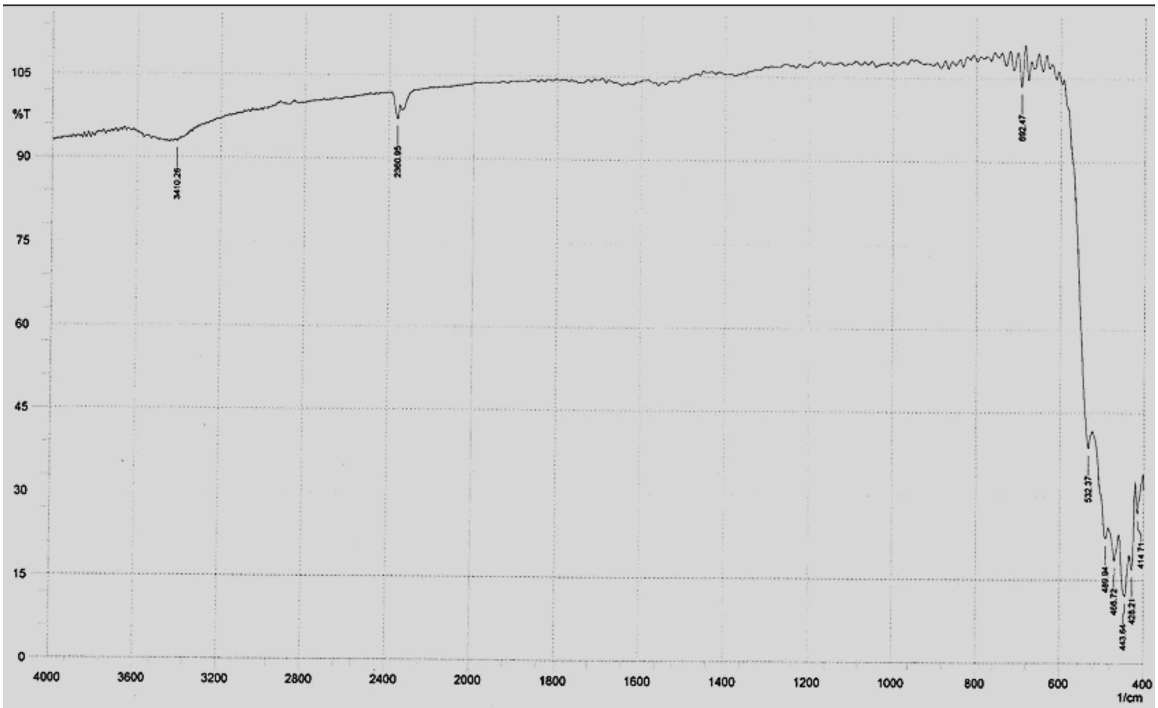


Figure 1. Infrared spectrum for zinc oxide.

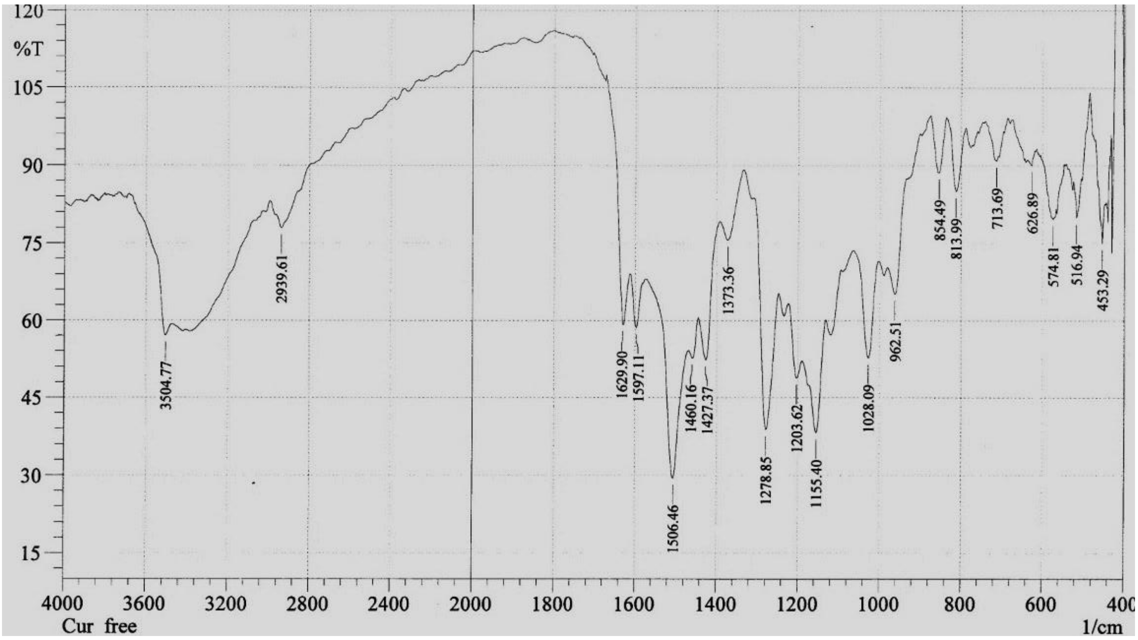


Figure 2. Infrared spectrum for free curcumin.

Infrared Spectrum of Nanohybrid Curcumin Cur-ZnO:

The results shown in Figure 3 indicate the success of the intercalation of curcumin preservative between the layers of zinc oxide through the occurrence of overlaps between the bands and shifts suffered by some bands as well as the emergence of new bands and can be explained by the most important bands shown in this spectrum. Noting the appearance of a band at the frequency 3506 cm^{-1} which indicates the vibration of the stretch of the hydroxyl bond (O-H) overlapping with the stretch of the aromatic C-H bond. While at the frequency 2974 cm^{-1} the appearance of the absorption band of the C-H aliphatic group has suffered a shift towards a higher frequency after it was noticed at the frequency 2939 cm^{-1}.

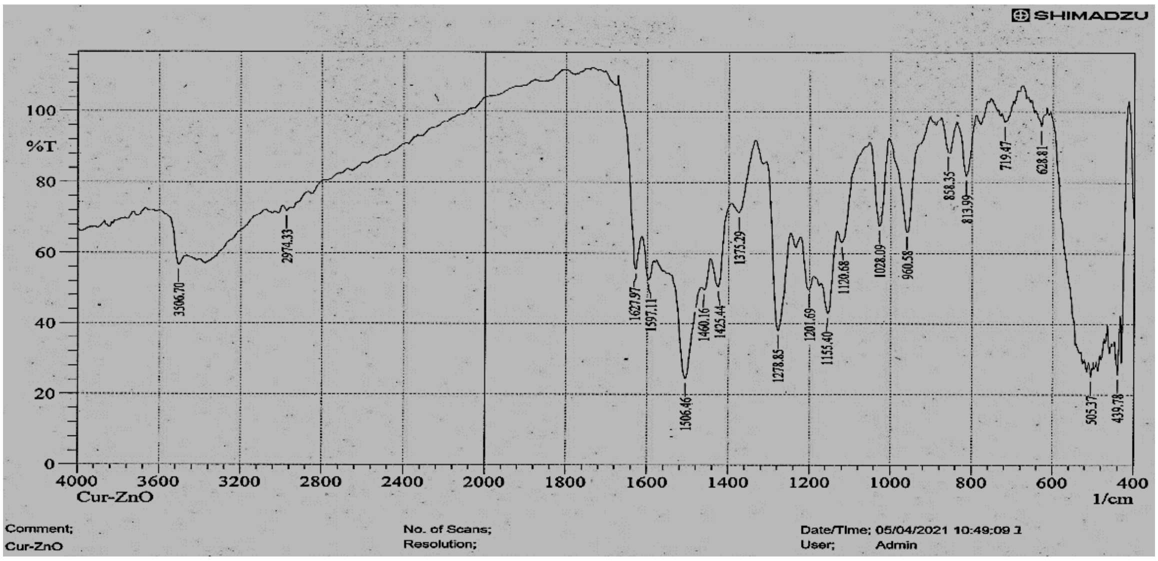


Figure 3. Infrared spectrum for nanohybrid curcumin Cur-ZnO.

Characterization by Using X-Ray Diffraction Spectrum (XRD)

The XRD of both Cur-ZnO nanohybrid curcumin and zinc oxide layers was performed to check the difference in layer thickness before and after the process of inserting curcumin between the layers of zinc oxide using Bragg's law. Figure 4 shows the XRD spectrum of zinc oxide and shows the diffraction of planes (100) at 2θ of 31.29° and with a crystal distance (*d*) equal to 0.281 nm, the plane (002) at (2θ) of 34.82° with a crystal distance equal to 0.259 nm, and the plane (101) appeared at 2θ of 36.29° and has a crystal distance equal to 0.247 nm [21].

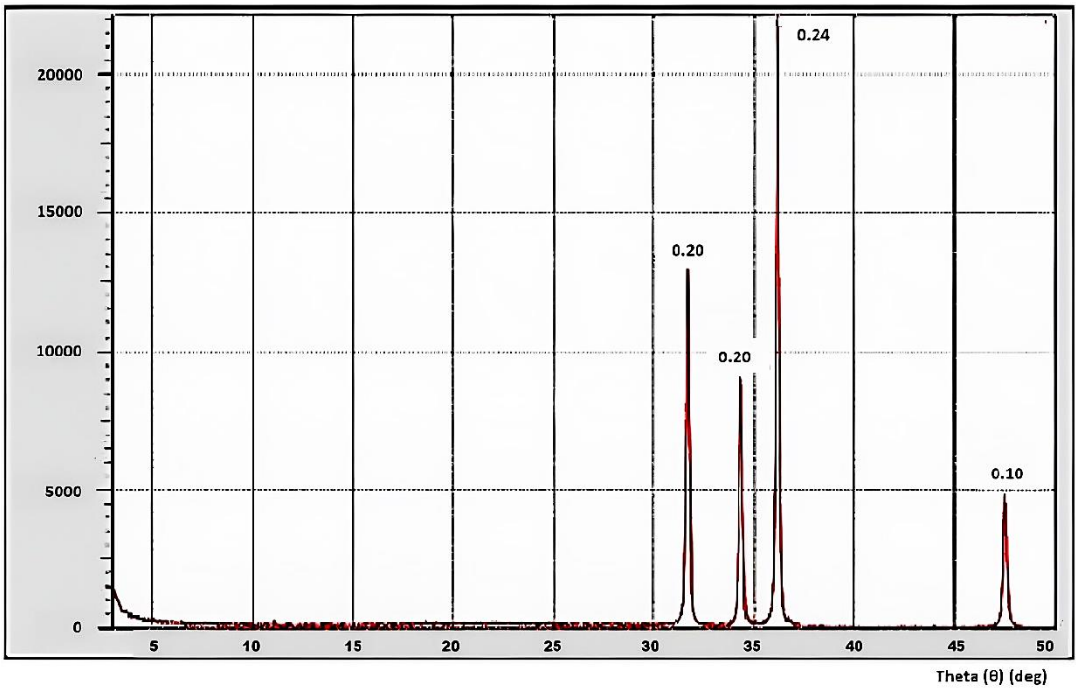


Figure 4. X-ray diffraction (XRD) spectrum for ZnO.

When the ion exchange between the layers of zinc oxide and free-curcumin occurs a diffraction plane (003) appeared at the 2θ of 14.46° with a crystal distance of 0.61 nm, which indicates the success of intercalation and the formation of a nanohybrid curcumin Cur-ZnO as shown in Figure 5 while it indicates that the plane (006) was overlapped with the plane (100) at 2θ of 31.29.

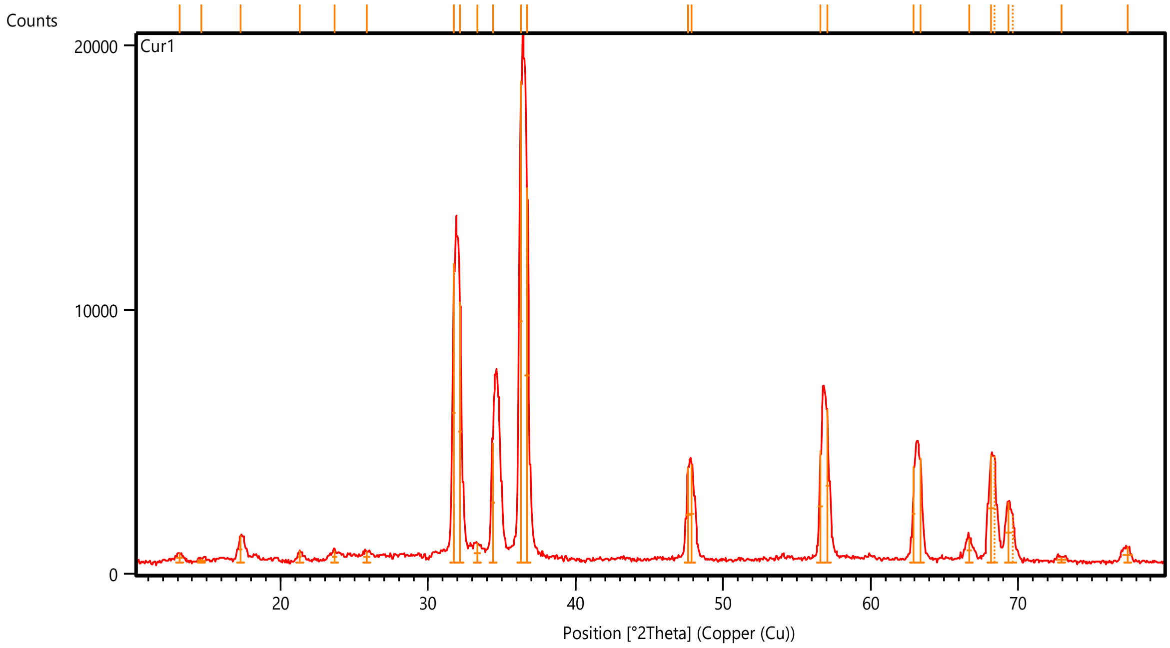
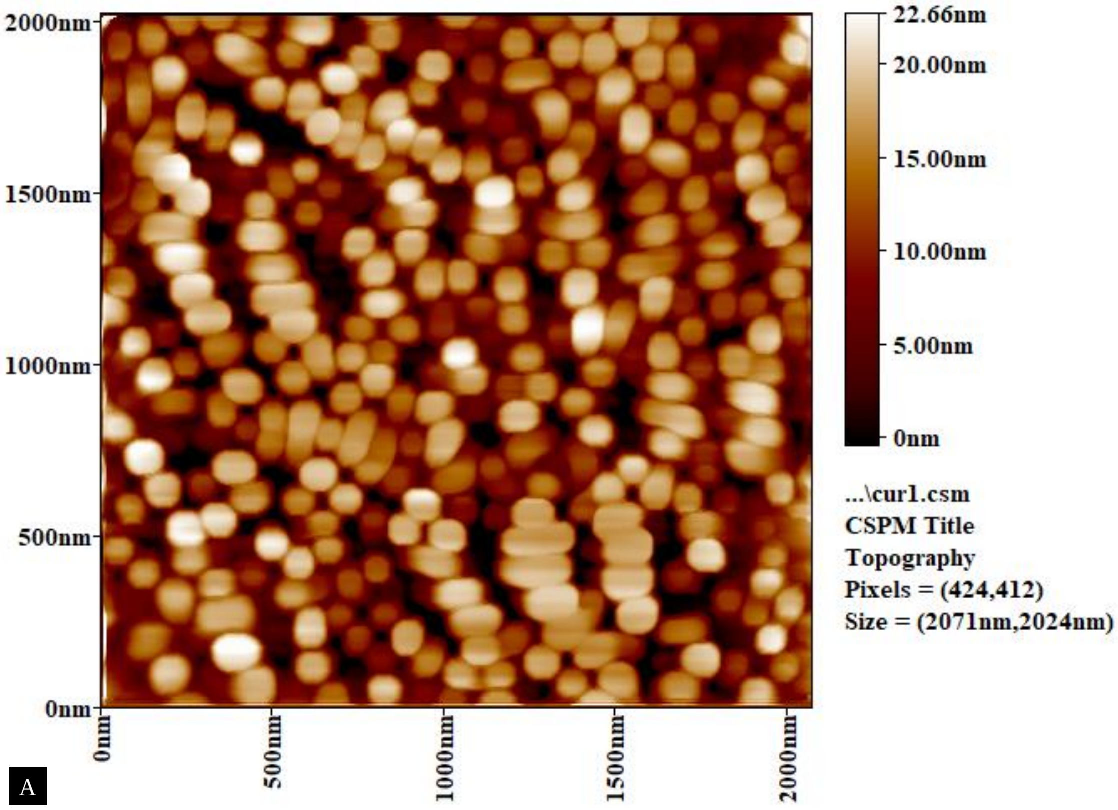


Figure 5. X-ray diffraction (XRD) spectrum (XRD) for nanohybrid curcumin Cur-ZnO.

Characterization by Using Atomic Force Microscope (AFM)

The outer surface of the nanohybrid curcumin Cur-ZnO has been studied. As shown in Figure 6A, a two-dimensional image of the preservative showed fine assemblies of rodile shapes. A three-dimensional image of a section of the surface of the nano preservative shows that the height of the particles assemblies was around 22.66 nm, indicating the motion of a hybrid nano preservative from free curcumin and zinc oxide.



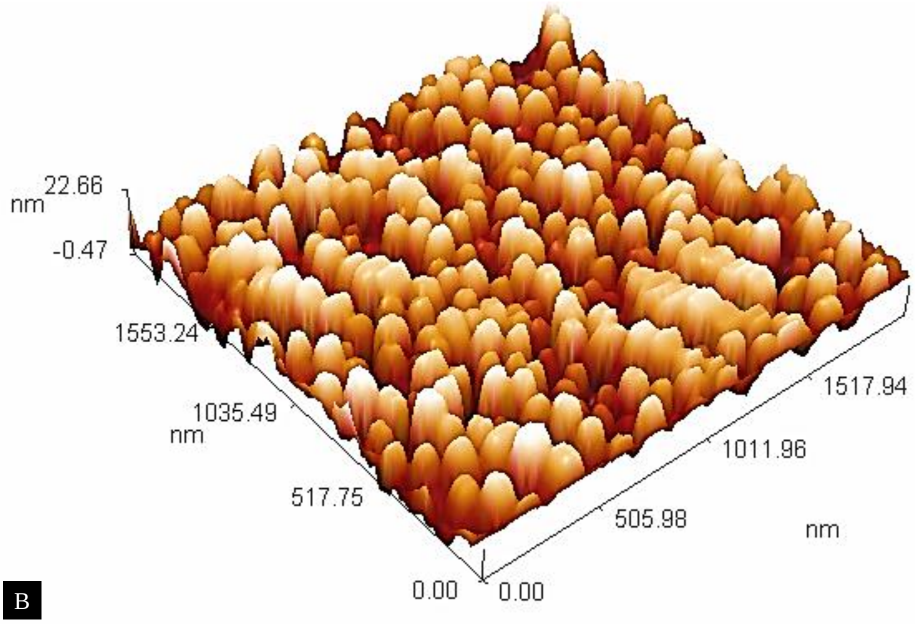


Figure 6. (A) Two-dimensional image of Cur-ZnO. (B) Three-dimensional image of a section of the surface of Cur-ZnO.

Table 1 demonstrates that the particle size of the nanohybrid curcumin Cur-ZnO is 68.07 nm. The process of preparing the nanohybrid curcumin led to the acquisition of nanoparticles with diameters confined between 15 and 100 nm, and the highest percentage of these nanoparticles was 7.12% for particle with a diameter of 90 nm while the lowest percentage for practical was 1.1% for particle with a diameter of 20 nm.

Table 1. Diameters, sizes and assemblies of nanohybrid curcumin Cur-ZnO using atomic force microscope.

Avg. Diameter:68.07 nm					**<=10% Diameter:30.00 nm**			
<=50% Diameter:65.00 nm					***<=90% Diameter:100.00 nm***			
Diameter (nm)<	*Volume (%)*	*Cumulation (%)*	*Diameter (nm)<*	*Volume (%)*	*Cumulation (%)*	*Diameter (nm)<*	*Volume (%)*	*Cumulation (%)*
15.00	1.64	1.64	55.00	4.66	33.70	95.00	5.21	82.19
20.00	1.10	2.74	60.00	5.75	39.45	100.00	3.56	85.75
25.00	3.29	6.03	65.00	4.38	43.84	105.00	4.93	90.68
30.00	3.01	9.04	70.00	6.58	50.41	110.00	3.84	94.52
35.00	4.38	13.42	75.00	6.58	56.99	115.00	4.38	98.90
40.00	3.29	16.71	80.00	6.30	63.29	120.00	1.10	100.00
45.00	6.58	23.29	85.00	6.58	69.86			
50.00	5.75	29.04	90.00	7.12	76.99			

Characterization by Using Scanning Electron Microscopy (SEM)

The outer surface of nanohybrid curcumin Cur-ZnO as well as the surface of the zinc oxide layers were studied using a scanning electron microscope. Figure 7 illustrates the scanning electron micrograph of the layers of zinc oxide where clearly defined hexagonal shapes can be seen in which the leaves of oxide appear superimposed on top of each other in irregular shapes and sizes as indicated by Bashi et al. [24].

In the nanohybrid curcumin some of these shapes have turned into shapes like scattered pieces of wood as well as the formation of compositions with high porosity when the formation of Cur-ZnO nano-curcumin resulting from the direct interaction of zinc oxide layers with free curcumin. This indicates the success of inserting curcumin into the layers of zinc oxide as shown in Figure 8.

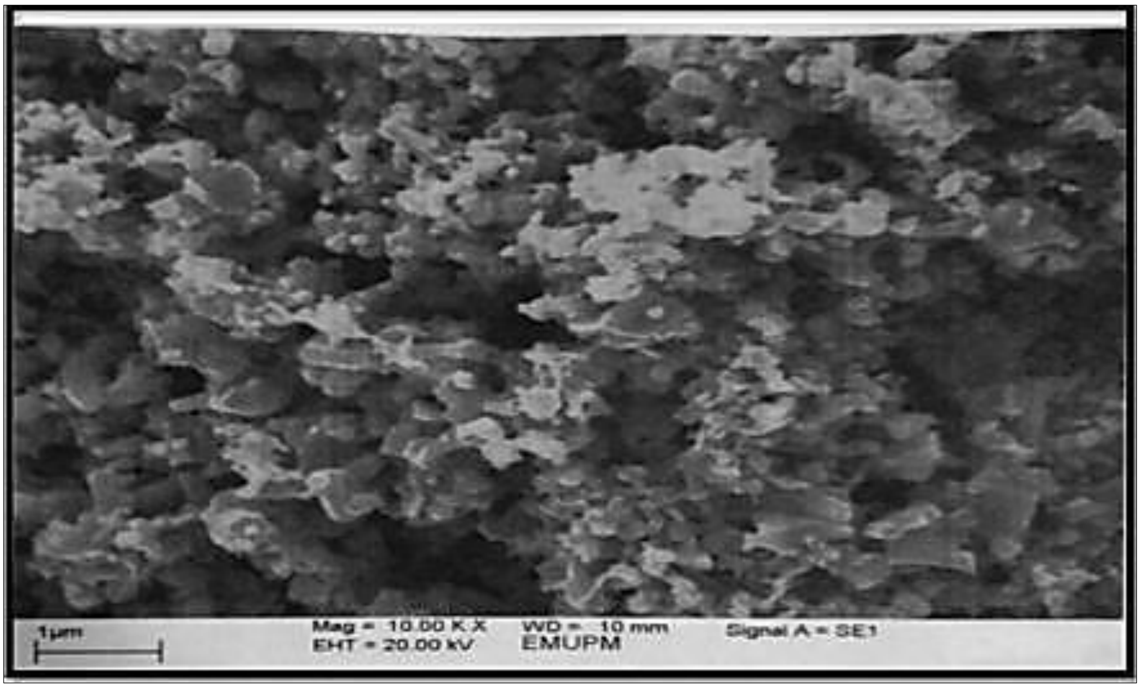


Figure 7. Scanning electron micrograph of zinc oxide layers.

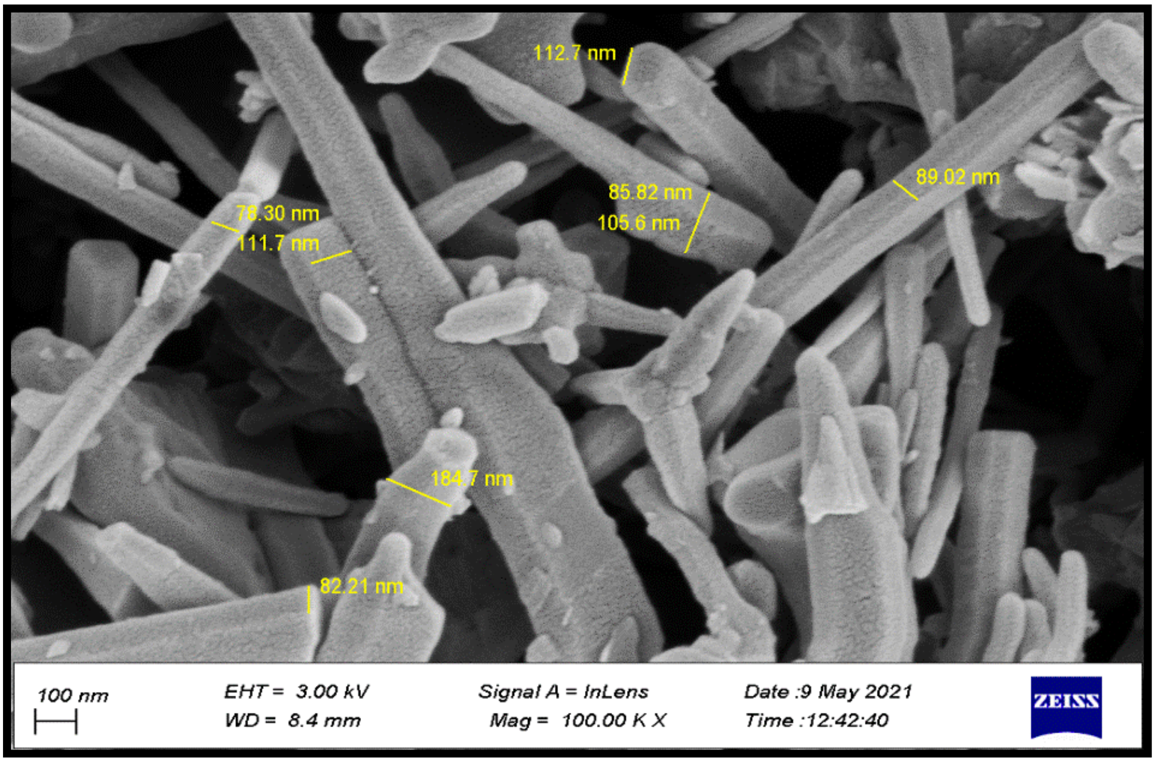


Figure 8. Scanning electron micrograph for nanohybrid curcumin Cur-ZnO.

Precise Element Analysis in Curcumin Nanohybrid

The results of analysis of the elements are shown in Table 2. Free curcumin contained percentages of carbon, hydrogen and nitrogen amounting to 54.55%, 6.59%, and 0%, respectively. The percentage of these elements in nanohybrid curcumin Cur-ZnO were 20.97%, 1.94%, and 0%, respectively. It is clear from these results that the ratio of loaded curcumin between the layers of zinc oxide reached 38.44%.

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Zinc oxide and other oxides are important negative ion exchangers due to their ability to ion exchange, interaction and absorption of biological molecules such as proteins and enzymes, as well as being non-toxic and with good biocompatibility [25].

Table 2. Accurate analysis of elements carbon, hydrogen and nitrogen in nanohybrid curcumin

Compound	Carbon	Hydrogen %	Nitrogen %	Loaded Curcumin %
free curcumin	54.55	6.59	0	
Curcumin nanohybrid	20.97	1.94	0	38.44

Evaluation of Inhibition Effect of Nanohybrid Curcumin Cur-ZnO Against Some Pathogenic and Food-Spoilage Bacteria

The inhibitory effectiveness of nanohybrid curcumin and free curcumin towards 10 isolates of pathogenic and food-spoilage bacteria was studied. It is noted through the results of the current study that nanohybrid curcumin (Cur-ZnO) in concentrations of 0.1, 0.2, 0.3, 0.4, 1.9, 3.8, 5.8, and 7.7 mg/mL and free curcumin in concentrations of 0.2, 0.5, 0.7, 1, 5, 10, 15, 20 mg/mL, have an inhibitory effect against both gram-positive and gram-negative bacteria. As shown in Table 3 and Figure 9, the inhibition zone nanohybrid curcumin (Cur-ZnO) and free curcumin (Cur), reached against *S. aureus* (48, 45) mm for (Cur-ZnO) and for free curcumin (Cur) at a concentration of 20 mg/mL, respectively, and bacteria *B. cereus* (46, 44) respectively, while the diameter of the inhibition zone of isolates *Klebsilla pneumonia, E. coli, E. coli O157, Salmonella cholera, Acintobacter boumannii, Proteus hauseri Comlex, Entero gergoviae* and *Hevani alvei* of the hybrid nanopreservative (Cur-ZnO) at a concentration of 7.7 mg/mL were 28, 31, 44, 42, 30, 43, 44, and 40 mm), respectively, while the free curcumin (Cur) with a concentration 20 mg/mL, the diameters were 24, 25, 40, 39, 27, 40, 42, 37, mm for bacterial isolates, respectively. The difference in the sensitivity of the gram-positive bacteria to gram-negative bacteria may be due to the difference in the structure of the cell wall of each of them, which makes *E. coli* more resistant, and this is consistent with what was stated by Hinton and Ingram [7]. It also agrees with Altunatmaz et al. [26], who mentioned that curcumin was more effective on gram-positive pathogenic bacteria than on gram-negative bacteria. This is because the structure of the gram-negative cell wall is more complex where the layer of lipopolysaccharides, which gives it more resistance, as well as the presence of the periplasmic space between the cytoplasmic membrane and the cell wall, which is more pronounced in gram-negative bacteria [4].

Teow et al. [16] pointed out that curcumin has shown strong antibacterial activity and other microorganism and therapeutic conditions in the past 50 years, as it has been marketed globally as a healthy dietary supplement mainly because of its antioxidant and anti-inflammatory properties, in addition to the high potential to develop it into an antibiotic against *Staphylococcus aureus* and other bacterial strains in the future.

Table 3. Inhibition effect of curcumin nanohybrid Cur-ZnO and Cur-free against some species of pathogenic bacteria and food-spoilage bacteria

Bacterial isolates	**Clear zone of preservatives (mm)**															
	Curcumin nanohybrid (Cur-ZnO) concentration mg/mL								***Free curcumin (Cur) concentration mg/mL***							
	0.2	*0.5*	*0.7*	*1*	*5*	*10*	*15*	*20*	*0.2*	*0.5*	*0.7*	*1*	*5*	*10*	*15*	*20*
	Actual concentration of Nano preservative															
	0.1	0.2	0.3	0.4	1.9	3.8	5.8	7.7								
Klebsilla pneumonia	14	15	16	16	18	20	24	28	15	15	16	16	18	20	22	24
E. coli	10	14	18	19	21	24	27	31	-	13	13	14	17	20	23	26
E. coli O157	21	21	22	23	29	35	40	44	17	18	19	21	26	31	35	40

Staphylococcus aurous	25	25	28	32	38	41	45	48	20	20	22	26	30	34	40	45
Salmonella cholera	16	17	18	20	23	28	36	42	-	-	15	16	21	27	34	39
Bacillus cereus	27	27	28	28	33	38	42	46	18	19	19	20	28	33	39	44
Acintobacter boumannii	16	17	17	18	20	24	27	30	13	13	14	15	17	20	23	27
Proteus hauseri Comlex	20	20	21	22	27	32	37	41	-	20	21	22	27	32	36	40
Entero gergoviae	18	18	20	21	27	34	38	42	18	19	19	20	26	32	37	42
Hevani alvei	17	18	19	20	21	26	32	38	-	-	14	15	20	25	31	37

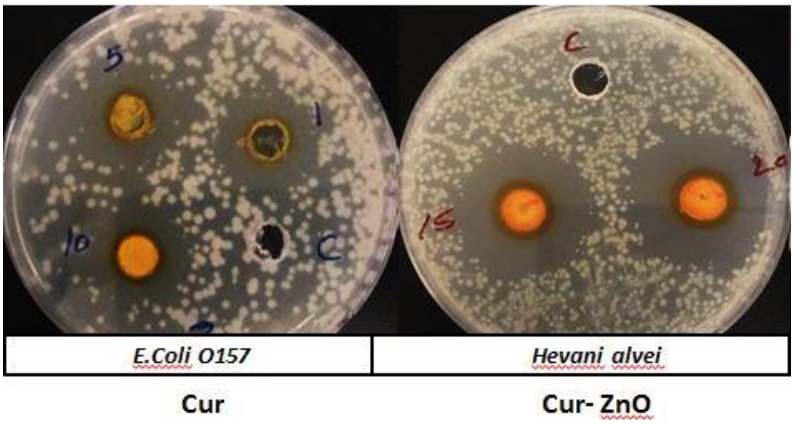

Figure 9. The inhibitory activity of nanohybrid curcumin (Cur-ZnO) and free curcumin (Cur) against some types of bacteria.

The results obtained by Altunatmaz et al. [26] indicated that curcumin was found to have anti-bacterial efficacy against many types of pathological and food-poisoning bacteria where the minimum inhibitory concentration (MICs) for *Listeria monocytogenes*, *S. aureus* was 125 µg/mL, while MICs for *E. coli O157:H7* and *Salmonella typhimurium* was 250 µg/mL.

Masson et al. [27] pointed out that the gram-negative and gram-positive bacteria change their morphological characteristics significantly due to the high density of negative charges on the surface of these cells, and therefore it can be concluded that these negative charges allow the absorption of a greater amount of positively charged nanocurcumin, which leads to changes in the permeability and structure of the cell walls of these bacteria.

There are three mechanisms to understand and explain the ability of curcumin to inhibit the growth of bacteria, the first of which is the binding of curcumin to FtsZ proteins, thus preventing the synthesis of FtsZ filaments, which consist mainly of the protein tubulin, which plays an essential and unique role in regulating cell division, which in turn prevents the formation of the Z ring, which ultimately leads to inhibition of cellular movement and reproduction of bacteria [28]. As for the second mechanism referred to by Mun et al. [17], curcumin increases the sensitivity of the cell wall of bacteria to antibiotics, the basis of which is based on β-lactam, such as penicillin and methicillin, while the third mechanism indicates that curcumin targets the cell wall and the cytoplasmic membrane of the bacteria cell, which leads to disturbances in them and the creation of holes and then finally the decomposition of the cell wall occurs [29].

CONCLUSION

The anti-microbial activity of free curcumin (Cur) and nanohybrid curcumin (Cur-ZnO) can be concluded from the results obtained. These forms of preservatives can be promising factors for their use as natural antimicrobials. Curcumin nanoparticles have also been given higher effectiveness as antimicrobials because of the unique properties of these compounds, nanocomposites react in a different way compared to their reactions when their size is normal, as this size allows them a high surface area and the spread of charges on the surface, which leads to a change in all physical, chemical, electrical, optical and magnetic properties. The results also showed that nanohybrid curcumin loaded with zinc oxide (Cur-ZnO) at ratio 38.44% gave higher efficacy than free-curcumin.

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