

# Inhibitory effect of nanocomposites on *E. coli* and *Trichophyton mentagrophytes*, *Candida albicans*

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## Abstract

The aim of the research is to synthesize cobalt oxide with Aloe vera and nickel oxide combined with turmeric extract, *Curcuma longa*. This study also compares them with the combined nanomaterials with *Cinnamomum verum* extract and *Zingiber officinale* extract characterize by Artificial roads nanoparticle and growth inhibition nanoparticles green synthesis method for *E. coli* bacteria and *Trichophyton mentagrophytes*, *Candida albicans* and for each substance. Their properties were according to the two methods. The results found that all the NPs were nano-sized with good properties. According to the XRD tests, the results were observed in the drawing, in conformity with the global measurements and nanomaterials 37.02 (111, 44.6 200), 62.5 (220). As for the SEM examination, the shapes of rocky, corky particles, Spherical and semi-spherical shapes in the form of scales or semi-crusts, and the particles appear size 25 nm TEM dimensions. The sizes of the nanoparticles range from 0.5-40 nm. The FTIR test for examining effective chemical bonds was between 500-4000 wavenumber (1/m). It was also found from the ZP examination that the material is not lumpy. The nickel oxide and cobalt oxide nanomaterials from the green synthesis method were more competent than the nanomaterials from the Artificial roads method in inhibiting *E. coli* bacteria isolates. It is a concentration of (100) M%. Two nanocomposites were combined at a concentration of 0.1% M. The results showed that the Aloe vera with cobalt oxide solution and the ginger extract with nickel oxide solution, observed the average diameters of inhibition of bacterial growth for both of them (47) mm, which is more efficient than the extracts of other types of plants in inhibiting *E. coli* bacteria isolates. Here, the average inhibition diameters of the nanocomposites cobalt oxide solution with aloe vera and nickel oxide with turmeric extract were (29) mm, while the average inhibition diameters of the two nanocomposites, cobalt oxide solution with aloe vera and nickel oxide with ginger extract. Also, the average diameter of inhibition for bacterial growth (38) mm, when the average diameters of the inhibition of the nanomaterial were with the extract of the plant of the study, the results were not encouraging, and the nanomaterial did not appear in the tests encouragingly. The nickel and cobalt oxide solutions can influence significantly the growth of fungi in terms of inhibition, as it was found that there was no fungal growth *Trichophyton mentagrophytes*, and they also had impacts on the inhibition of fungal growth *albicans*. Nanosolutions with cinnamon plant cinnamons, average inhibition diameter (35) mm, nanomaterial with ginger extract, average inhibition diameter (23) mm.

**Keywords:** FRIT, XRD, UV, SEM, TEM, , *E. coli*, *Trichophyton mentagrophytes*, *Candida albicans*, Co O, Aloe vera, , ZETA POTENTIAL.

## INTRODUCTION

The term nanotechnology covers the design and functional structure construction with a characteristic dimension at least measured in nanometers, using different processes synthesize metal nanoparticles to reach the level of individual atoms and molecules (Pal, Jana, Manna, Mohanta, & Manavalan, 2011).

New methods are green synthesis in the manufacture of high quality nanomaterials compared to synthetic nanoparticles, Also, low cost (Eugenio et al., 2016) pathogens can penetrate and cause infection in the human body (Cianci et al., 2013).

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This includes the E.coli bacteria, which have the ability to cause infection of wounds and burns due to their transfer from their normal parts, where they infect the urinary tract, causing urinary tract infections because they can produce two types of external toxins. Candida albicans is a wide spread species infecting the skin in of any part of the body, its cells has oval shape of pseudophypha, as they form the preliminary helping it to injure the cover of the cornea (Jawetz, Melnick, Adelberg, Sales, & Salvaggio, 1996).

## Material and methods

The first method is preparing nickel oxide NANOPARTICAL STRUCTUR Preparation of nickel nitrate with sodium hydroxide N4(Saraev et al., 2005).

Preparation of (1M) molar of solutions the following:

A. sodium hydroxide NaOH

4g of NaOH is taken dissolved in 100ml of water.

B. nickel nitrate (Ni(NO<sub>3</sub>)<sub>2</sub>).

One molar, Prepared % 14.5 g, (Ni(NO<sub>3</sub>)<sub>2</sub>). the solution by following the precipitate if it is present until it disappears and monitoring the color . On their preparation, solutions A and B are mixed comparing the color to settle for some time, according to the solution by looking at the light passing through the tube through the window. The formation of a heavy precipitate, which is the salt formed, was observed.

The second method is to prepare the nickel oxide GREEN NANPRACTICAL

NiCl<sub>2</sub>·6H<sub>2</sub>O with turmeric extract N7 (Carnes, M., & Steigerwald, M. 2009)

A. A solution extract of turmeric

Preparing 1%, at 50 ° C with spiral motion by magnetic pole to dissolve and filtered by cotton in a glass funnel and the process was repeated. according to the solution by the precipitate if it was present until it disappeared and the watching color by looking at the light passing through the tube.

B. Preparation of a solution of nickel chloride (NiCl<sub>2</sub> 6H<sub>2</sub>O)

A semi-molarity of approximately % 14.5 was prepared and a half-gram was added to adjust the weight due to the loss of some substance in adhesion to the glass during preparation. On their preparation, A and B are mixed and for an hour at 40°C , according to the solution, by following the sediment if it is present until it does not more exist and watching the color by looking at the light passing through the tube through the window.

A. Nickel oxide with ginger N8 (Sanusi, S. B., Audu, Y., Hamza, I., Usman, A., & Makama, P. 2019)

1% of ginger and depending on the solution by the precipitate if it exists until it is not more apparent and examining the color by looking at the light passing through

the tube through the window.

B. Preparation of a solution of nickel chloride (NiCl<sub>2</sub> 6H<sub>2</sub>O)

Solution B is prepared as mentioned previously, and solution A is added to B in the same way as previously mentioned.

Nickel Chloride with cinnamon N3(Rahman & Noor, 2012)

A. Preparing a solution of the extract of the plant of the cinnamons

The weight of one gram of the cinnamons' powder was taken and dissolves and filtered by cotton in a glass funnel. The process was repeated on volume of water to 100 ml and mixing device and a sample was taken in the test tube for comparing the color to settles ,if it is present until it disappears and the color by looking at the light passing through the tube from through the window.

B. Preparation of nickel chloride (NiCl<sub>2</sub> 6H<sub>2</sub>O)(Carnes et al., 2009)

Solution B is prepared as previously mentioned and solution A is added to B in the same way as previously mentioned.

Preparation of cobalt oxide nanoparticles Method 1: N1 Artificial roads nanoparticle

A - To prepare 1M molar NaOH, take 4g of NaOH and dissolve 100ml of distilled water.

B- Preparation (CoO), a molarity of 18.5% of Co(NO<sub>3</sub>)<sub>2</sub> was prepared, at a temperature of 40 C with movement. The movement of spirochetes is by magnetic electrode inside the solution. After preparing solutions A and B, mix the solutions. Then 50% the quantity is taken to the oven for drying at a temperature of 200 ° C for an hour. Then it is taken out of the oven and left to cool outside the oven at room temperature for a whole day.

Green synthesis nanoparticles by plant extract : N2

A- How to prepare of Aloe vera plant solution

CoO, 1M molar solutions are prepared by Aloe vera solution A piece of the white pulp of the leaves of the aloe vera plant is taken, 10 gm weight is added to 100 ml of water.

B- Solution B is prepared as mentioned previously, and solution A is added to B in the same way as previously mentioned.

Bacterial Pathogen Isolates

The swab was taken and the bacteria were diagnosed by Vitek2 compact system.

Diagnosed yeasts:

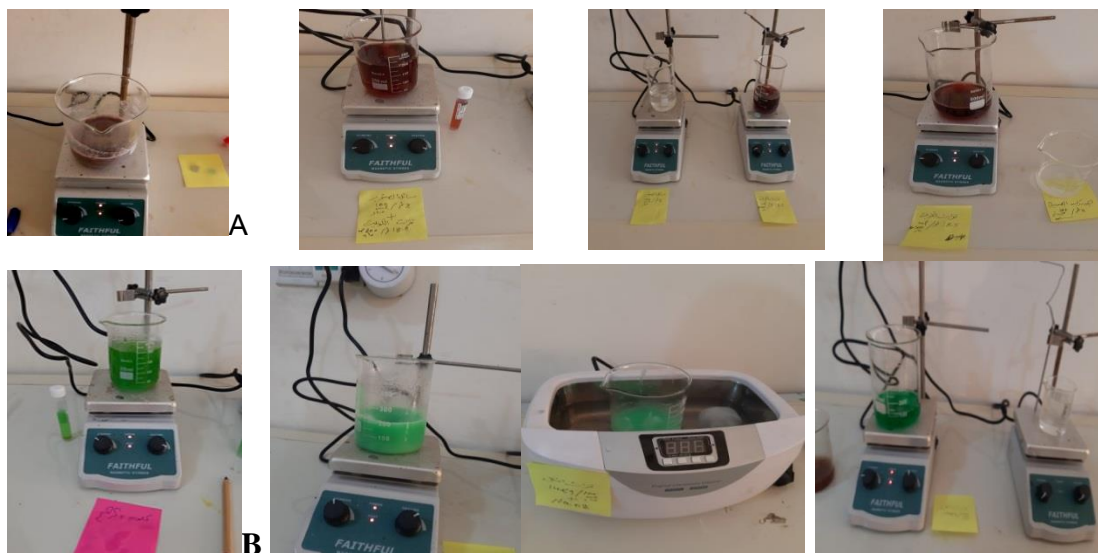
We diagnosed yeasts and isolated them according the microscopic features of the shape, size and color of conidia.

Germ tube test

We conducted this test based on Collee, Duguid, Fraser, and Marmion (1989) to differentiate between species of the genus Candida Spp.



A. Preparation of NiCl<sub>2</sub>·6H<sub>2</sub>O with cinnamons. B. Preparation of NiCl<sub>2</sub>·6H<sub>2</sub>O with ginger solution. C. Prepare NiCl<sub>2</sub>·6H<sub>2</sub>O with a solution of turmeric.



A. Co(NO<sub>3</sub>)<sub>2</sub> mixed with *Aloe vera* plant solution to obtain a Co(NO<sub>3</sub>)<sub>2</sub> solution with *Aloe vera*.  
 A- Co(NO<sub>3</sub>)<sub>2</sub> with NaOH, where it mixes and appears thick, cobalt oxide salt

### Results and discussions

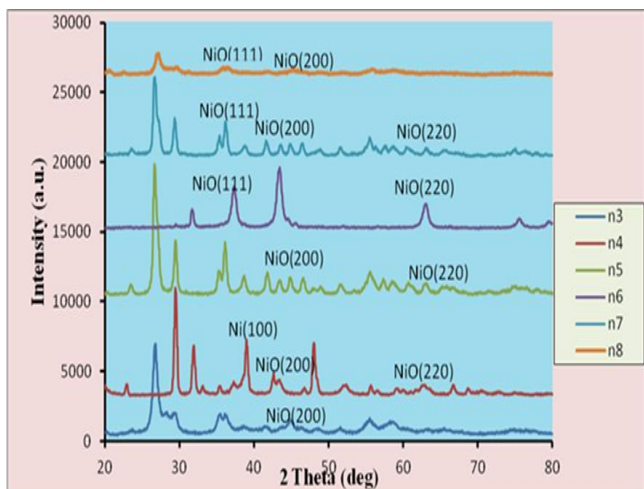
The results of the nanomaterial tests of two methods.

The XRD test is a test to the existence of nanomaterials. Table (1) shows is nanomaterials were 37.02 (111, 44.6 200)), 62.5 (220) in each of the materials NT3, NT7, NT8 N1, N2, and NT4 of two methods.

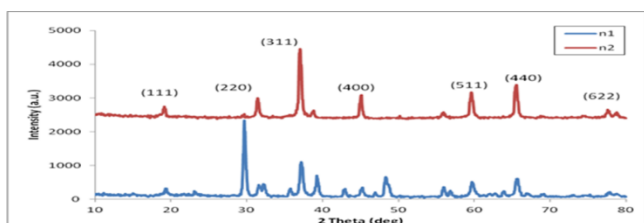
Table No. (1) the Miller's treatment values for cobalt and nickel compounds.

31	220	Co O
38	311	Co O
45	400	Co O
39.36	100	NiO
44.6	200	NiO
62.5	220	NiO

International Measurements2 Theta (deg) (	Miller's treatment	compoudn
27	102	Co OH
27	103	Co OH

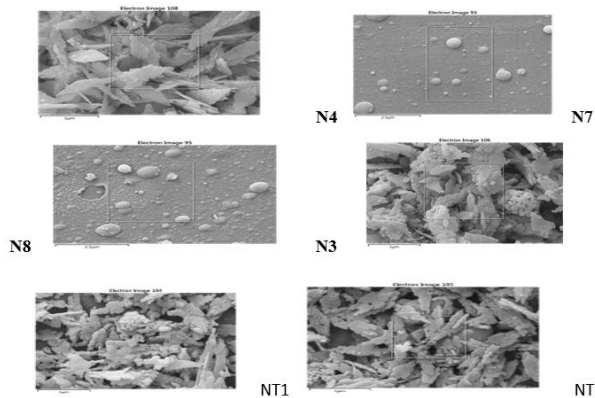


The results of the xrd examination for nickel oxide N4 of the first method. And nickel oxide with turmeric, cinnamon and ginger, N3, N7 N8 prepared by the nanoparticles green synthesis.



The results of the xrd examination for cobalt oxide N1 prepared by the first method. And cobalt oxide with aloe vera extract N2 prepared by the second method.

1. The results of the SEM test of the morphological properties to find the size of the nanoparticle

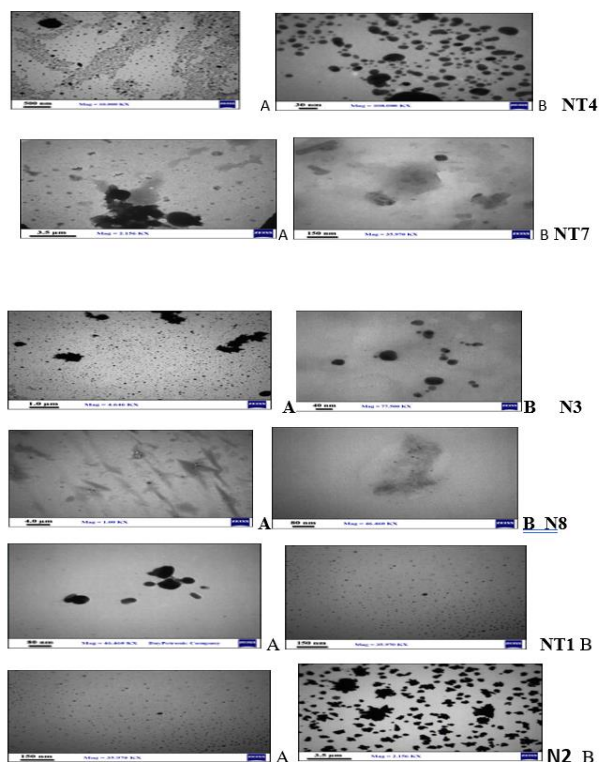


The results of the SEM examination test the morphological properties of cobalt oxide to find the size of the nanoparticle NT1, N3, N4, N7, N8, NT2.

NT3 material appears rocky, almost spherical shapes with dimensions not more than 400-500 nm at the drawing scale and these particles seem with dimensions of 25 nm less than 100 nm. NT4 figure shows the particle shell shape with a size of 10 nm aggregating to a size of 300 nm for

nanoparticles. Figure NT7 and NT8, are the particles in spherical as pebbles with a size is 10 nm, aggregating to up to 2.5  $\mu\text{m}$  for nanoparticles (Jalill 2016).

2. Results of the TEM examination



3. The results of the TEM examination of nickel oxide solution first method N4 NT1 for cobalt oxide, nickel oxide solution NT7, N8, N3, N2, prepared by the second method.

Figure NT3 ,A was 1.5  $\mu\text{m}$ . In B, the nanoparticle drawing scale is 40 nm .Figure NT4, the largest image scale A was 500 nm were single and large spherical interconnected. Based on scale in the image B, the drawing scale was 30 nm. Different sizes of nanoparticles range from 0.5-30 nm. Figure NT7 ,A was 3.5  $\mu\text{m}$  with single interconnected and large spherical objects. Based on the scale in the image B, the drawing scale was 150 nm. The different sizes of nanoparticles range from 0.5-1 nm. Figure NT8 , A was 4.0  $\mu\text{m}$  as single, interconnected and large spherical bodies. The Image B shows about 80 nm. And different sizes of nanoparticles range from 0.5-15 nm.

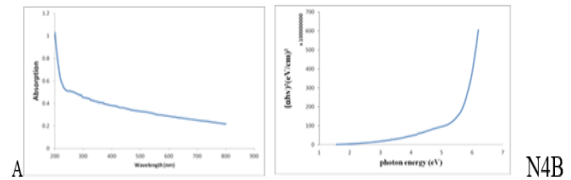
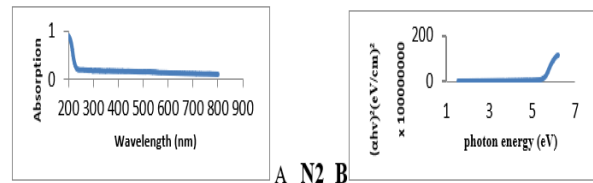
4. UV examination Graph A Absorbance of a solution of nickel oxide NT4

The first method

It is noted that the material has a high absorbance at 200 - 236 nm at more than 236 nm it has a very low absorbance (high permeability) which is the range from 236 - 800 nm. Ggraph B is the energy gap, which is important in electronic transitions, is an optical property, so if the material is

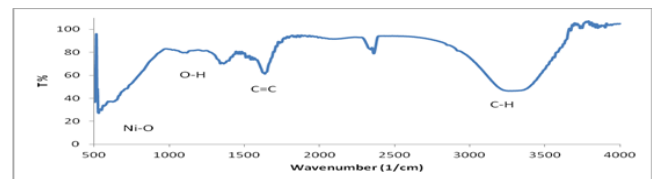
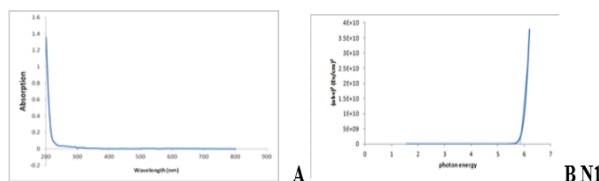
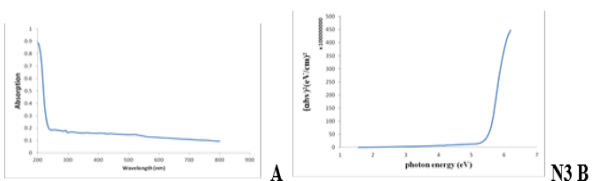
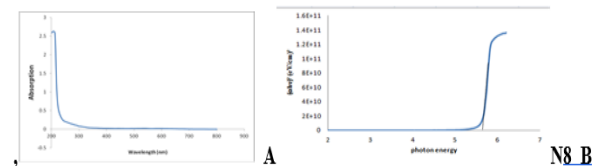
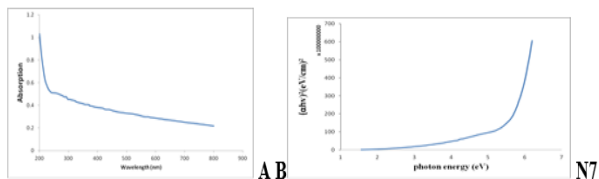
transparent or opaque, it is not transparent. If the transmittance is high, it indicates that the material has high transparency, and it is measured in units  $(\alpha h\nu)^2$  (eV/cm)<sup>2</sup>. The energy gap value is 3.5 eV, and it is a semiconducting material that achieves the oxide of the nanomaterials.

The examination of UV absorbance and energy gap, cobalt oxide solution and nickel oxide NT3, N2, NT7, NT8. The second method Graph A indicates that the material is transparent with high transmittance and very low absorbance in the range 236-800nm. The homogeneity of the material completely and the deviation of the absorption towards the short waves, which indicates the formation of very small nanoparticles. Chart B. The energy gap is of high value as it is a semiconductor like metal oxides. In the NT3 sample graph, the energy gap value is 4.3 eV, which is a semiconductor material that realizes the oxide of the nanomaterials. As for the graph of the sample NT7, the value of the energy gap is 5.3 eV, which is a semiconducting material, which achieves the oxide of the nanomaterials. As for the graph of the sample NT8, the value of the energy gap is 5.6 eV, which is a semiconducting material that achieves the oxide of the nanomaterials. These results are identical to what the researcher reached (Abd, Ali, & Hussein, 2016).

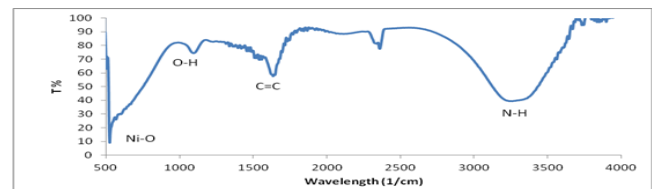


UV graph is the nickel oxide NT4, N1 artificial roads nanoparticle and the nanoparticles green synthesis N3, N2, N8, NT7 .

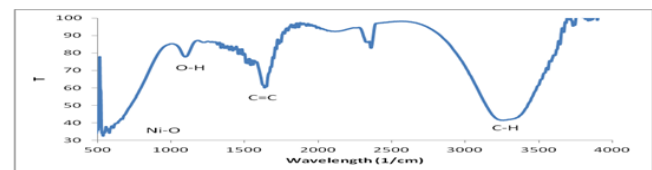
5. FT-ir is determine the effective chemical bonds. It is an analysis of effective bonds where the effective metallic bonds are between the reciprocal of a wavelength measured in centimeters between 500-4000 Wavenumber ( 1/m)(Shuihab & Khalf, 2018).



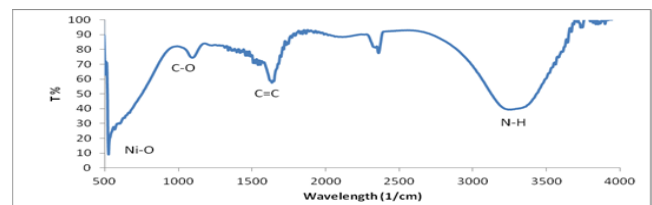
NT4



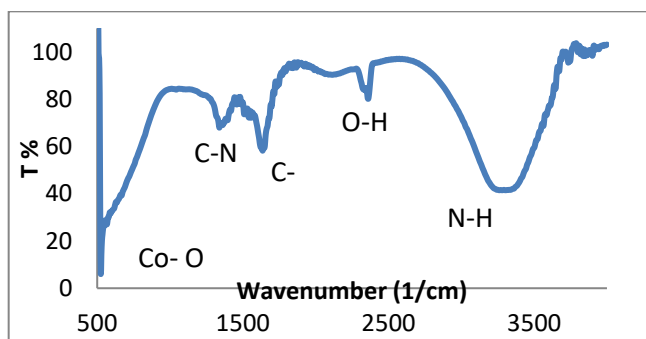
NT3



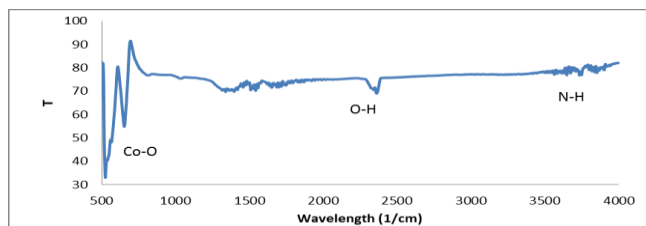
NT7



NT8



NT1



N2

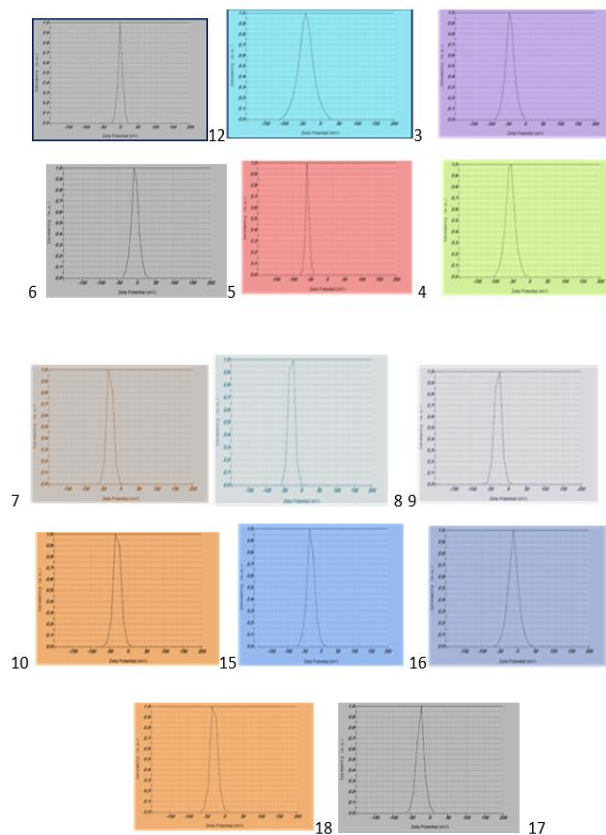
FT-IR for the solution N1, N4 and N4, N7, N8.

Examination of zeta potential (Malvern Instruments, 2012)

The Zeta examination shown in Table No. (2) shows a positive repulsive value, as positive charges. Yet, the negative values are the repulsive negative charges, mv(25.1 -28.7,-27.7, -27.5, -28.5, -17.8) is observed, respectively. The nanomaterials are not agglomerated.

Table No. (2) the zeta potential test.

Electrophoretic Mobility	Z. P	No.
0.000007 cm <sup>2</sup> / vs	0.9 mv	1
-0003 cm <sup>2</sup> / vs	- 38.6 mv	2
-0.000339 cm <sup>2</sup> / vs	-43.6 mv	3
-0.000425 cm <sup>2</sup> / vs	-54.7 mv	4
-0.000444 cm <sup>2</sup> / vs	-57.3 mv	5
-0.000038 cm <sup>2</sup> /vs	-4.9 mv	6
-0.000223 cm <sup>2</sup> /vs	-28.7 mv	7
-0.000215 cm <sup>2</sup> / vs	-27.7 mv	8
-0.000213 cm <sup>2</sup> /vs	-27.5 mv	9
-0.000221 cm <sup>2</sup> /vs	-28.5 mv	10
-0.000138 cm <sup>2</sup> /vs	-17.8 mv	14
-0.000234 cm <sup>2</sup> /vs	-30.1 mv	15
-0.00087 cm <sup>2</sup> /vs	-11.2 mv	16
-0.000221 cm <sup>2</sup> /vs	-28.5 mv	17
-0.000195 cm <sup>2</sup> /vs	-25.1mv	18



The antifungal activity of nanocomposites combining cobalt oxide with nickel oxide nanocomposites in inhibiting the growth of bacteria and fungi:

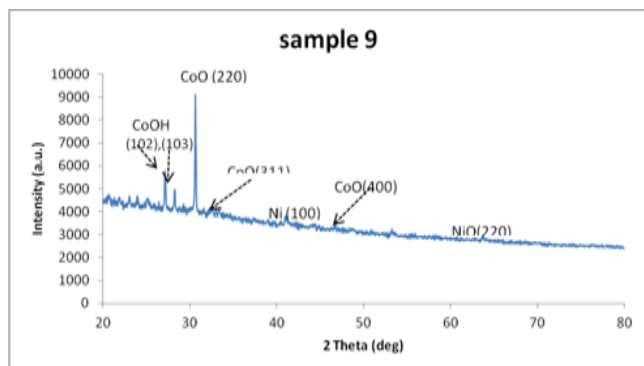
The effect of nano-solutions on inhibiting bacterial and fungal growth was tested, as the combined nano-solutions were mixed according to Mambin in Table (3) below.

Table (3) shows the mixing of two methods, in gradual and inverse proportions.

No.	Compound name	%M	Compound name	%M
1	<b>Co(NO<sub>3</sub>)<sub>2</sub> with NaOH</b>	0.0	<b>(Ni(NO<sub>3</sub>)<sub>2</sub>)+ NaOH</b>	100
2		25		75
3		50		50
4		75		25
5		100		0.0
6	<b>Co(NO<sub>3</sub>)<sub>2</sub> solution with Aloe vera.</b>	0	<b>NiCl<sub>2</sub>·6H<sub>2</sub>O solution with turmeric</b>	100
7		25		75
8		50		50
9		75		25
10		100		0

15		75	<b>Ni(NO<sub>3</sub>)<sub>2</sub> solution with ginger</b>	25
16		50		50
17		25		75
18		0.0		100

1. The examinations are carried out according to the Hashim (2012) method.
2. XRD examined the existence of nanocomposites and on the Bragg angle peaks in the graphs. It uses international standards GCDP or ASTEM. The graph is CoO, which is the predominant cobalt oxide.



XRD assay format for solution of embedded nanomaterials  
It is noted from the graph that the cobalt compound is dominant with the nickel compound and this applies to the results of the research (Abd et al., 2016; Kalyani & Gurunathan, 2016).

1. SEM is examination

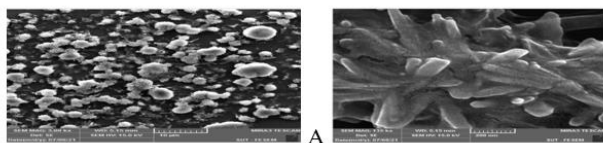


Figure showing the result of the SEM examination of a solution of two nanomaterials Nanocomposites

3. SEM examination image shows A represents a nanocomposites deposited on the glass, with a black background and pink particles, with dimensions of 200 nm at the scale of the drawing, and these particles appear in the dimensions of the drawing scale 50-90 nm less than 100 nm. Image B is a large with dimensions of 10 μm.

4. TEM is a test of the shape and size of nanocomposites and a scale for the nanoparticle.

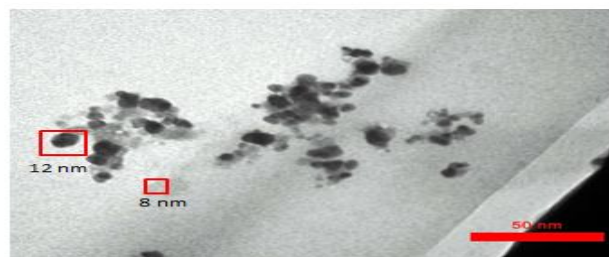


Figure showing the TEM assay of two nanomaterial's Nano composites

The TEM examination shows the size of the scale of the drawing in image at 50 nm, and by measuring the nanoparticles, The very smallest of the universally recognized nanoparticles is called quantum dot (Poole Jr & Owens, 2003).

5. The FT-ir tests checking and determination of effective chemical bonds. It is an analysis of effective bonds where the effective metallic bonds are between the reciprocal of the wavelength measured in centimeters between 500-4000 of the drawing.

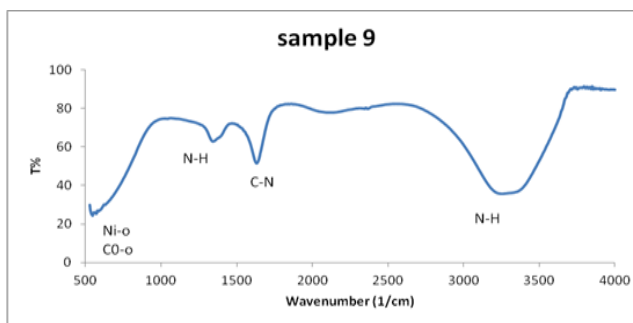
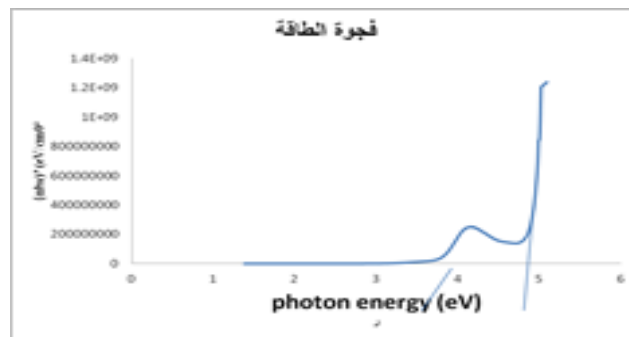


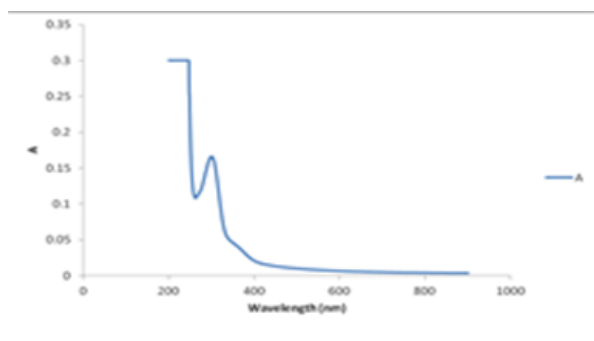
Figure showing the FT-ir of two embedded nanomaterials

FT-ir examined through the bonds site in the table(Enders, North, Fensore, Velez-Alvarez, & Allen, 2021). The drawing shows bond sites of the metal oxides Co-O and Ni-O are at 532.

6. The UV is an absorptions spectrum versus photon energy



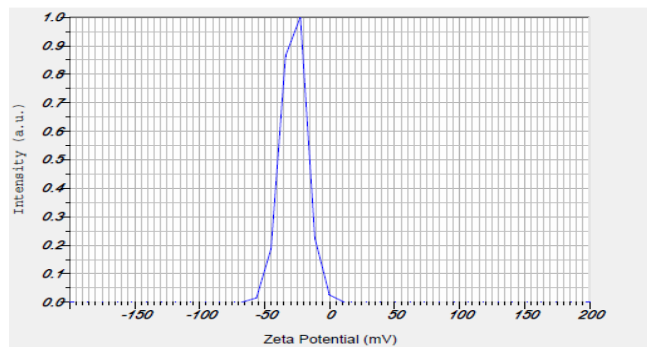
A



B

UV form tests. Through the results, it is noted that the energy gap, which is the conduction bundle to the equivalence bundle, is photon energy 3.8ev. The value of the energy gap appeared when converting to nanomaterials, as shown in graph A. As for the absorbance, B shows that the plasmolayer is at 200-400 wavelength (nm) and this is evidence be nanomaterial.

1. Zeta potential determines the particle size and surface charge known as zeta potential dynamic light scattering.



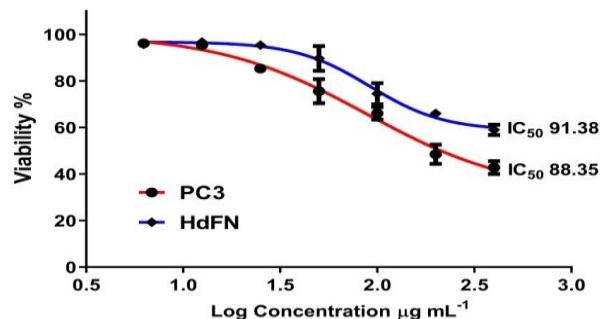
The form of zeta potential, where the results indicate that the material is not agglomerated

Peak No.	Zeta Potential	Electrophoretic Mobility
9	-27.5 mv	-0.000213 cm <sup>2</sup> /vs

It is noticed from the graph that there is an amount of negative value, which is the presence of discordant negative skeins. The graph Zeta Potential is -27.5 mv, and this is a small value so that no agglomeration occurs.

7. MTT: Measuring cell vitality and its resistance to cytotoxicity assay by measuring the number of live cells and dead cells. Through the graph, the results of the toxicity measurement were shown. The toxicity MTT result is through the relationship between the concentration of the solution, expressed in logarithms, for ease of drawing of log concentration  $\mu\text{g.ml}$ , horizontal axis and its relationship

with the rate of dead cells % viability. The inhibitory solution for the growth of E.coli bacteria is N7 and N2. Graph of PC3 prostate cancer cells and normal cells in the body, HdFN, as it was observed from the result of the graph based on the value of 50% of the cells killed, symbolized by IC 50 (the potential to kill 50% of cells) as follows:



The graph shows cobalt oxide solution with aloe vera extract and nickel oxide solution with turmeric extract showing the toxicity result of MTT normal cells in the body HdFN and the amount of cancer cells symbolized by PC3.

Table No. (3) the concentrations of the substance with the mean of dead cells for three samples and SD. The MTT cobalt oxide solution with aloe vera extract and nickel oxide with turmeric extract.

Concen.	PC3		HdFN	
	Mean	SD	Mean	SD
400.00	42.79	2.79	58.94	2.23
200.00	48.52	4.18	65.97	1.10
100.00	66.05	2.70	74.48	4.57
50.00	75.54	5.21	89.64	5.30
25.00	85.30	1.75	95.37	0.90
12.50	95.33	0.79	96.64	0.70
6.25	96.10	1.71	95.68	0.41

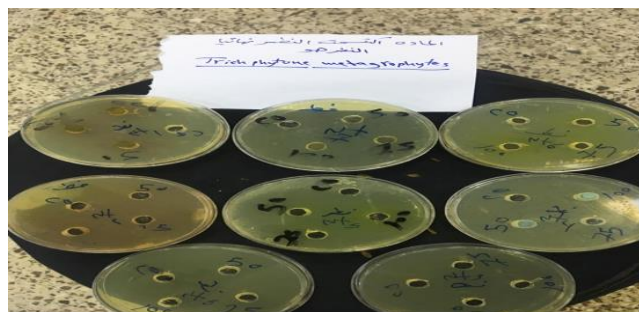
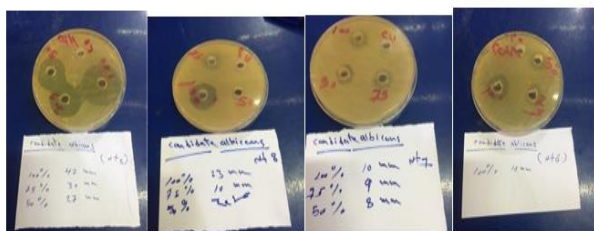
N7 and N2 inhibits the growth of bacteria and shows that the higher the concentration of the substance, the lower the percentage of dead cells. This means that the solution gives better results at lower concentrations and the better the solutions are diluted. The nanoparticles are less agglomerated and spaced, and the nanomaterial is non-toxic, and this matches the results reached by the researcher (Pinto, Daina, Sadocco, Neto, & Trindade, 2013).

The effect of nanomaterials on fungal growth:

The influence nickel oxide solutions on the growth of fungi is significant inhibitory, with no Trichophyton mentagrophytes. Also, Candida albicans with 13,10,23,35 mm respectively at 100% M concentration.

Table No. (3) the concentration of the artificial roads nanoparticle and the nanoparticles green synthesis and its effect on the fungal growth of Candida albicans, and it appears from the table that there is no growth and 100% inhibition of Trichophyton mentagrophytes.

The length of the diameter of the damping area mm		concentration%	Plant type	method
<i>Trichophyton mentagrophytes</i>	<i>Candida albicans</i>			
0.0	0.0	25	NT4 :N1	The first method
0.0	0.0	50		
0.0	0.0	75		
0.0	16	100		
0.0	0.0	25	<i>Curcuma longa</i>	The second method
0.0	8	50		
0.0	9	75		
0.0	10	100		
0.0	0.0	0.0	<i>Zingiber officinale</i>	
0.0	0.0	50		
0.0	10	75		
0.0	23	100		
0.0	0.0	25	<i>Cinnamomum verum</i>	
0.0	0.0	50		
0.0	14	75		
0.0	35	100		
0.0	0.0	25	<i>Aloe vera</i>	
0.0	33	50		
0.0	33	75		
0.0	45	100		
There is no fungal growth = 0.0				

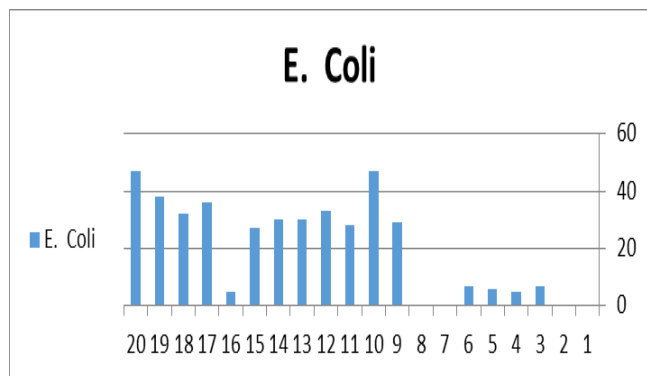


The nanomaterial significantly influenced the inhibition of the growth of fungus by varied concentrations of nano solutions N1,N2,N3,N4,N5,N6,N7,N8.

Effect of nanosolutions on growth inhibition of E.coli . bacteria

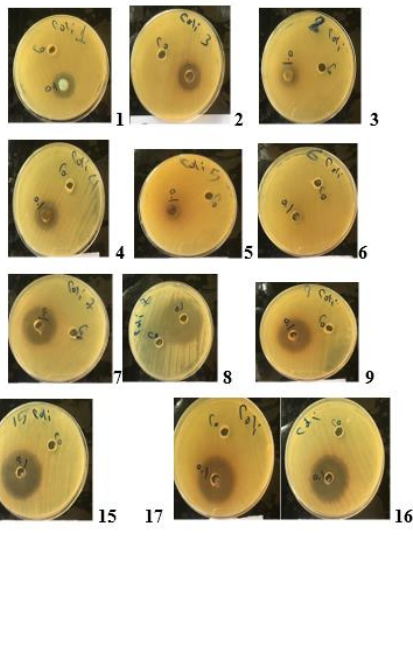
Table No. (4) shows the effect of dilute nanosolutions at 0.1M% concentration for all series of combined and inverted nanosolutions. N1,N2,N3,N4,N7,N8. The diameter of the growth inhibition zone for E.Coli bacteria is shown. It was observed that the solutions prepared by the first method, which are numbered 1,2,3,4,5,6, had the least effect in inhibiting bacterial growth compared to the other solutions by the second method. Nickel oxide solution with turmeric 50%

had the most average diameter of the E. Coli bacteria growth inhibition area of 47 mm, while the average diameter of the N2 with nickel oxide with ginger was 38 mm. The rest of the solutions show the measurements for the inhibition area of growth respectively, 28,33,36,32,29 mm are solutions consisting of a compact nanomaterial, as shown in the graph.



The graph shows the percentage of bacterial growth in all the combined nano-solutions

Codes	E. Coli
CON.	0.1 M
1	7
2	5
3	6



The picture shows the zone of inhibition using nanomaterials of cobalt oxide with nickel oxide (1,2,3,4,5,6,7,8,9,10,15,16,17,18) for the growth of Ecoli bacteria according to Table No. ( 4 ).

Table No. (4) Inhibiting the bacterial growth rate of E.Coli

Codes	E. Coli
CON.	0.1 M
1	7
2	5
3	6
4	7
5	0
6	0

7	29
8	47
9	28
10	33
15	36
16	32
17	38
18	47

### Conclusions

1. The plant extract with CoO nanoparticles and the extract with NiO from the second method showed more efficiency than the nanoparticles of the first method .
2. A N2 shows more efficiency than a solution of N8, as well as N7 , and N3, as the solution of nickel oxide with cinnamons did not show encouraging nano results, in inhibiting Ecoli bacteria isolates.
3. The nanomaterial significantly influenced the inhibition of growth of two types fungi.

### Recommendations

This work recommends the following:

1. Studying the possibility of using cobalt oxide nanomaterials with aloe vera and nickel oxide with ginger, against bacterial, other fungal and parasitic pathogens, and within standardization and quality control controls.

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