Original Article

Comparison of using Nanoparticle Solutions from Various Plant Extracts to Inhibit the Growth of Certain Pathogenic Bacteria and Fungi

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Abstract

Objectives: This study aims to compare the nano-solution made from cinnamon extract with the effect of the nano-solution made from turmeric mixed with the nano-solution made from aloe vera in inhibiting the growth of fungi and bacteria. The importance of the study is due to the urgent need to find materials to treat pathogenic bacteria quickly, especially bacteria that are difficult to treat and at low costs. **Methodology:** The study is scientific, medical, and applied. The nanomaterials were observed when examining the nano solution made from plant extracts. They were tested on bacteria isolated from collecting samples by wound swabs using a sterile swab and then transferred to the laboratory for cultivation on culture media. Bacteria and fungi were isolated and examined. The isolates are *Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Enterobacter cloacae, Stenotrophomonas maltophilia, Acinetobacter baumannii, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa*, which infect the skin from patients' wounds in some hospitals. **Results:** It was noted that the effect of the solutions manufactured using concentrations of (75, 50, 25, 100)% m of the nano-turmeric extract solution, where the average ranged at a concentration of 100% between 12-23 mm, was less effective than the solutions manufactured from the nano-cinnamon extract, where the average diameters of the inhibitory zones in the bacterial culture reached 23-60 mm. It was also found that the nano-turmeric extract solution mixed with the nano-aloe vera extract solution was more effective than the nano-cinnamon extract solution mixed with the nano-aloe vera extract solution manufactured at concentrations of (1.0, 0.05, 0.07, 0.01)% m. It was also found that the average diameters of the inhibitory zones of the cultured bacteria treated with the mixed nano-solutions at a concentration of (0.07% m) ranged between 30-47 mm. These nano solutions manufactured with plant extracts are more effective than industrially manufactured nano solutions. It was found from treating these manufactured nano solutions with a culture of pathogenic fungi isolated from wounds, including *Candida albicans*, that the average diameters of the inhibition zones for all solutions ranged between 10-35 mm at a concentration of 100%. As for *Trichophyton mentagrophytes*, all solutions inhibited the growth of fungi, and no increase was observed due to the treatment. All solutions inhibited growth by 100%, and no fungal growth was observed. The cytotoxicity of the nano solutions was tested, and they were non-toxic. It was possible to prepare fast and effective nano solutions to treat bacteria and fungi quickly and cheaply. **Conclusions:** Solutions made from nano-turmeric extract mixed with nano-aloe vera extract solution can be used to treat bacterial wound infections as well as fungal infections. **Recommendations:** The research contributes to the rapid treatment of bacterial infections and reduces costs, the use of medications, and the occurrence of complications among patients, preventing them from staying in hospitals for a long time.

Keywords: *Staphylococcus Epidermidis*, *Staphylococcus Aureus*, *Enterobacter Cloacae*, *Stenotrophomonas Maltophilia*, *Acinetobacter Baumannii*, *Pseudomonas Aeruginosa*, Cinnamomum Verum, *Candida Albicans*, *Trichophyton Mentagrophytes.*

Introduction

The widespread prevalence of bacterial infections has gained global attention regarding diseases and epidemics and the pursuit of new methods to obtain rapid treatments for diseases.[1] Medical science has made extensive use of nanotechnology.[2] The search for alternatives to treat bacterial infections was prompted by the high antibiotic resistance exhibited by many bacterial species.[3] Plants are used as medicine because they contain phenols and other chemical substances that are useful in treating

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bacterial illnesses[4] and resist fungal and bacterial growth.[5] Among the plants often used to treat different diseases is Cinnamomum verum.[6] Furthermore, a medicinal plant extensively utilised in oils or powder form to treat various ailments is Cinnamomum verum.[7] Considered a main plant for wound healing,[8] aloe vera also can tolerate several dangerous germs.[9] Medically, Curcuma longa is used to cure diseases caused by germs.[10] Many medicinal treatments use turmeric, as its active components are very prevalent.[11] Active chemical components found in turmeric have been shown by earlier research.[12] Antibacterial resistance to antibiotics[13] is caused by their ability to develop enzymes that interfere with the way antibiotics operate[14,15] or produce toxins that encapsulate them and prevent antibiotics from completing their job.[15] The need to use nanomaterials[16] to fight bacteria with high degrees of resistance to antibiotics to treat many illnesses and their usage and manufacturing Most of the bacteria in wounds are antibiotic-resistant.[17] Investigating nanoparticles' effects helped to juxtapose these results with prior studies.[14,18] As previous studies have shown, nanomaterials restrict fungal development[19] and inhibit several harmful bacteria.[20] Most bacterial and fungal diseases affect skin wounds because they touch their environment directly.[21]

Research Questions

The effect of nano-solutions manufactured by green synthesis or synthetic methods and their effectiveness with plant extract solutions prepared in the form of aqueous extracts on pathogenic bacteria and fungi that infect wounds in the skin of hospital patients.

Rationale of Study

The possibility of using solutions made from nano-turmeric extract mixed with nano-Aloe vera extract solution and cinnamon extract to treat bacterial wound infections as well as fungal infections in the rapid treatment of bacterial infections and reducing costs, use of medications, and the occurrence of complications among patients and preventing them from staying in hospitals for a long time.

Method Section

Wound swabs were collected from hospitalised patients, cultured on MacConkey and blood agar, and incubated for 18-24 h at 37°C. Isolates were diagnosed based on morphological characteristics of the growing colonies and biochemical tests. Using the Vitek2 compact device,[22,23] Cobalt oxide was prepared.[24,25] Nickel oxide was prepared[26,27] using green synthesis[28,29] and chemical synthesis methods. Nanomaterials were prepared from Cinnamomum verum extract.[20]

A nickel oxide solution with nano turmeric was prepared[30] by mixing a 1% turmeric solution using a magnetic stirrer with a 1 M nickel chloride solution (NiCl2·6H2O) %14.5. After mixing, the precipitate disappeared, and the colour stabilized at a temperature gradually raised from 30°C to 70°C to obtain nano nickel oxide. The cinnamon solution was prepared in the same way.

Cobalt oxide was prepared from Aloe vera extract.[31] 10% aloe vera solution was mixed with cobalt nitrate solution ($Co(NO_3)_2$) at 18.5% and left until the precipitate disappeared and the color stabilized to obtain nano cobalt oxide. After preparing nickel oxide and cobalt oxide, the nano solutions were mixed at 50°C for an hour with gradual and reverse ratios. The nanomaterials were then examined using XRD, SEM, TEM, UV, FTIR, and ZP tests. Cytotoxicity tests were conducted on nickel and cobalt oxide solutions.[27] Bacteria were cultured for 24-48 hours and fungi for 40 days in specific media for each type. They were then treated with nano solutions, and the results were recorded.

Result

It was observed from the results of the examination of the solutions made from nickel oxide and cobalt oxide that there were nanoparticles. The XRD test results were: 38.95 (111), 38.95 (100), 38.95 (101), 42.6 (200), 42.6 (100), 62.45 (220). As for the examination of the nano solution made from turmeric extract, the results were 38.55 (111), 38.55 (100), 41.6 (200), 43.45 (100), 44.8 (200), and 60.3 (220). These results were consistent with international standards. Figure (1) illustrates these findings.[32]

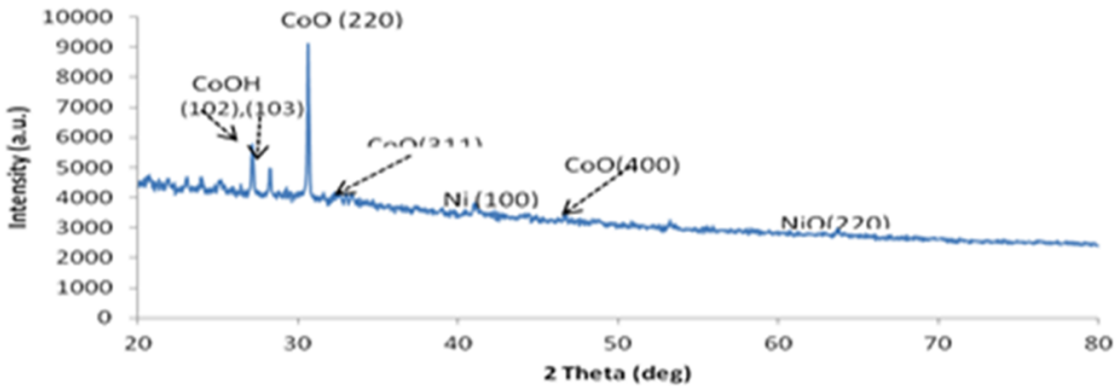

Figure 1: XRD Examination K Nano Solution with Turmeric Extract and D Nano with *Aloe Vera* Extract.

As for the SEM examination, the nano solutions were examined, representing material D; the particle shapes are rocky or flinty, spherical, and nearly spherical. These particles appear with dimensions of 25 nm, smaller than 100 nm. The K particles appear spherical like stones, being 10 nm, and a size of up to 2.5 µm for the nanoparticles.[33] Figure (2) shows these results.

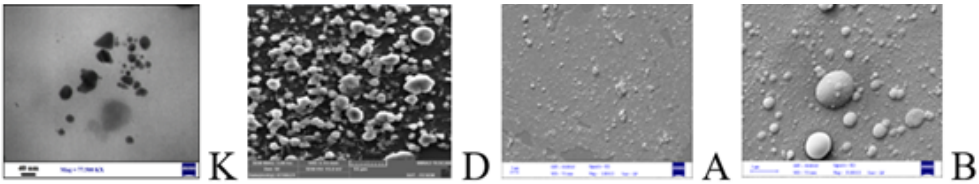

Figure 2: SEM Examination Results of the Two Nano Solutions A = Nano Solution *Aloe Vera* Extract, K = Nano Solution Turmeric Extract, D = Cinnamon Solution B= Nano Solution Prepared Synthetically.

The TEM examination is a test to determine the nanoparticle's size and form before deposition.[34] D nanoparticles of 1.5 µm, consisting of the largest, single spherical entities joined to each other. Based on the scale, the nanoparticle size was 40 nm. Various sizes of nanoparticles were observed, ranging from 0.5 to 40 nm. K of 3.5 µm, consisting of large, single spherical entities joined to each other. Various were observed, ranging from 0.5-1 nm. Aggregates were observed, and the nanoparticle size did not exceed 0.5 nm.[34] Figure (3) illustrates these findings.

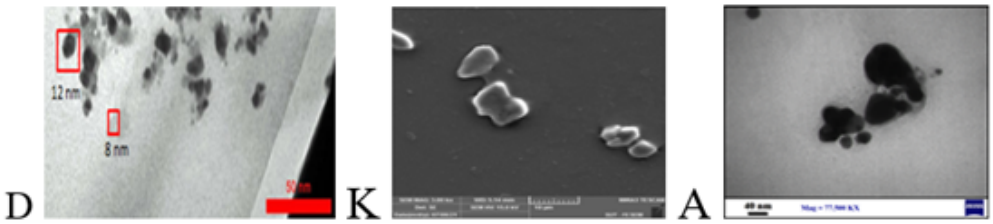

Figure 3: TEM Examination Results of the Two Nano Solutions A=Nano Solution *Aloe Vera*, K= Nano Solution Turmeric Extract, D= Nano Cinnamon Extract Solution).

The FTIR examination identifies active chemical bonds. solution of A showed bonds Co-O at (±50 (500 to 694)). The wavelength 1315 was at the C-N bond level, 2400 at the O-H bond, and 3500 at the N-H bond, based on the vibration table in the source. C, O, and H bonds indicate the presence of metal oxides. As the nano material is diluted, the particles with firm charges attract and repel, preventing aggregation, and the material settles at the bottom of the container during preparation.[19] Figure (4) shows these results.

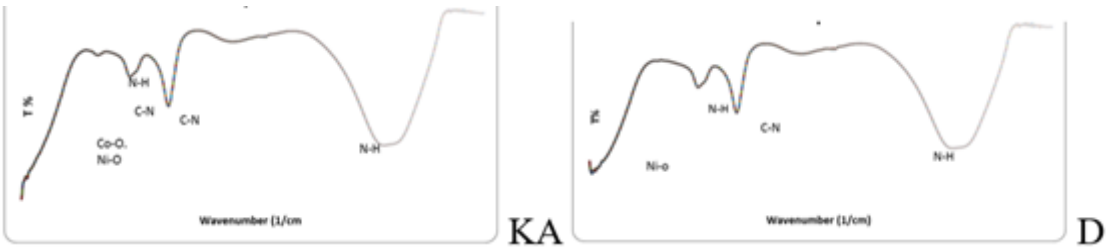

Figure 4: Shows the FT-IR Examination (K.A =Nano solution Aloe vera extract and nano solution turmeric extract, D = nano solution with cinnamon extract).

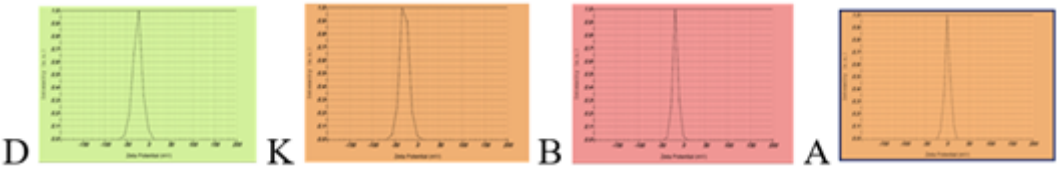

Figure 5: Shows the Zeta Potential Examination of the Nano Solutions, Indicating that the Materials are Non-aggregated. D = Nano Cinnamon Solution, K = Nano Turmeric Solution, A = Nano Aloe Vera Solution, B = Nano Solution Prepared Synthetically.

The ZP examination was conducted.[35] The ZP examination determined the particle size, surface charge (negative and positive), and the repulsion and attraction between charges based on the Zeta potential factor, the solutions were non-aggregated and clear. Figures (5) and Table (1) illustrate these findings.

Table 1: Shows the Examination Values of the Manufactured Nano Solutions.

Peak No.	Zeta Potential	Electrophoretic Mobility
Bchemical	0.9 mv	0.000007 cm2/vs
Acactus	-4.9 mv	-0.000038 cm2/vs
DStudents	-25.1mv	-0.000195 cm2/vs
Kturmeric	-28.5 mv	-0.000221 cm2/vs

The Zeta Potential examination values of the nano solutions indicate that the materials are non-aggregated. D = nano cinnamon solution, K = nano turmeric solution, A = nano Aloe vera solution, B = nano solution prepared synthetically.

The UV examination absorption coefficient, determining the energy gap.

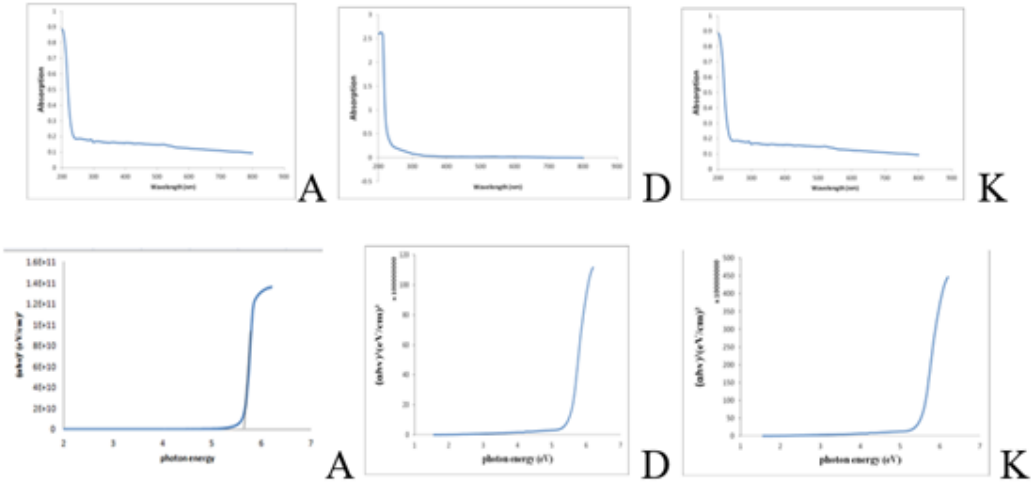

Figure 6: Shows the UV Examination of the Nano Solutions, Indicating that the Materials are Non-aggregated. D = Nano Cinnamon Solution, K = Nano Turmeric Solution, A = Nano Aloe Vera Solution.

Figure (6) The graph shows that the solution is very homogeneous and light between 236 and 800 nm. The energy gap value in the graph is 3.8 eV, and D's is 4.3 eV, but K's is 5.3 eV, indicating it is a semiconductor and achieves the oxide of the nano material.

The effectiveness of nano plant extracts is due to the synergy between their chemical contents and the nanoparticles in the nano solution.[32]

Table 2: Shows the Average Diameter of the Bacterial Growth Inhibitory Zone Using the Manufactured Nano Solutions.

Nanomaterial	K.NP.= NiO					D.NP.= CoO					KA.NP.=NiO + CoO				
Type of Bacteria	CONC.%M					CONC.%M					CONC.%M				
	B	25	50	75	100	B	25	50	75	100	B	0.01	0.05	0.07	0.1
Acinetobacter Baumannii	0	0	0	20	32	0	0	11	13	25	0	0	15	33	35
Escherichia coli	0	0	0	22	**23**	0	0	17	33	34	0	0	20	41	04
Enterobacter cloacae	0	0	0	12	22	0	0	16	34	35	0	0	20	37	42
Klebsiella pp	0	0	0	18	02	0	0	01	03	04	0	0	02	32	45
Pseudomonas aeruginosa	0	0	0	01	12	0	0	11	12	23	0	0	8	26	35
Staphylococcus aureus	0	0	0	20	22	0	0	0	0	**60**	0	0	20	42	**47**
Stenotrophomonas maltophilia	0	0	0	81	24	0	0	81	91	30	0	0	28	40	30

K = Nickel oxide turmeric solution, D = Nickel oxide cinnamon solution, A = Aloe vera extract with Cobalt oxide solution, B= Nano solution prepared synthetically.

The results demonstrated that the cobalt oxide solution with Cinnamomum verum had inhibition zone diameters of (0.0, 0.0, 0.0, 60, 11, 13, 23) mm, indicating its lower effect. However, its on S. aureus was 60 mm on average. The D cinnamon solution showed high efficacy against various bacterial types.[36] Nickel chloride with cinnamon extract showed effectiveness.[31] Its efficacy on Ps. aeruginosa was observed at 100, 75, and 50% M concentrations. As noted in previous studies, the nano turmeric extract showed an inhibitory effect on pathogenic bacteria due to its chemical compounds that inhibit bacterial growth.[37]

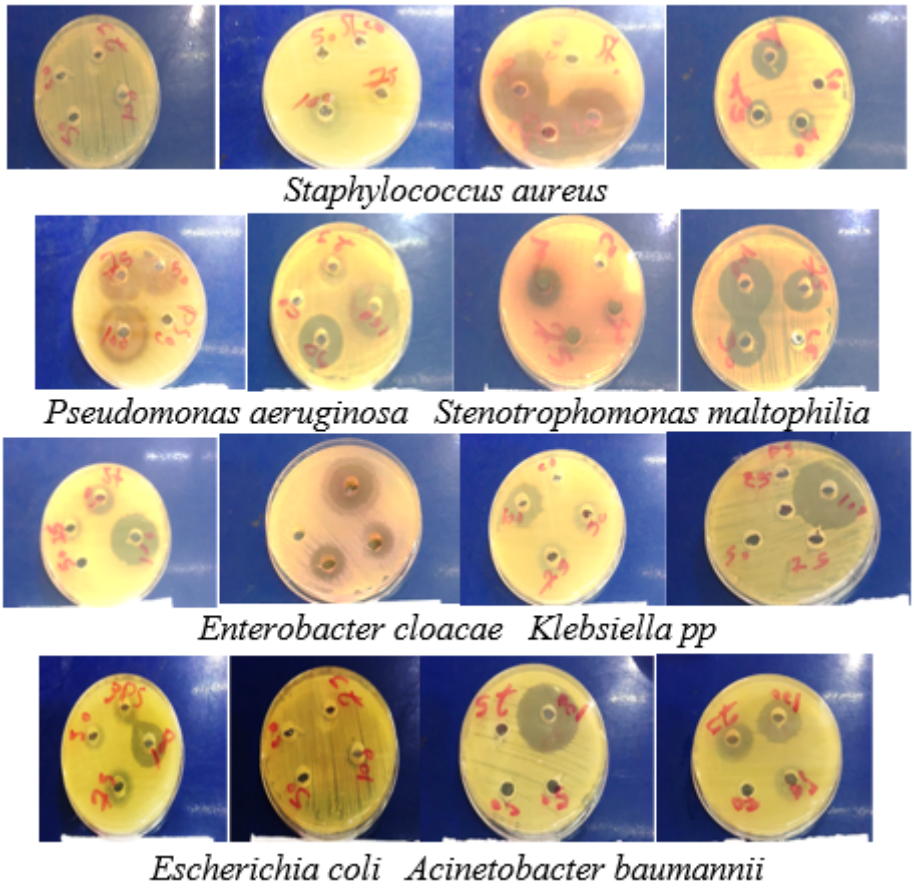

Figure 7: Shows the Average Diameter of the Bacterial Growth Inhibitory Zone using the Manufactured Nano Solutions. K = Nickel Oxide Turmeric Solution, D = Nickel Oxide Cinnamon Solution, A = Nano Solution with Aloe Vera Extract, B = Nano Prepared Synthetically.

Table 3: Shows the Average Diameter of the Fungal Growth Inhibition Zone Using the Manufactured Nano Solutions.

Nanomaterial	D.NP.= NiO					K.NP.= CoO					KANP.=NiO + CoO				
Type of Fungi	CONC. %M					CONC. %M					CONC. %M				
	B	25	50	75	100	B	25	50	75	100	B	0.1	0.01	0.05	0.07
Candida albicans	16	0	0	14	35	25	0	8	9	10	20	45	0	35	33
Trichophyton mentagrophytes	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO

K = Nickel oxide turmeric solution, D = Nickel oxide cinnamon solution, A = Aloe vera extract with Cobalt oxide solution, B = Nano solution prepared synthetically, K.A = Mixed cobalt oxide and nickel oxide solution, NO growth = No fungal growth.

The K solution, nickel chloride with turmeric, inhibited bacterial growth at concentrations of 100, 75, and 50% M. The nano cinnamon extract had high efficacy due to its many chemical compounds[38] that synergize with nano materials to quickly inhibit bacterial growth. The nano turmeric extract also inhibited the development of all isolated bacterial types.[39] Table 2, Figure 7.

The treatment of nano solutions in fungal cultures, as shown in Table 3, showed that the nano cinnamon solution had an inhibition rate of 35 mm for *Candida albicans* with a 100% M concentration. This was more effective than the inhibition zone diameter of the fungal treated with the nano turmeric extract solution, which was 10 mm at a concentration of 100% M. Figure (8).

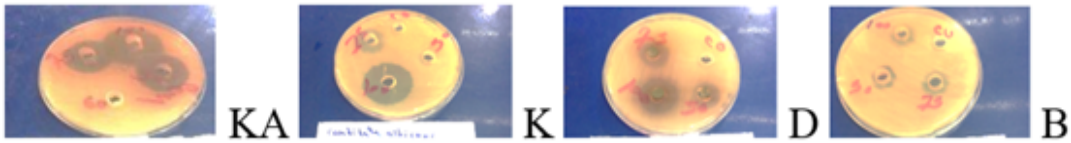

Figure 8: Shows the Average Diameters of the Inhibition Zones for *Candida Albicans*. It was Noticed that B = Nano Solution Prepared Synthetically, D = Nickel Oxide Cinnamon Solution, K = Nickel Oxide Turmeric Solution, A = Cobalt Oxide Solution with Aloe Vera Extract, K.A = Mixed Cobalt Oxide and Nickel Oxide Solution.

As shown in Figure 9, it was observed that the nano solutions for *Trichophyton mentagrophytes* had a full inhibition rate, with no fungal growth. This is explained by the chemical composition of the plants, which is essential in working in concert with the nanomaterials to limit the growth of fungi.[40] Cinnamomum verum is a medicinal plant with chemical compounds synergising with nano materials to inhibit fungal and bacterial growth.[41]

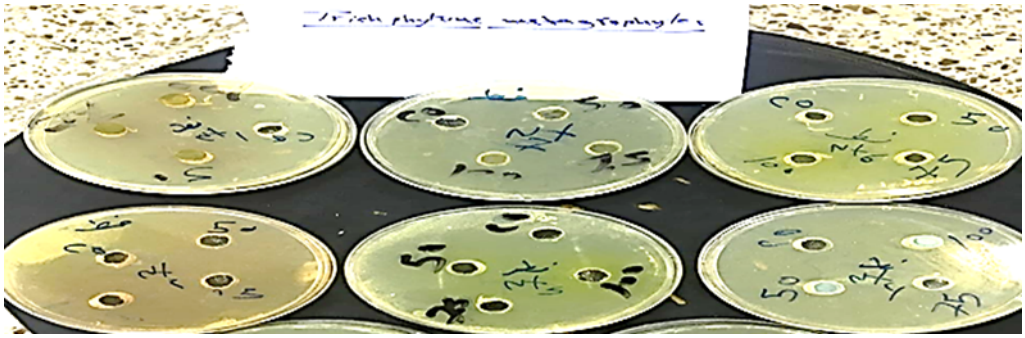

Figure 9: Displays the Inhibitory Zone Average Diameters for *Trichophyton Mentagrophytes*. B = Nano Solution Prepared Synthetically, D = Nickel Oxide Cinnamon Solution, K = Nickel Oxide Turmeric Solution, A = Aloe Vera Extract with Cobalt Oxide Solution, and K.A = Mixed Cobalt Oxide and Nickel Oxide Solution. No Fungal Growth was Observed (NO growth).

The cytotoxicity MTT test, counts the number of living and dead cells to assess cell viability and resistance to toxins.[42,43] The MTT assay (metabolic dye [3-(4,5 Dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide]) was employed to gauge the nano solution's cytotoxicity after the two nanomaterials were combined. The solutions were non-toxic to the most inhibitory bacterial growth solution.[44,45] Table (4), Figure (10).

Table 4: Presents a Statistical Study of the Numbers of PC3 Cancer Cells And Normal HdFN and the Impact of the Treatments on Cell Growth Rates.

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

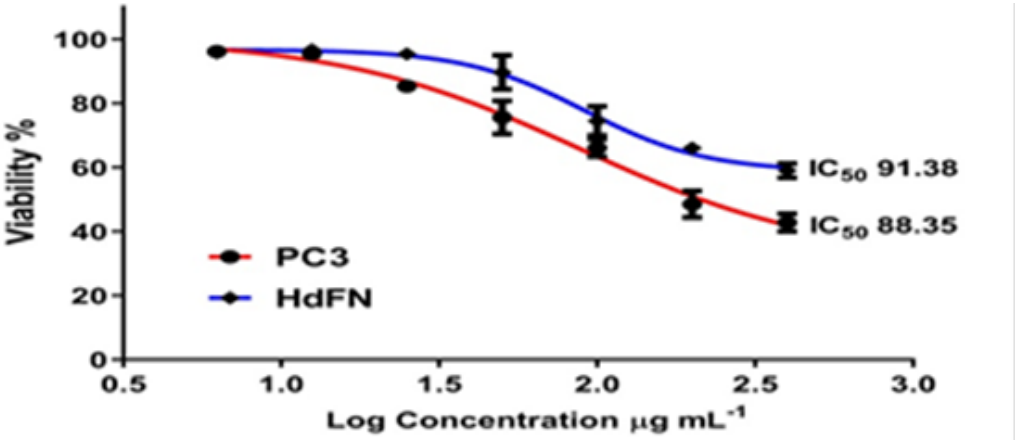

Figure 10: Displays the IC50 Cell Killing Ratio, and the Number of PC3 Cancer Cells Killed by the Nanosolutions, their Ratio to the Number of Normal HdFN Cells.

Laboratory mice were treated to investigate the effect of produced nano materials on bacteria-infecting wounds. Three replicates were taken for each treatment. The method of infection involved creating wounds on the

back of the animal, cleaning the wound area, and then applying a bacterial isolate sample to induce disease. The infection lasted two days until swelling, redness, congestion, and pus appeared in the infected area. The treatment involved drying the solution and mixing 9.5 grams of glycerin (a simple fat with no chemical effect) with 0.5 grams of the previously dried solution powder, which had the highest bacterial growth inhibition rate at a concentration of 0.07% M. The dried powder was ground using a ceramic mortar, and a portion was taken for treatment three times a day. It was observed that the nano materials provided therapeutic results within a few hours of treatment, causing the wound to harden and the redness, swelling, and pus to disappear (Figure 11). The mice were monitored for three days and left under observation until the tenth day to monitor the effect of different nano materials. The mice remained alive, indicating that the nano material was non-toxic. The rapid wound healing was attributed to the nano materials being in the quantum dot size range (5-12 nm), which can penetrate bacterial walls, enter bacterial metabolic pathways, and disrupt bacterial DNA and RNA, killing the bacteria quickly within hours. These nano materials synergize with the chemical content of plant extracts to exert their bactericidal effect within hours, as noted in previous studies.[46,47]

Figure 11: Shows the Results of Treating Laboratory Mice with Manufactured Nano Solutions, Indicating the Average Diameters of the Inhibition Zones for *Candida Albicans*. It was Observed that K = Nickel Oxide Turmeric Solution, D = Nickel Oxide Cinnamon Solution, A = Aloe Vera Extract with Cobalt Oxide Solution, B = Nano Solution Prepared Synthetically, K.A = Mixed Cobalt Oxide and Nickel Oxide Solute.

Implications

Solutions made from nano-turmeric extract mixed with nano-*Aloe vera* extract solution can be used to treat bacterial wound infections as well as fungal infections. The same applies to cinnamon extract, as the two solutions contribute to the rapid treatment of bacterial infections, reduce costs, use of medications, and complications among patients, and prevent them from staying in hospitals long. It was noted from the cytotoxicity test that the nano-solutions are not toxic.

Limitations

Through the study, it was shown that the nano-solutions prepared by the green synthesis method with extracts of turmeric, aloe vera, and cinnamon have high effectiveness at diluted concentrations against some bacteria and fungi in skin infections in wounds and are non-toxic.

Conclusions

The green synthesis method of the nickel oxide and cobalt oxide nano solutions before mixing the two solutions was more effective than the nano material prepared by the artificial method, which weakly inhibited bacterial and fungal growth.

- The MTT cytotoxicity assay showed that the solutions prepared for treating bacterial infections were non-toxic and highly effective at low concentrations.

Final Recommendations

- The use of nano nickel oxide solutions with turmeric mixed with nano cobalt oxide solutions with aloe vera for treating bacterial and fungal skin infections, as they are non-toxic.
- The use of EDX, SEM analysis, and STEM (Scanning Tunneling Electron Microscope), to observe all effects within and on the bacterial cell wall.

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