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Characterizations of Synthesized Nano Hybrid Octyl Gallate with ZnO LHS and Determined its Anti Microbial Activities

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Abstract
ZnO nanostructures were synthesized by sol-gel reaction between zinc oxide and (Octyl gallate), Octyl gallate Evolution was characterized by UV-VIS spectroscopy,), Fourier transform infrared (FT-IR) spectroscopy technique, X-ray diffraction (XRD), Scanning electron microscopy (SEM) and Atomic Force Microscope (AFM). A well crystalline fiber-like structure was formed. Controlled release was fitted with different modules. Antimicrobial activity tests were done against eight bacterial and three fungal isolates, results showed that nano hybrid composite was efficient in inhibiting both gram positive and gram negative bacteria as well as the fungal isolates used in this study.
Keywords: ZnO; nano materials; antimicrobial activity; intercalations.

I. INTRODUCTION

ZnO has Zinc Oxide Layered hydroxides are a 2D material One of the versatile properties of layered hydroxide (ZLHs) has the ion exchange property. ZnO gust different anions such as Folic Acid and mixed solution of the two anions (2,4dichloro phenoxy acetate+4Chloro phenoxy acetate) [1,2] as a host which substituted small anion such as H_2O, nitrate, carbonate etc. ZnO intercalations are similar to layered double hydroxide [3]. ZnO nanoparticles loaded active packaging against foodborne pathogens[4], ZnO nanostructures can be synthesized into s variety of morphologies including nanowire, Nano rods, tetrapod's, Nano belts, Nano flowers etc.. Can obtain with wet chemistry [5-14]. Therefore, many efforts are concentrated on the synthesis of ZnO materials [15-23]. Among those methods based on physical and chemical technologies to synthesize ZnO particles, organic complexion additives are always used to control the growth of the crystals [23-30]. Addition of organic active molecules will give certain stability in the synthesized nanoparticles. So, it is necessary to find some effective methods to induce the conversion of the Zn complex into ZnO-organic nanoparticles. ZnO, fiber-like materials from the decomposition of bis (acetyl acetonat) zinc fibers at 110 °C in the presence of superheated steam was obtained [31], Octyl gallate is phenolic compound widely used in the food industries as antioxidant [32] In this work we report the use of Octyl Gallate (OG) as a template to intercalating with ZnO LHs to form stable complex" Octyl Gallate- ZnO (OG-Zinc)", nanoparticle via a simple hydrothermal synthesis (at 40 ^oC). The resulted composite was characterized structural and controlled release and bioactivities against some gram positive and negative bacteria's, to show the different activities between free octyl gallate freely used as food preservative, ZnO nanoparticles without any organic materials and the synthesized Nano composite (OG-Zinc). But, to the best of our knowledge, there is no report in the open online publications on the intercalation of Octayl Galate in the anionic form with ZnO.

MATERIALS AND METHODS

ZnO and OG purchased from sigma, were used without further purification. 50ml Solution of (0.05, 0.1, 0.2 M) of OG was prepared. This solution was mixed with the solution prepared of 1gr of ZnO in 50ml de-ionized water, in a conical flask, formation of gel suspension started, aging at the 40 ^oC for 18 hours, cooled, centrifuged and wash for four times with de-ionized water, dried in oven at the 50 ^oC, grinding and kept in a sample.
Antimicrobial activity:

a- Antibacterial activity:

Eight bacterial isolates included five gram positive and three gram negative were used to test the antibacterial activity of the Nano hybrid compound zinc oxide-octyl gallate (ZnO-OG) as well as the free octyl gallate (OG). Agar well diffusion method [33] was used to evaluate the antibacterial activity on Muller –Hinton agar followed by incubation at 37 ^oC for 24 hrs.

b- Antifungal activity :

20mg/ml of ZnO-OG was used to test the antifungal activity of this Nano hybrid compound against three fungal isolates. *Aspergillums Plavus ,aspergillus parasiticus* and *penicillium expansum*. The fungal isolates were grown on potato dextrose agar (PDA) at 28 ^oC or four days.

II. CHARACTERIZATIONS

Powder (PXRD) was obtained with a Shimadzu XRD-6000 powder diffractometer using (λ=1.540562 Å) at 40 kV and 30 mA a scan rate of 1 min. /degrees. Fourier Transform Infrared (FTIR) spectra were recorded by using a spectrophotometer Perkin-Elmer 1725X Japan in the range of 4000-400 cm-1. The surface morphology and bulk structure of the sample were observed by scanning electron microscope (SEM) model vga-easy and AFM microscope AFM model, AA3000, Advanced Angstrom Inc- USA .

III. RESULTS AND DISCUSSION

The evidence for the phase structure of the prepared sample was obtained by XRD pattern, as shown in Fig. (1). All the diffraction peaks can be indexed to those of hexagonal ZnO. After refinement, the lattice constants, a=3.251 Å, c=5.210 Å, were obtained, which is very close to the reported value for ZnO (a=3.253 Å, c =5.209, JCPDS card, No.80-0075). The average particle size was estimated to be 70 nm based on the Scherer equation, D ¼ Kλ βdcosθ, here K is shaping factor of average crystallite, λ is wavelength for the Kα1 (1.54056 Å), β is full width at half-maximum of the diffraction line and θ is Bragg's angle. Fig. (2) XRD patterns for the OG-Zinc. The first basic reflection corresponding to the highest d -value gives information about the interlayer distance. (2.58) nm. of the synthesized nano sheet thin films made up of nano fibers progressing of the intensities of the harmony (003) clearly this sharp peak was moving in the direction of small angle. In the sample of ZnO Fig.1, there is no diffractions appear in this region of XRD pattern, that is as good indication for the intercalations of OG within Zinc oxide to synthesizing OG-Zinc. That the parameter c= 3 (d003) =7.7nm approximately. The (006) and (009) were appearing at d=1.38 and 0.58 nm respectively.

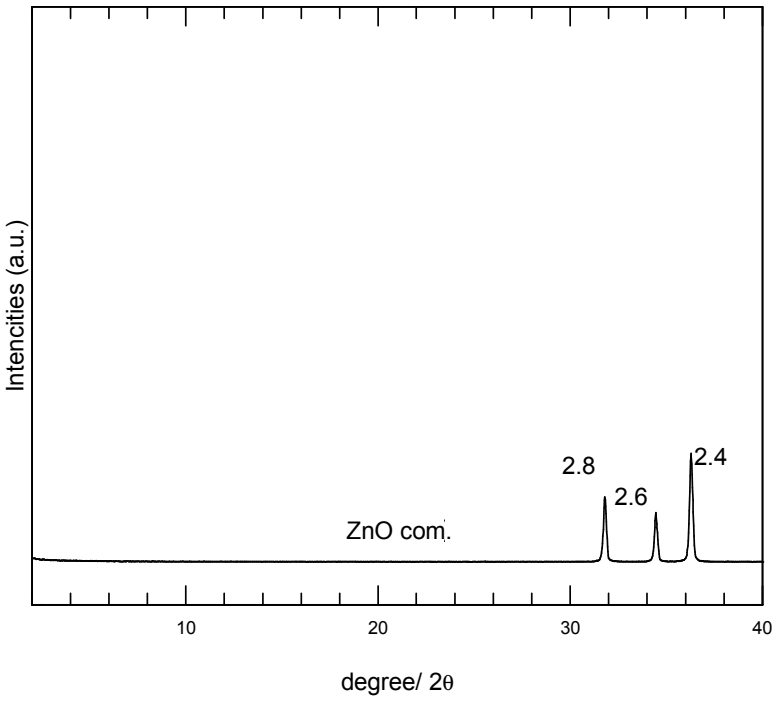


Fig. [1] PXRD of ZnO shows the three harmonies characteristic of Zinc oxide

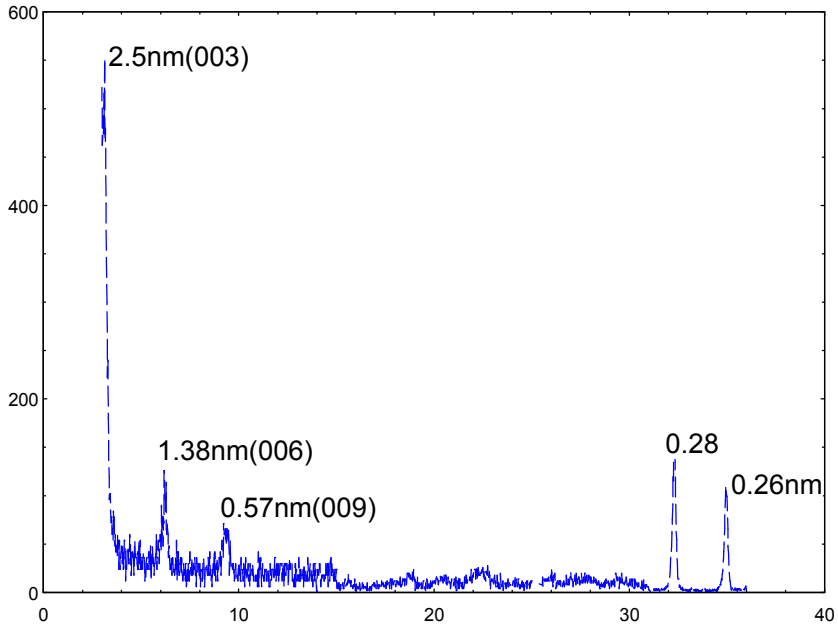


Fig.2] PXRD pattern spectrum shows the OG-Zinc Nano hybrid

Surface morphology studies:

Fig. [3] Shows AFM images of the ZnO layered and the nano particles synthesized with OG-Zinc, The ZnO sheet with a mean size of (70nm). The AFM observation is in good agreement with the data obtained by xrd technique. The SEM image of ZnO fig. [4B] shows small particles clearly indicated the hexagonal lattice, but in the case of (OG-Zinc) the SEM fig [4A] shows a different morphology a fiber –like structure resulted from the intercalation of OG with ZnO. This was a good indication to the intercalation done.

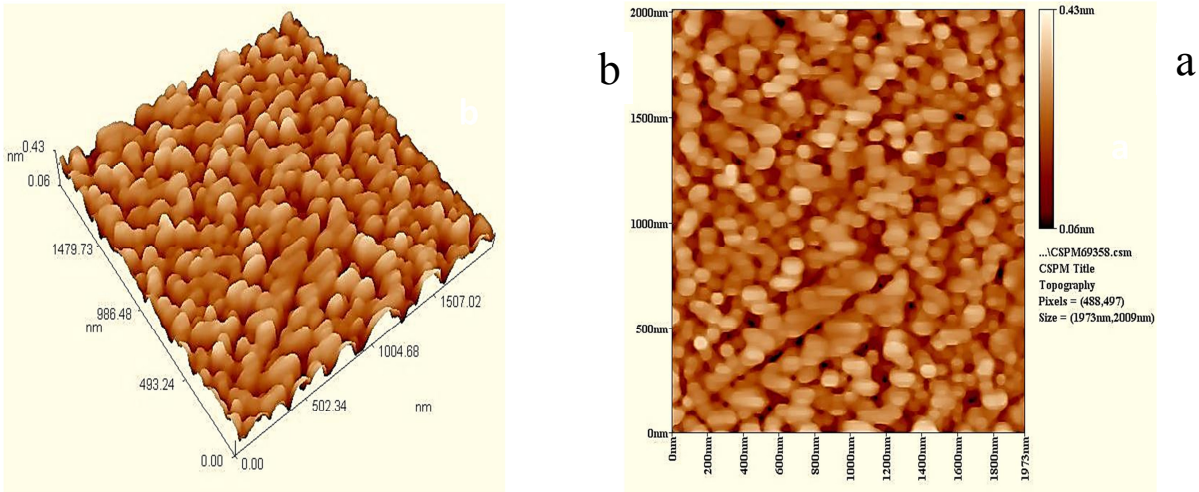
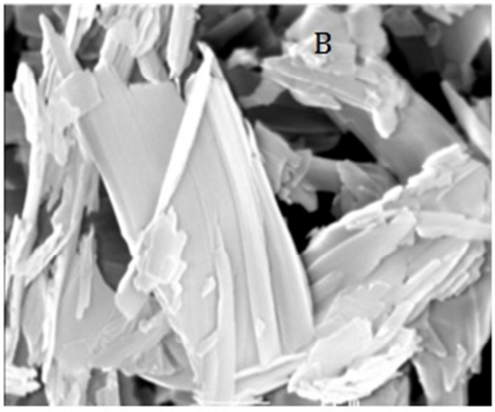


Fig. [3] Shows AFM images of the ZnO layered and the nano particles synthesized with the OG-Zinc(a=two dimensional, b=three dimensional).

B



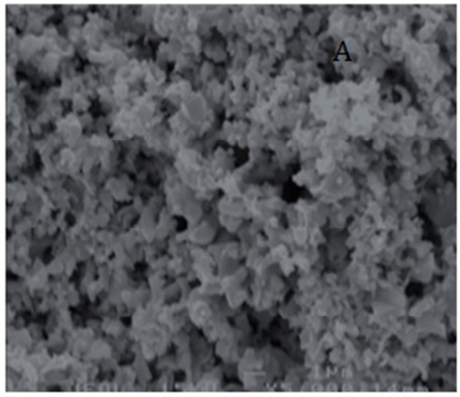


Fig. [4] A- SEM image (B) of (OG-Zinc) Nano hybrid shows the fibers- like structure, SEM image (A) shows hexagonal crystals of ZnO.

Controlled release of OG into aqueous solutions:
De-ionized water and carbonate aqueous solution; (0.005M) and (0.05M) were used to study the effect of the release media on the release rate of OG from OG-Zinc nano particles, as show in fig (5).

The accumulated OG released into de-ionized water increased with contact time for the first. The release was found to be almost complete with the first (23) min. and the accumulations reached 90% in the acidic pH=2 However. At the same time the release in alkaline media pH=13 is a 85% solution equilibrium was achieved at around(20)min. the amount of accumulated OG released as well the release rate increased with increasing initial concentrations of the carbonate ion in the aqueous solution. That the CO_3^{-2} ions are ion-exchanged with the anions on to the nanocomposite surface and at the same time the anions, OG are then released into the solution. The process slowed down as the time proceeded, due to the reduction of CO_3^{-2} ion concentration and the formation of a new "nanocomposite" with carbonate as the counter anion. The release obey the pseudo second order kinetic model, $(t / Ct = 1/k_2CT^2 + t / CT)$, with $r^2=1$ and $K_{2=}0.0282$.

Fig [5] shows the % Release of OG from the layer of Zinc hydroxide

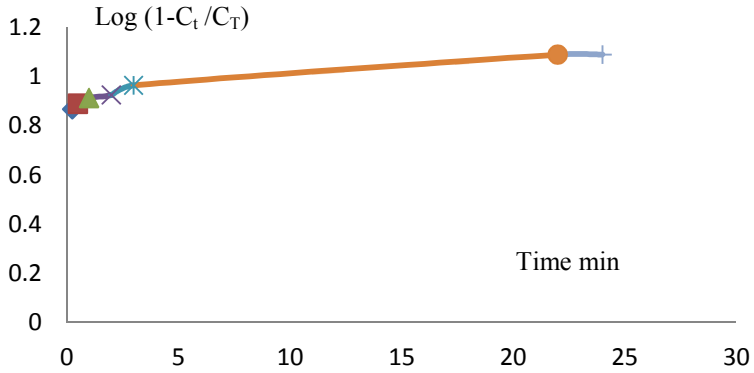


Fig [6] 1st order kinetic model of the release of OG from Zinc hydroxide to the carbonate media
(Log $(1-C_t /C_T) = k_1t /2.303$)

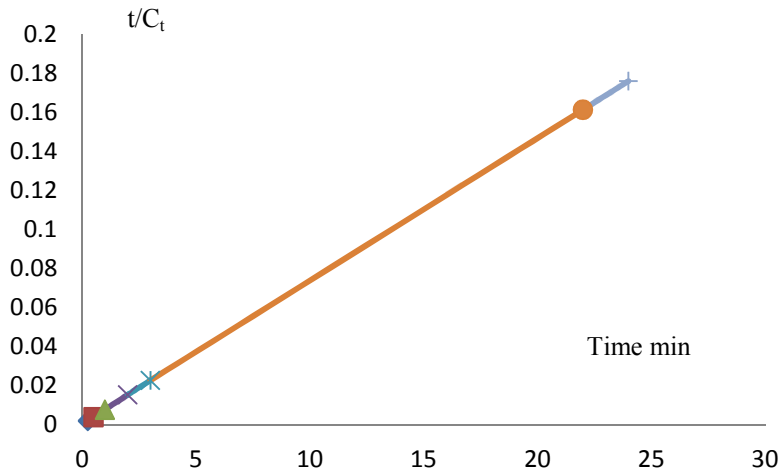


Fig. [7] 2nd order kinetic model of the release of OG to the carbonate media ($t / C_t = 1/k_2C_T^2 + t / C_T$)

The FTIR technique

The FTIR spectrums of the hybrid is a complement of the xrd results and provide further evidence for the intercalations in addition, some absorption bands are slightly shifted due to the interaction of both the anion (Fig. 8) and the host layer (Fig.8). The typical broad absorption bands of zinc layer hydroxide superposing with the anion hydroxide observed and the water molecules at 3540 cm^{-1} and the band due to the (C–O-H) stretching vibration of C-H stretching in the organic chain at 2932 cm^{-1}, the asymmetric and symmetric stretching of C=O appears at 1700 cm^{-1}. The bands at 1470 cm^{-1} attributed to C=C vibrations of the aromatic ring. The bands at about 1200 cm^{-1} are due to C–H twist vibrations. In the OG-Zinc Nano composites (fig.10) shows a combination spectrum of both the host ZnO and the guest anions, the bands located at 1467 cm^{-1} due to the stretching vibration of C=C in the aromatic ring and the band 1409 $cm^{-1}$are due to CH_3 bending . The presence of carboxylate group, COO can be deduced by the observation of bands at 1712 are due to C=O stretching , the band at 1583 are due to C-O-H bending , 783 and 893 cm^{-1} are due to symmetric and anti-symmetric vibrations of the COO- group in the OG intercalated in the inter layers of ZnO. Bands at 2400–2365 cm^{-1} are corresponded to C–H stretching mode. Which are attributed to asymmetric and symmetric vibration, respectively [34 -39] whereas a band at around 1037, 1352 cm^{-1} is corresponded to (–C-0-) stretching vibration.

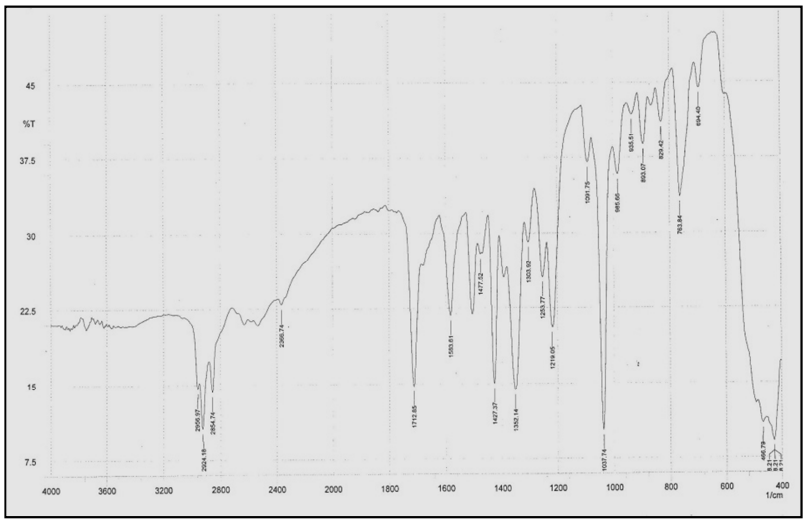


Fig [8a] shows FTIR spectra of OG as commercial compound

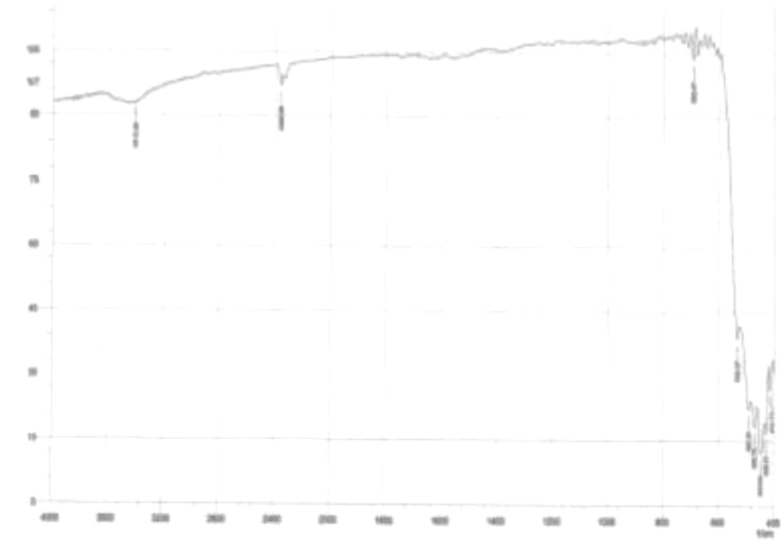


Fig [18b] shows FTIR spectra of ZnO as commercial compound.

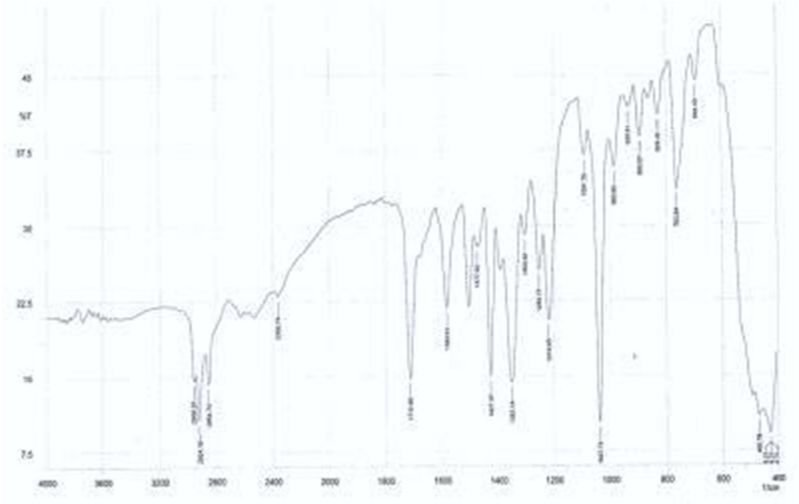


Fig. [8c] shows FTIR spectra of OG-Zinc Nano hybrid composites.

Antimicrobial activities:

Antibacterial activity:

Results obtained from tables (1, 2) showed that both gram positive and gram negative bacteria were sensitive to the octyl gallate as free molecule and Nano hybrid (ZNO-OG).

In case of gram positive bacteria ZNO-OG exhibited highest antibacterial activity agaist *Bacillus Subtilis* with an inhibition zone (15mm) at 15mg/ml concentration, whereas minimal activity was shown against *Listeria monocytogens* type 10403s with inhibition zone (12mm) table(1)

Minimum inhibitory concentration were 500µg/ml for *staphylococcus aureus* , *L. monocytogens* 10403S and *B.Cereus*. while it was 250 µg /ml for *B. subtilis and L. monocytogens* (local).

Octyl gallate is a phenolic compound. The antimicrobial activity of the phenolic compounds is related to the inactivation of cellular enzymes, so it depends either on the penetration of these compounds to the micro-organisms cells or on the changes in the permeability of microorganisms membranes [40]

In case of gram negative bacteria, results in table (2) showed that ZNO-OG exhibited highest antibacterial activity against *Pseudomonas aeruginosa* with an inhibition zone of 14.5mm at 15 µg /ml concentration, whereas lowest activity was obtained against *E.coli* with an inhibition zone of 9.5 mm at the same concentration

Generally, it seemed that gram negative bacteria is more resistant to the (ZnO-OG) in comparing with gram positive. This may be due to the complexity gram negative cell wall which composed of a thin layer of peptidoglycan, outer membrane, lipopolysaccharide and lipoproteins [41].

Antifungal activity

Results in table (3) showed that the Nano hybrid compound (ZnO-OG) was more efficient in inhibiting the

fungal isolates *A.flavors* and *A. parasitic us* than free OG, with an inhibition percent of (90.9 &100) %, respectively while the free OG gave highest inhibition percent against *P. expansum*.

Octalyl gallate is a wide spectrum antifungal [42] .Gallants compounds exhibited their antifungal activity by an acting as a surfactant [43,44].

Table [1] shows the diameter of inhibitions of Nano composite ZnO-OG and free OG against different gram negative bacteria

Table [1] shows the diameter of inhibitions of Nano composite ZnO-OG and free OG against different gram negative bacteria.

Bacterial type	Compounds studied	Diameters of inhibition (mm) Concentration mg/ml								Average
		0.1	0.25	0.5	1	5	10	15	Control 0.1	
S.aureus	ZnO-OG	0	0	6.5	7.5	10.5	12	14	17	11.52
	OG	0	12	15	16.5	20	27.5	32	17	
L.monocytogenes Wild type 10403s	ZnO-OG	0	0	6.5	8.5	8.5	10.5	12	22.5	12.93
	OG	0	13	15	17.5	22	22.5	26	22.5	
L.monocytogenes Local	ZnO-OG	0	5.5	10.5	10	11	12.5	13.5	24	12.71
	OG	0	9	11	15	18.5	20	22	24	
B.cereus	ZnO-OG	0	0	6.5	7	11	11.5	13	25	15.62
	OG	0	10	14.5	16.5	36	37	37	25	
B.subtilis	ZnO-OG	0	6	7.5	10	12.5	14	15	20	12.56
	OG	0	12	14	14.5	16.5	20	20	20	
Average		0	6.75	9.75	12.3	16.65	18.65	20.45	721.	

Compounds Studied	ZnO-OG	OG
Average	9.47	17.26

LSD for bacteria = 0.28, for compounds = 0.18, for concentration = 0.36, for interaction = 1.13

Table [2] shows the diameter of inhibitions of nanocomposite ZnO-OG and free OG against different gram positive bacteria

Bacterial type	Compounds studied	Diameters of inhibition (mm) Concentration mg/ml								Average
		0.1	0.25	0.5	1	5	10	15	Control 0.1	
E.coli ATCC 25922	ZnO-OG	0	0	0	7	7.5	9	9.5	13	6.15
	OG	0	0	5	6.5	6.5	9.5	11	13	
S.typhi	ZnO-OG	0	0	0	6.5	9	10	11.5	18.5	7.78
	OG	0	0	6	7.5	10.5	11.5	15	18.5	
P.aeruginosa	ZnO-OG	0	0	7	8.5	10	13	14.5	18	10.43
	OG	0	6.5	10	12.5	14.5	15.5	19	18	
Average		0	10.08	4.66	8.08	9.66	11.41	13.41	16.66	

Compounds Studied	ZnO-OG	OG
Average	7.20	9.04

LSD for bacteria = 0.38, for compounds = 0.31, for concentration = 0.63, for interaction = 1.54

Table [3] shows the diameter of inhibitions of Nano composite (ZnO-OG) and free OG against different gram negative bacteria

Compounds Studied / Fungal type	Inhibition rate %	
	ZnO-OG	OG
A.flavus	**90.0**	**83.2**
A.parasiticus	**100**	**84.8**
P.expensum	**96.6**	**100**

LSD for bacteria = 0.28, for compounds = 0.18, for concentration = 0.36, for interaction = 1.13AA

Conclusion

Intercalation of OG within ZnO is possible. XRD characterization revealed large interlayer distances caused by the OG intercalation. A maximum gallery height of (2.5) nm was observed for the intercalate OG-Zinc. This confirms that not only surface adsorption, but the (intercalation) of the OG-Zinc has taken place. These results are of great importance to the field of chemical admixtures in bio inorganic composites. The fact this type of antimicrobial molecules was intercalated into ZnO phases.

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