Original Article

Comparison of the Effect of Combined Ginger Extract Nanosolutions on Growth Inhibition of Bacterial Isolates Causing Wound Infections

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Abstract

Objectives: The aim of the study is to prepare safe, non-toxic nanomaterials and use them as alternatives in treating different types of bacteria, especially bacteria resistant to a large number of antibiotics commonly used in wound treatments in hospitals. Two nanocomposites were used, both of which were prepared using green synthesis. **Methodology:** Nickel nitrate with ginger and cobalt nitrate with *Aloe vera* were used as starting materials to prepare nanosolvents by the green synthesis method because it is a fast method for producing the nano solution. Additionally, many physical analyses and measurements were carried out without producing toxic waste and without requiring complex techniques. **Results:** The XRD showed that the films of the prepared solutions, deposited on glass by the drop casting method (nickel oxide with cobalt oxide), have a cubic phase and a polycrystalline structure. The morphological surface study of the nanocomposites by scanning electron microscopy showed that the particles were 127 nm in size. TEM (Transmission Electron Microscopy) revealed that the shape of the particles was spherical and semi-spherical and the average particle size was between 12-20 nm. The formation of NiO and CoO was confirmed in a high-purity phase by FT-IR spectra. The UV-Visible spectrum was used, where the band gap of the solution of the two nanomaterials (nickel oxide with cobalt oxide) was 2.75 eV. The effectiveness of the synthesized nanomaterial towards antibacterial activity was also determined. Here, good effectiveness was recorded against *P. alcaligenes, Escherichia coli, Klebsiella pneumoniae, Staphylococcus epidermidis, Staphylococcus aureus, Enterobacter cloacae, P. Putida, Stenotrophomonas maltophilia, Acinetobacter baumannii, Pseudomonas aeruginosa.* **Conclusion:** The synthesized nanoparticles can be used as a treatment against bacterial species that have multiple resistance to antibiotics in skin infections resulting from wounds.

Keyword: SEM, TEM, UV, FTIR, ZP, XRD, MTT, Extract of Zingiber Officinale and Aleo Vera.

Introduction

Bacterial resistance to antibiotics is a common problem nowadays[1] due to the excessive use of drugs by individuals without consulting a physician[2], which led to the emergence of high resistance strains.[3] This has resulted in the spread of antibiotic-resistant bacterial infections and caused a difficulty of treatment for them.[4] The technology of manufacturing nanomaterials has emerged to solve the problems associated with the spread of bacterial infections that are difficult to treat. There are several methods of manufacturing nanomaterials that have been developed across all fields of industry for the medical, pharmaceutical, plant and animal technological fields of all kinds. One common method is the green synthesis of plants, as well as the chemical method used in our research.[5]

Chemical compounds in plants help in the formation of nanoparticles by acting as oxidizing and reducing agents.[6,7] Nickel oxide (NiO) is a semiconductor. It is used in many photovoltaic applications because it is positively charged with a wide energy band gap of 3.6 to 4 eV.[6] Cobalt oxide CoO has a band gap of 2 eV. It can be prepared by chemical and physical methods.[8] This study used the extract of ginger Zingiber officinale and *Aloe vera* to prepare nanosolutions. Ginger is a medicinal plant used to expel gases, cramps, flatulence and menstruation.[9] *Aloe vera* has many uses in treating wounds and fighting fungi, mold and bacteria. It is also used as an analgesic for inflammation and skin ulcers because aloe vera is an

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antiseptic and disinfectant.[10]

Materials and Method

Pathological bacteria were isolated from wound swabs of inpatients[11] in hospitals: *P. alcaligenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis, Staphylococcus aureus* , *Enterobacter cloacae* , *P. Putida* , *Stenotrophomonas maltophilia , Acinetobacter baumannii* , *Pseudomonas aeruginosa*, which are the most common in wound injuries.[12] These isolates were cultured on media.[1] It was diagnosed and tested for antibiotics and the response to some types of antibiotics was in different proportions[13] where bacterial samples were diagnosed using the Vitek 2 compact system as in Table No. (1) and Table No. (2). The nanomaterials were manufactured by a chemical method, where molar nickel chloride was made. The weight was 14.5% (NiCl2·6H2O), and mixed with a solution of 40% molar sodium hydroxide NaOH molar 1M using the magnetic stirrer. The temperature was gradually raised from 30 ° C to 70 ° C. The green synthesis method[14], with 1% of the ginger plant extract[15] is prepared and mixed with 14.5% of nickel chloride (NiCl2·6H2O).) aqueous solution for an hour at a temperature of 50± ° C with the electromagnetic mixer. Then, the color of the resulting mixture was compared from time to time with the sample that was taken from the solution until the color of the nanosolution became stable and the precipitate disappeared. In the same way, cobalt oxide was synthesized with aloe vera nanoextract by the green synthesis method[12] from a solution weighing 18.5 g of molar cobalt nitrate ($Co(NO3)_2$) which was dissolved directly in 100 ml distilled water and mixed with molar sodium hydroxide solution prepared as above. A 10% *Aloe vera* extract solution was prepared and mixed with a 1M cobalt nitrate solution ($Co(NO3)_2$) following the method described above.[16] Also, the two solutions were mixed following the same steps used previously to synthesize cobalt oxide via green synthesis. The combined solutions were prepared by taking graduated and reverse ratios of 25%, 50%, 75% and 100% of the solutions using the same method as before in a water bath at ±50 °C. The antibacterial efficacy of these solutions was evaluated by testing their inhibitory effects on the growth of bacteria isolated from wound swabs, comparing results to untreated isolates. Additionally, a sensitivity test was conducted using antibiotics available in hospitals.

Results and Discussion

Nanomaterials were manufactured and nickel oxide nanomaterials were observed after XRD (X-ray diffraction) analysis of *Zingiber officinale* extract at 18.6(001), 35.85(100), 35.85(111), 45.1(200), 27(102), 27(103), 31(220), 38(311), 45(400), 39.36(100), 44.6(200). These peaks conform to international standards. The TEM (Transmission Electron Microscopy) examination showed rocky and floral shapes of the nanoparticles at sedimentation, while SEM (Scanning Electron Microscopy) revealed nanoparticle size post-sedimentation. The UV-visible analysis (UV examination) indicated the energy gap and absorption characteristics of the nanocomposite. FTIR (Fourier Transform Infrared Spectroscopy) analysis revealed chemical bonds and interactions between chemical elements. The ZP (zeta potential) test showed non-agglomeration of molecular charges.

Cobalt oxide materials, synthesized from Aloe vera extract as described by Saruchi *et al.*[17], were analyzed by XRD with peaks at 37.02 (111), 62.5 (220), and 44.6 (200), all conforming to international standards. TEM and the nanoparticle appear in sizes ranging from 0.5-40 nm. The SEM test shows the cork, spherical shapes with dimensions of 25 nm. FTIR identified effective chemical bonds which were between 500-4000 wavenumber (1/m). ZP testing confirmed non-lumpiness of the material, while nanomaterials resulting from mixing two nucleated substances were also examined. The XRD examination showed the presence of nickel oxide and cobalt oxide, which conforms to international standards of Miller's coefficient. Two peaks were observed at 102 and 103 (2 Theta = 27°), with SEM showing particle shapes around 50–90 nm, not exceeding 200 nm. TEM measurements identified particles as 5–12 nm in size, termed quantum dots. ZP analysis indicated blending of cobalt oxide and nickel oxide nanomaterials.[18] Cobalt oxide with nanomaterial nickel oxide nanomaterials were observed after XRD examinations of ginger extract 18.6(001), 35.85(100), 35.85(111), 45.1(200), 27(102), 27(103), 31(220), 38(311), 45(400), 39.36(100), 44.6(200) which conform to international standards. Also, the TEM examination showed the shape and size of the nanoparticles in rocky and floral shapes at sedimentation. The SEM examination shows the size of the nanoparticles after sedimentation. The UV examination revealed the energy and absorption gap. The FTIR examination reveals the chemical bonds between atoms and the presence of chemical elements.[19] The ZP examination depicts the electrical charges and shows the non-agglomeration of the material molecules as shown in Figures (1,2,3,4,5.6).

The materials were also tested for antibiotic response, and results showed variable effectiveness across several antibiotics.[20] Bacterial samples were identified using the Fatak 2 device, as shown in Tables 1 and 2.

Table 1: The Chemical Examination to Diagnose Bacterial Species.

Type of Bacteria	IND	MRVP	CIT	CAT	OXI	CoA	TSI	Gas	H2S
P. alcaligenes	-	- -	+	+	+	0	R/R	-	-
Escherichia coli	-	+ -	-	+	-	0	Y/Z	-	-
Klebsiella pneumoniae	-	- +	+	+	-	0	Y/Z	+	-
Staphylococcus epidermidis	-	- -	-	+	-	-	Y/Z	-	+
Staphylococcus aureus	+	- -	-	+	-	+	Y/Z	+	-
Enterobacter cloacae	-	- +	+	+	-	0	Y/Z	+	-
P. Putida	-	- -	+	+	-	0	R/R	-	-
Stenotrophomonas maltophilia	-	- -	V	+	-	0	R/R	-	-
Acinetobacter baumannii	-	- -	+	+	-	0	R/R	-	-
Pseudomonas aeruginosa	-	- -	+	+	+	0	R/R	-	-

Test results of missed 2 using Vitek2 compact system, IND= Indole, MRVP= red instance Voges- Proskauer, CIT=citrate,=CAT catalase, =OXI oxidase andCoA =coagulant enzyme, = TSI detector Trisugar.

Table 2: The Percentage of Sensitivity Types of Bacteria Antibiotics.

Antibiotics Type of Bacteria	Cfm %	IMP %	TE %	E %	Cip %	AK %	C %	VA %	Amp %	THIS %
Stenotrophomonas maltophilia	0	0	100	0	100	100	100	0	0	0
Staphylococcus aureus	0	90	0	41	25	90	50	66	50	0
Pseudomonas aeruginosa	0	83	0	0	0	86	0	0	0	0
Escherichia coli	0	100	0	0	66	100	100	0	0	0
Enterobacter cloacae	0	100	0	0	100	100	0	100	0	0
Dog for a pp	0	94	0	0	80	100	75	0	0	0
Acinetobacter Baumanni	0	100	100	0	0	100	0	0	0	0

Amp= Ampicillin, Cip= Ciprofloxacin, AK= Amikacin, TE= Tetracycline ,DA= Daptomycin ,IMP= Imipenem ,E= Erythromycin ,C= Chloramphenicol ,VA= Vancomycin Cfm= Cefixime

Isolated types of bacterial swabs from wounds were cultured using nano-manufactured solutions *of nickel oxide nanoparticles with ginger extract* NP.= NiO, cobalt oxide nanoparticles with aloe vera extract NP.= CoO and cobalt oxide nanoparticles with nickel oxide (NP = NiO + CoO) in combination with aloe vera extract and ginger. It was noted after taking measurements of bacterial culture growth that the chemically manufactured materials served as control solutions. There was no significant effect on bacterial growth inhibition with the synthesized nanosolutions.[21] There was an effect on growth inhibition at a rate of three implants per concentration. The inhibition zones of bacterial species were very high[22] compared to the inhibition diameters of antibiotics[23] shown in Table 2, where the inhibition diameters did not exceed 2-3 mm and were with different inhibition ratios relative to the total samples collected.[24]

Table 3: The Rates of Diameters of the Diagonal Areas of Bacterial Growth Using Nanosolutions.

Nanomaterial	NP.= NiO					NP.= CoO					NP.=NiO + CoO				
Type of Bacteria	CONC. %M					CONC. % M					CONC. % M				
	Cont.	25	50	75	100	Cont	25	50	75	100	Cont.	0.1	0.01	0.05	0.07
Stenotrophomonas maltophilia	5	11	20	30	34	6	8	11	13	25	8	42	14	15	42
Staphylococcus aureus	2	13	35	37	47	3	9	33	33	45	9	40	35	33	45
Pseudomonas aeruginosa	1	8	12	33	43	1	11	22	34	35	7	39	02	30	45
Escherichia coli	0	9	0	02	03	1	8	01	03	46	11	40	02	02	49
Enterobacter cloacae	4	11	0	12	33	3	11	27	30	42	5	35	8	02	45
Dog for a pp	2	9	20	33	45	3	7	24	03	40	9	33	02	02	43
Acinetobacter Baumanni	1	11	81	24	28	2	9	81	91	45	4	33	14	18	47
P. alcaligenes	0	10	23	25	35	0	4	22	30	33	2	40	28	30	45

CONC. = conctration, %M molar centigrade, chemically synthesized nanoparticlesCont.= control, nickel oxide nanoparticles, ginger plant extract NP.=NiO, cobalt oxide nanoparticles, aloe vera extractNP.=CoO, cobalt oxide nanoparticles with nickel oxide NP.=NiO + CoO of aloe vera plant extract with ginger plant extract.

Table 3 depicts that the concentrations of the solutions at M 100% were more effective in inhibiting bacterial growth with ginger extract,[25] and the same effect was observed with *Aloe vera* extract on all types of bacteria. The diameters of the inhibition zones ranged from 40-47 mm, while the other bacterial species had 30-40 mm. These results indicate that the effectiveness of nanomaterials manufactured by the green synthesis method[26] is higher compared to the inhibition results of nanomaterials manufactured by chemical method.[15] It was noted that the concentrations of the manufactured nanomaterials were mixed and combined with the solution Nanocomposite. The results of lining for all bacterial species were high 0.1, 0.07 M%. as in Figure 6 due to the presence of two nanomaterials with very small sizes ranging between 5–12 nm, which is called a quantum dot.[27]

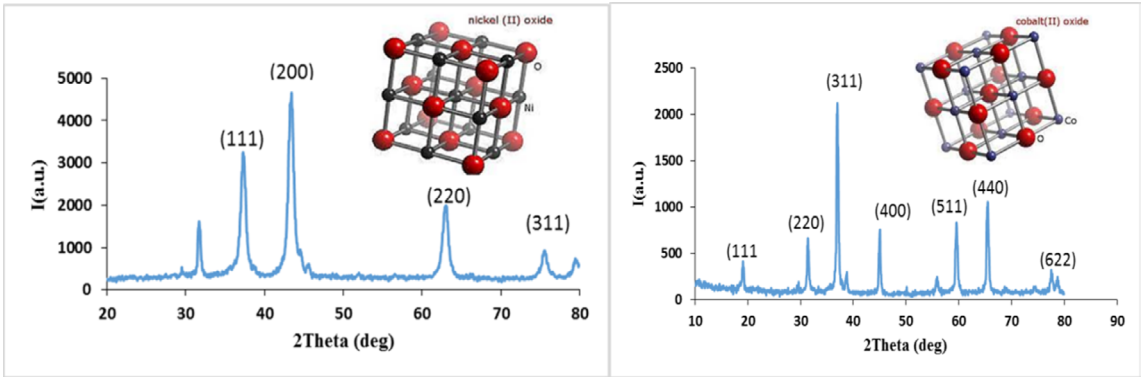

Figure 1: XRD Pattern of NiO, CoO Films.

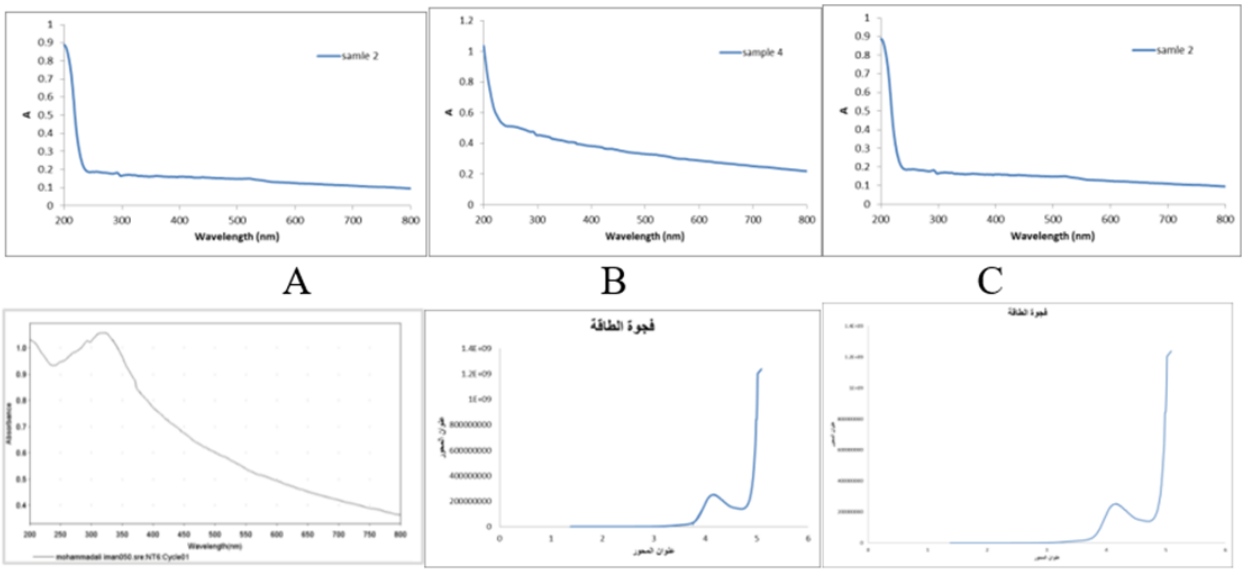

Figure 2: The UV Examination Showing the Energy Gap and Absorption of Nanocomposites of Nanosolutions Indicates that Sample C Represents a Mixture of two Solutions, Combining Cobalt Oxide with Nickel Oxide Nanometer Solutions.

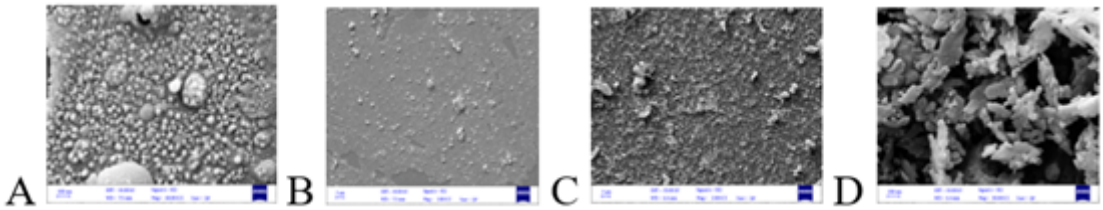

Figure 3: The SEM Examination of the Nanosolutions Shows that Sample C Represents a Mixture of Two Solutions, Combining Cobalt Oxide with Nickel Oxide Nanoparticles, While Sample D Represents the Nanomaterial Manufactured by Chemical Methods.

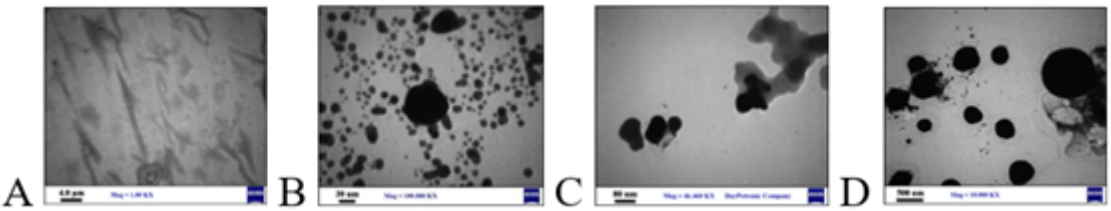

Figure 4: The TEM Examination of the Nanosolutions Shows that Sample C Represents a Mixture of Two Solutions, Combining Cobalt Oxide with Nickel Oxide Nanoparticles, While Sample D Represents the Nanomaterial Manufactured by Chemical Methods.

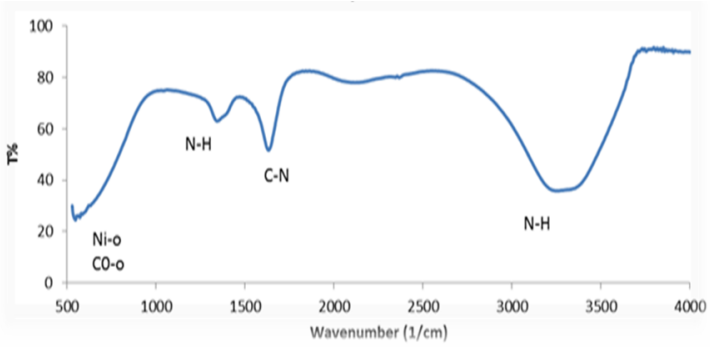

Figure 5: FTir Examination between Chemical Bonds between Atoms and the Presence of Chemical Elements of Nanomaterials.

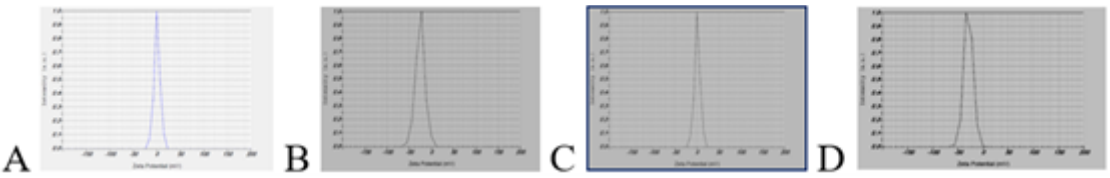

Figure 6: ZP Examination of Nanosolutions while C is the Combination of Two Solutions Combined with Cobalt Oxide Solution with a Solution of Nickel Oxide Nanoparticles D Represents the Nanomaterial Manufactured by Chemical Method.

It was observed from the ZP examination shown in Figure 6 and Table 4 that the nanocolates are lumpy and transparent.

Table 4: Shows ZP Measurements for Manufactured Nanoanalyzers.

Peak No.	Zeta Potential	Electrophoretic Mobility
A	-28.7 mV	-0.000223 cm2/vs
B	0.9 mV	0.000007 cm2/vs
C	- 38.6 mV	-0003 cm2/vs
D	-25.1mV	-0.000195 cm2/vs

Stefanowicz-Hajduk and Ochocka[28] determined the toxicological properties. Cytological toxicity assay MTT is a method using materials (metabolic dye [3-(4,5 Dimethyl thiazol-2-yl)-2,$5-] diphenylterazolium$bromide), in which the toxicity of the nanosolution results from the mixing of the two nanomaterials is measured. It was noted that the solutions are non-toxic as in Table 5.

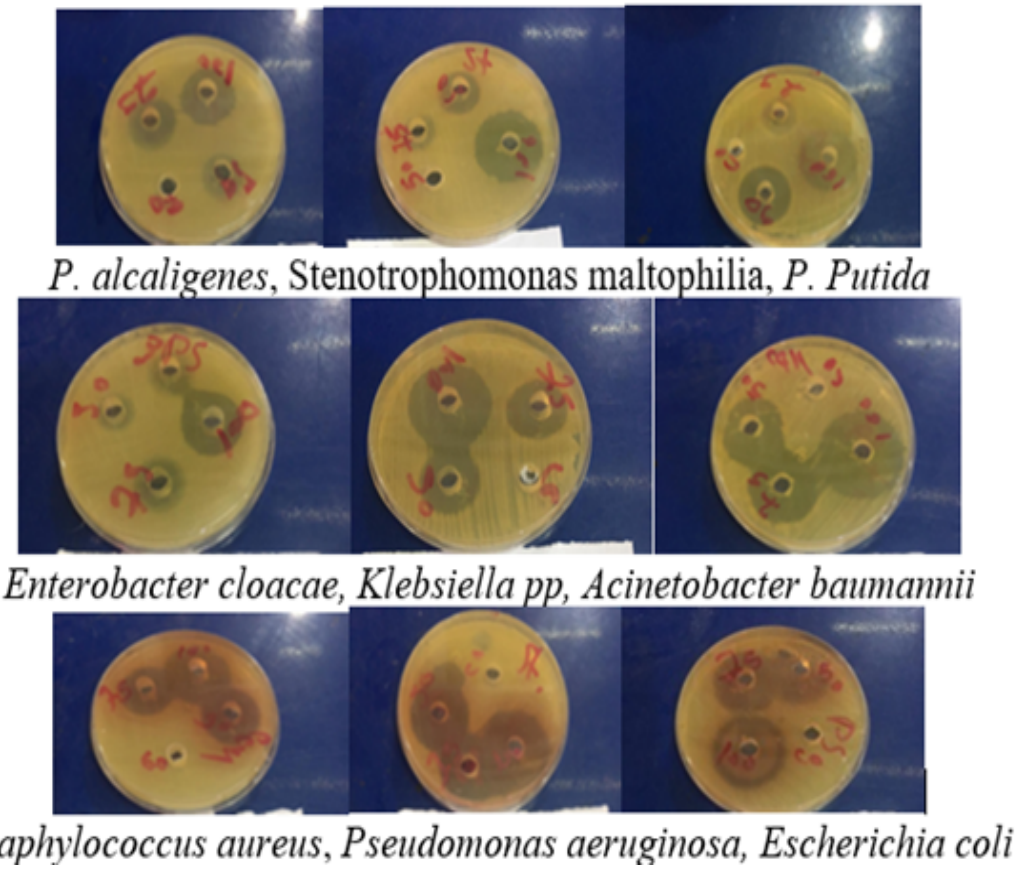

Figure 7: The Results of the Rates of the Diameters of the Growth Inhibition Zones. The Concentrations of Nanosolutions for some Bacterial Species, Representing the Nanomaterial Manufactured by Chemical Methods, were Compared with those Manufactured by the Green Synthesis Method, Showing the Effectiveness of Solutions Containing a Mixture of Two Solutions, Combined with the Solution of Nickel Oxide Nano.

The graph shows the effect of the solution on different cells and the mean number of dead cells is symbolized by Mean, and the amount of deviation is symbolized by SD. MTT: the effect of the solution on different cells. The average number of dead cells is symbolized by Mean, and the amount of deviation is symbolized by SD. The difference in values IC50 was observed, which indicates that the substance is not toxic.

Table 5: Shows the Rates of Normal Cell Numbers and Cancer Cells for Cytotoxicity Testing.

Type of Cell	PC3		Target	
Concen.	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

These solutions were also tested on test animals,[22] after conducting the process of drying the solutions and converting them into powder. Then these dried materials were taken and mixed with Vaseline 9.5 g with 0.5 g of powder for the dried solution. Three laboratory mice were used for each nanosolution treatment. The animal's back hair was shaved and a wound was made. Then the isolated bacteria were taken, and each type of bacteria was infected, taking into account the control sample, where wounds were treated with petroleum jelly only and compared to a sample treated with Fusidin ointment.[26] The animals were left under observation for every 12 hours, where it was seen that the animals that were treated with nanosolutions fully recovered within less than 12 hours, where signs of disappearance of skin congestion and stiffness of the wound appeared for all treatments except for the control sample. The wound was treated with petroleum jelly only, and a sample was treated with a substance of Fusidin ointment. Healing was not achieved within this period. The condition of the animals was observed over ten days post-treatment, with no cases of toxicity noted, as shown in Figure 7.

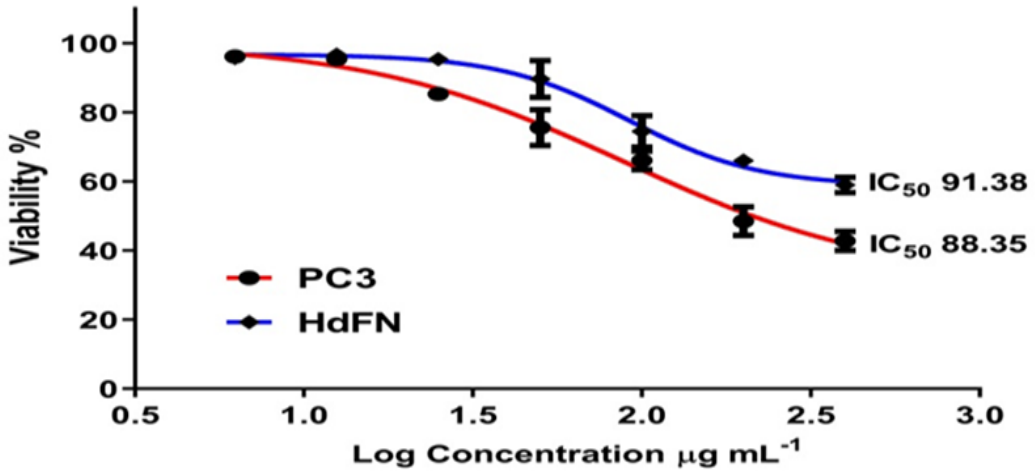

Figure 8: The Results of Treating Mice Using Different Concentrations of Nanosolutions for Various Bacterial Species: A Represents the Treatment with Nanomaterials Manufactured by Chemical Methods. B Shows the Treatment with Nanoscale Nickel Oxide Solution. C Represents Treatment with Cobalt Oxide Solution. D Represents Treatment with a Mixture of Cobalt Oxide and Nickel Oxide Nanosolutions.

Conclusions

The MTT assay results showed that the prepared solutions were non-toxic and highly effective, especially when diluted to low concentrations.

Overall Recommendations

This work recommends the following:

1. Studying the possibility of using these extracts against pathogens inside the living body, especially the oral area, to document the validity of what was reported about other non-dermal therapeutic uses of these extracts.
2. Determining the optimal duration for treatment when used within 12-hour intervals and assessing the effectiveness of the nanomaterials on bacterial cells. This recommended study could involve SEM microscopy and analysis with STEM microscopy to observe the effects on the bacterial cell wall and internal structures, helping to determine the rate of nanomaterial entry into bacterial cells.

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