

Green Synthesis of NiO-CoO Nanocomposites and its Antibacterial Activity

Assistant Professor Dr. Asmaa Ahmed Hatem Sultan*

Middle Technical University,
Technical Medical Institute,
Baghdad, Iraq.

Rana Jalal Shaker

Department of Biological Sciences,
Tikrit University,
Tikrit, Iraq.

Abstract: This research aims to manufacture and characterize green synthesis in this study, nano-solutions were prepared by the green synthesis method, using nickel nitrate with saffron and cobalt nitrate with aloe vera as raw materials. Then various physical analyses and measurements were carried out, such as XRD, SEM, TEM, UV-Vis, and FTIR. The X-ray diffraction (XRD) study showed the films, which were deposited on a glass substrate by drop casting method, have a polycrystalline structure with a cubic phase. The morphological surface of the Nano composites was studied by a scanning electron microscope; the particles were of irregular shapes and microscopic sizes, with an average diameter of about 127 nm. From the results of the transmission electron microscope, it is found that the shape of the particles is spherical and the average size of the particles ranged between 12-20 nm. NiO-CoO formation with high purity phase confirmed by Fourier transform infrared (FT-IR) spectra. UV-Vis spectrum was used to study the optical properties, where the bandgap of the Nanocomposites (nickel oxide with cobalt oxide) was 2.75 eV. The Nanocomposites are investigated against the anti-bacterial activity of *Staphylococcus aureus* and *Pseudomonas aerogenes*, where it recorded good efficacy and within the permissible specifications. It can be used as a treatment against bacterial species that have multiple resistance to antibiotics in skin infections caused by burns.

Keywords: Green synthesis, burns, FRIT, XRD, UV, SEM, TEM, NiO, Co O, aloe vera, staphylococcus aureus and pseudomonas aerogenes

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I. INTRODUCTION

A. Field of Invention

In this study, two nanocomposites were prepared by the method of green synthesis (green syntheses) instead of chemical and physical methods because it is a fast method in the production of nanosolution. It does not leave toxic residues and does not need complex techniques as it provides large quantities of nanomaterial at a low cost of high economic quality as it can be used in many applications unlike other methods [1].

B. Background of the invention

The advent of nanotechnology has led to the integration of nanomaterials into many consumer products and in industry, communications, agriculture, and medicine where nanoparticles receive special attention in the medical and biological fields [2]; [3]. Nanomedicine is a combination of nanotechnology and medicine that plays a crucial role in various applications such as drug delivery, antifungal activity, antiviral activity, antioxidant and anti-cancer, and antibacterial such as bacteria that infect burn patients. It was found that some of the burn patients who are hospitalized for treatment are infected

*Correspondence concerning this article should be addressed to Assistant Professor Dr. Asmaa Ahmed Hatem Sultan, Middle Technical University, Technical Medical Institute, Baghdad, Iraq. E-mail: assma2222@mtu.edu.iq

with the bacteria, *Staph. aureus*, *P. aeruginosa*, burns are a common phenomenon and occur everywhere. So, we must make sure that the environment that surrounds us is safe, as burns are divided into fourth- degrees (superficial burns that affect the skin and muscle tissue, so they are simple and called first-degree burns, while burns in which damage reaches some layers under the skin are called second-degree burns). While there are burns up to all layers of the skin, these are third-degree burns, but when the burn reaches the bones and muscles, they are called fourth-degree burns) so the treatment depends on the severity of the burn that modern treatments have contributed to improving results. In 2008, antibiotics were taken through a vein for those with large burns, but the emergence of the problem of antibiotic resistance causes a risk of transmission of infection and rapid infections, leading to reduction in the improvement of survival rates among those infected. Thus, studies resorted to the use of nanomaterials because of their wide effect as an antibacterial antibacterial, where it was found that the synergistic action of the nanomaterial with antibiotics has a great effect because the nanoparticles have properties that differ from their analogs in the normal case. Micro (bulk) due to the change in physical, chemical, thermal, electrical and mechanical properties [4]; [5]; [6]; [7]; [8] and several traditional methods are used in their preparation, but there is a constant quest in the scientific community to develop these methods and reach a less toxic, environmentally friendly, cost-effective and clean method for the synthesis of nanoparticles, where the method of green synthesis using plant extracts appeared and became more common because it is environmentally friendly and inexpensive. Plant extracts contain active substances in Biologics such as starch, proteins, terpenoids, phenolic acids, alkaloids, polysaccharides and polyphenols. By acting as oxidative and reducing agents, these chemicals help in the formation of nanoparticles [9]; [10]; [11]. Nickel oxide (NiO) from semiconductors (positively charged) with a wide power gap of 3.6 to 4 volts is used in many photovoltaic applications[10]; [12]. Cobalt oxide is in the form of an olive-green crystalline powder in the case. unblemished, but is often found in an impurity state and is dark gray. It is easily oxidized to cobalt(III) hydroxide and has a gap in the 2-electron volt band that can be prepared by chemical and physical methods[13]. In this study, saffron plant extract (*Saffron Crocus sativus L.*) was used. Also, the cactus plant (*Aloe perfoliata vera*) is in the preparation of nanosolutions, the saffron plant is a medicine used to expel gases, spasms, and flatulence, and menstruation. It enhances blood flow in menstruation and was used by Europeans in the Middle Ages to treat respiratory infec-

tions and disorders such as cough and to treat whooping cough, colds, scarlet fever, smallpox, cancer, lack of oxygen, and asthma and saffron had other goals including blood disorders, insomnia, paralysis, heart disease, stomach disorders, gout, and chronic metrorrhagia, which is Antidepressant also used to expel intestinal worms, sedative drugs for neurological and psychiatric conditions, medicines used to stimulate the urinary secretion and many other drugs[14]. The cactus plant has been used by the Egyptians for 6000 years to heal wounds, heal infections and relieve burns and ulcers, and nowadays it is used to combat bacteria, mold, fungi, and viruses because the aloe vera plant is antiseptic and sterile [13].

C. General Description of the Invention

The nano solution was prepared by the green synthesis method, which is considered one of the most important methods used in the preparation of nanoparticles because it is a cost-effective, safe, and non-toxic synthesis method. Here, nickel nitrate $\text{Ni}(\text{NO}_3)_2$ was used with saffron plant extract and cobalt nitrate $\text{Co}(\text{NO}_3)_2$ with aloe vera extract and distilled water as raw materials in the preparation of the combined nano solution of nickel oxide and cobalt oxide respectively in the first stage. Also, 14.5 g of nickel nitrate was dissolved in 100 ml of distilled water with stirring by magnetic stirrer for an hour at a temperature starting from 30 to 70 degrees gradually until the disappearance of the precipitate to obtain a homogeneous solution, after which the saffron extract was prepared by dissolving 1 gram by 100 ml of distilled water at a temperature of 30 to 700 degrees gradually raise the temperature to facilitate the exit of chemicals in the plant at extraction Celsius and for 60 minutes as shown in Figure 1. Then filter the plant extract with cotton to get rid of impurities After preparing the two solutions, the saffron extract solution is added to the nickel nitrate solution gradually with continuous stirring and at the same temperature of preparation and for an hour until the color change occurs with the disappearance of the precipitate permanently, and this indicates The occurrence of interaction and the formation of nanoparticles[15].

The second stage includes the preparation of cobalt oxide nano, 18.5 g of cobalt nitrate was dissolved directly in 100 ml of distilled water with continuous stirring and at a temperature starting from 30 to 70 and for an hour. Then, the aloe vera extract was prepared by taking a weight of 10 g of locally grown aloe vera leaf pulp and dissolved in 100 ml of distilled water and then placing on the stirrer Magnetic for an hour at a temperature of 30 degrees was gradually raised to 70 degrees and then filtered also using medical cotton, after preparing the two

solutions, aloe vera extract was added to the cobalt nitrate solution gradually with continuous stirring and at a temperature of 70 degrees and for an hour to obtain a cobalt oxide nano solution as in Figure 2. In the third stage, the two solutions of nickel oxide are mixed with cobalt oxide at a rate of 25% and 75% respectively to form a nanocomposite to study its properties and the effect of its bacterial activity [16]; [17].

II. MATERIAL AND METHODS OF PREPARATION

A. Examination of the Effect of the Prepared Nanosolution on the Growth of Bacteria

The diffusion method of drilling (Agar-well diffusion, method) was used, as the surface of the medium Mueller-Hinton Agar was inoculated by 0.1 ml of bacterial stuck that was disseminated by the sterile culture diffuser. Then holes were made in this medium by a sterile cork piercer with a diameter of 5 mm and by two holes in the first dish to study the effect of the solution at a concentration of 0.1 molar and 4 pits for the second dish in order to study the effect of reducing the concentration on bacterial growth and in concentrations (0.01,0.05,0.07). One of these pits is used as a control agent added to 0.1 ml of distilled water after which the dishes were incubated at a temperature of 37°C for 24 hours to watch the growth inhibition areas around these pits in diameters of bacterial inhibition areas given in mm units.

III. RESULTS AND DISCUSSION (APPLICATIONS) X-RAY DIFFRACTION ANALYSIS

Figure 3 shows the X-ray diffraction pattern of nickel oxide nanoparticles prepared by the green synthesis method and deposited on glass bases by placing 5 drops by drop casting method. The XRD spectrum shows three distinct peaks with diffraction angles = 37.02, 44.2, 65.5, and 75 that correspond to the crystalline planes (111), (200), (220) (311) respectively and these results are consistent with the source [12]. The diffraction pattern of nickel oxide nanoparticles is compatible with the international card (JCP2_47-1049). The tests also indicated that it had a polycrystalline structure, indicating that it was in the cubic phase and there was no trace of other materials.

Figure 4 shows the X-ray diffraction examination of cobalt oxide particles prepared using *Aleo vera* plant extract to confirm the presence of nanomaterial and precipitate on glass bases by placing 5 drops and by drop-casting method. The results showed that cobalt oxide nanoparti-

cles are polycrystalline and with cubic structure, and this corresponds to the source [1] and at diffraction angles (19.1, 31.8, 45.6, 59.2, 65.4, 79.3) and crystalline levels (111), (220), (400), (511), (440), (622) respectively.

After confirming the identity of nickel oxide and cobalt oxide, an X-ray diffraction examination was performed for the mixture consisting of nickel oxide with cobalt oxide by 25% and 75%, respectively, prepared by the green synthesis method of saffron extract and aloe vera extract and deposited on glass bases with a clarification of 5 drops by drop-by-drop method). The results show that the cobalt compound is predominant with the presence of nickel compound and the appearance of two peaks of cobalt hydroxide CoOH with crystalline levels (102) and (103) at a diffraction angle of 27° respectively as shown in Figure 5 and this is consistent with the results of the research [18]; [19].

Figure 6 represents the images of the scanning electron microscope SEM at the magnification power KX3.00 of the compound (nickel oxide and cobalt oxide) deposited on glass by drop casting method and prepared by green synthesis method. It is noted from the images that the particles were of irregular shapes and microsize due to agglomeration in the material, which led to the formation of particles not exceeding 127 nanometers in diameter.

Figure 7 represents the image of the electron microscope (TEM) of the solution (nickel oxide and cobalt oxide) where it was found that the shape of the particles is spherical and semi-spherical and the size of the particles ranges between 20-5 nm and the presence of sizes between 5 to 12. This indicates the state of (quantum dot). The existing agglomerations can be attributed to the presence of proteins that surround the particles [20], [21].

Figure 8 shows the FTIR spectra of the solution of nanomaterials (nickel oxide and cobalt oxide prepared by plant synthesis with a range of 500-4000 cm⁻¹) where the absorption peaks and their positions are due to the chemical composition, crystalline nature, and morphology of the material. C-N Bond, 1600 cm⁻¹ C-N Insistence, 3200 cm⁻¹ N-H Bond, 1080 CM⁻¹. The presence of N-H confirms the presence of plant matter in solution and the presence of plant is important in the process of oxidation and chromatic transformation in the nano solution [22]; [23]; [24]; [25]; [26]; [12].

A. Study of UV Visible Spectroscopy of Nanomaterial Solution (Nickel Oxide and Cobalt Oxide) and the Tauc Relationship

Figure 9 shows the optical absorption spectrum of the nickel oxide and cobalt nanoscale solution exam-

ined by UV-visible spectroscopy with a wavelength range (200-900 nm). We note from the figure the presence of the plasmon peak at the wavelength of 200-400 nm and this is evidence of the formation of the nanomaterial, while the second peak at the wavelength of 523 nm indicates the color of the material and the presence of two types of small and large particles, so there are two peaks for absorption, and this is confirmed by the SEM examination and the TEM examination. A decrease in absorbency was also observed with increasing wavelength, indicating that the prepared solution has high permeability in visible and near-infrared areas.

The optical energy gap was calculated using Taus' equation by drawing the graphical relationship between $(\alpha hv)^2$ and hv as in Figure 10. The energy gap is calculated when it is $0 (\alpha hv)^2 =$ from the area of the intersection of the tangent with the axis. Thus, the intersection point represents the energy gap and we note that the values of the optical energy gap E_g of the nanosolution (nickel oxide and cobalt oxide) were 2.75 electron volts.

B. Antibacterial Activity Test

The effectiveness of the antibacterial activity of the solution of nanomaterials (nickel oxide and cobalt oxide)

was prepared by the green synthesis method of saffron extract and aloe vera extract was examined respectively using the method of propagation of agar well against two bacterial strains, *Ps.aeruginosa*, *staph. aureus*. Three replicates were taken for each test sample and after the average diameters were taken, it was noted that the nanosolution inhibits the growth of bacteria by forming halos around the samples known as the inhibition zone. Figure 11 is the solution with a concentration of 0.1 M, the average diameter of the inhibition zone is (45) mm, which is a high inhibitory effect on the bacterial growth of *Staph. aureus*, and when the solution was diluted to concentrations (0.01, 0.05 0.07%) M. , the highest measurement of the diameter of the inhibition zone of *Staph. aureus* bacteria was found. *Staph.aureus* is has a concentration of 0.07 M % was an average diameter of 8 mm as shown in Table 1.

In the case of *Ps. aerogenes*, it was found that the effect of the solution at a concentration of 0.1 M inhibits bacterial growth by an average diameter of 44 mm and when the concentration is diluted (0.01, 0.05 0.07%) M. It is the highest amount for measuring the diameter of the inhibition zone at 0.07%M is at a diameter of 6 mm as shown in Figure 12 and Table 1.

TABLE 1
ANTIBACTERIAL ACTIVITY OF THE NANO SOLUTION IN DIFFERENT CONCENTRATIONS.

Inhibition zone of <i>Ps. aerogenes</i> (mm)	Inhibition zone of <i>Staph. aureus</i> (mm)	Concentration%
44	45	0.1
4	3	0.01
5	5	0.05
6	8	0.07

The areas formed around the wells are due to the direct interaction between the nanoparticles and the surface of the bacterial cell membrane This reaction leads to disruption of the cell membrane Inhibition zones vary depending on several different factors such as the type of bacteria, the concentration of the antibacterial agent, the surface area of the sample, the shape and size of the nanoparticles as well as the structural structure of the cell membrane where it caused the difference in activity against gram-positive and gram-negative bacteria[27]; [28]. The literature explains the possible pathways of action of nanoparticles against bacterial activity where nanoparticles can enter the cell through diffusion and cellular retention, affect mitochondrial activity in cytoplasm contact, cause the release of reactive oxygen species, and release metal ions that penetrate the membrane and reach the DNA, leading to nuclei damage and cell death [27]; [28]. The special mechanism of antibacterial action of

NPs is still unknown. So far, three specific pathways have been proposed: (i) rupture of the cell wall and membrane, (ii) penetration and damage within cells, and (iii) oxidative stress [29]; [30].

C. Effect of the two Nano-Combined Materials (nickel oxide with cobalt oxide) on *Ps. aeruginosa* and *Staph. aureus* Bacteria Inside the Body In Vivo

Half of the amount of nickel oxide solution and half of the amount of cobalt oxide solution are mixed and placed in the oven to dry at a temperature of 200 degrees for an hour. Then it was taken out of the oven and left to cool outside the oven at room temperature for a whole day so as not to turn into ash or charred inside the oven. To prepare the nanomaterial processed, 0.5g of nickel oxide powder and cobalt oxide were taken and mixed well with 9.5g of petroleum jelly. Three repeaters were taken and the dorsal part with the blade causing a burn. then one

group was infected with the bacteria *Staph. aureus* and the other was infected with the bacteria *Ps. aeruginosa* after removing part of the bacterial colony from the dish by swab and placing the bacteria on the skin of the mouse in the throat position in the dorsal part as shown in Figure 13. On the second day of infection redness occurred in the affected area with the appearance of festering patches inflamed causing infections loaded with abscesses of the purulent type, where each group was distributed inside a cage. The first group of mice was treated with petroleum jelly, where we noticed that the infections remained without improvement. Increase the area of the affected area and there was no response to Vaseline alone and some of them died and this indicates that Vaseline alone does not perform effectiveness against bacteria.

The other groups were treated with nanocomposites prepared by the green synthesis method of plant extracts *Saffron Crocus sativus L*, *Aloe perfoliata vera* with nickel nitrate and cobalt nitrate respectively after the treatment of burns injuries with nanocomposites. The response appeared on the first day of treatment, where the red color disappeared in the skin, swelling, swelling, swelling and lack of area of bacterial infection disappeared, and stiffness occurred in the area of infection, the response guide to treatment, and this proves the effectiveness of the prepared substance as shown in Figure 14.

D. Toxicity Measurement MTT

Figure 15 represents the close relationship between the concentration of the substance expressed in log concentration ($\mu\text{g.ml}$) and the viability rate of both PC3 prostate cancer cells and normal cells in the body HdFN, indicates that with an increase in the concentration of the substance with the decreases in the proportion of dead cells. This means that the solution gives better results at low concentrations. The more solutions are diluted, the better this means that the nanomaterial is less agglomerated and the nanoparticles are spaced apart. The IC50 cell killing ratio is 50% for cancer PC3 cells is 416.8 while its value for normal HDFN cells is 101.6. The difference between the two values is very large, and this indicates that the limits of killing cancer cells do not exceed the concentration of the substance is 416.8 and below. For normal cells, the limit of killing cells at a concentration of 101.6, meaning that the substance is non-toxic, and this is due to the content of the substance of the different compositions of compounds. Or it may be due to the source of the solution material, which contains (cobalt nitrate + cactus plant 75% and nickel nitrate + saffron plant 25%) as saffron and cactus plants contain many secondary compounds attributed to this result [30], [31].

E. Rewarding Description of Drawings and Shapes

- Figure 1: Saffron extract is on the left, nickel nitrate solution is in the middle, and nickel oxide nano oxide is on the right.
- Figure 2: Aloe vera plant solution on the left, a solution of cobalt nitrate in the middle, and a solution of cobalt oxide on the left.
- Figure 3: XRD pattern of NiO nanostructure
- Figure 4: XRD pattern of the nanostructure of CoO membranes
- Figure 5: XRD pattern of the nanostructure of a CoO compound with NiO.
- Figure 6: SEM image of the membrane of the nano solution (nickel oxide and cobalt oxide).
- Figure 7: TEM image of the solution of nanomaterials (nickel oxide and cobalt oxide)
- Figure 8: FTIR examination of the solution of nanomaterials (nickel oxide and cobalt oxide)
- Figure 9: Optical absorption spectrum of nanomaterial solution (nickel oxide and cobalt oxide)
- Figure 10: The energy of the band gap is estimated by drawing $(\alpha h\nu)^2$ versus photon energy.
- Figure 11: Shows the inhibition diameters growth of *Staph. aureus* at 0.1 M% on the left and diluted concentrations (0.01,0.05,0.07%) on the right.
- Figure 12: Inhibition diameters growth of *Ps. aerogenes* by 0.1 M% on the left and dilute concentrations (0.01,0.05,0.07%) on the right.
- Figure 13: Images of a mouse whose hair has been shaved dorsally and infected with *Staph. aureus* on the left and a shaved mouse infected with *Ps. aerogenes* on the right.
- Figure 14: Shows the condition of the mouse on the first day after taking the treatment and appears in response to treatment, the disappearance of red congestion of the skin, the disappearance of the tumor, and swelling.
- Figure 15 shows the result of toxicity MTT and normal cells in the body HdFN, the number of cancer cells denoted by PC3 for the embedded nanomaterial (nickel nitrate solution with saffron extract and cobalt nitrate with aloe vera extract).

IV. CONCLUSIONS

The preparation of the material by the method of green synthesis of nickel nitrate with saffron and cobalt nitrate with *aloe vera* is a simple, inexpensive, and environmentally friendly method. The prepared nanomaterial has proven its effectiveness against pathogenic bacteria. The response appeared on the infected mice on the first day of treatment, where the red color disappeared in the

skin, swelling, swelling, swelling, lack of area of bacterial infection, and stiffness occurred in the area of infection, evidence of response to treatment, and this proves the effectiveness of the prepared substance. From our findings, a solution of nanomaterials (nickel oxide and cobalt oxide) can be used in many biological applications. Get treatment in a short period, time within a day or fewer hours. It is possible to treat the most virulent and antibiotic-resistant bacteria available today.

V. RECOMMENDATIONS

Effect of Nanocomposites (Nickel Oxide-Cobalt Oxide) was prepared by Green Synthesis Method on Biological Applications. According to the first protective element, a solution of nickel oxide and a solution of cobalt

oxide were prepared, and then the two solutions were mixed in a certain proportion to obtain the final nanosolution. According to the first protective element, the final nanosolution had an inhibitory effect at 0.1 molar more than dilute concentrations where the average diameter of the inhibition zone was about 45 mm and 44 mm for Staph. aureus and Ps. aerogenes, respectively. According to the first protective element, the application procedure was carried out in two stages, the first is the preparation of the nanomaterial and the study of its properties and the second stage is to take a certain amount of nanosolution and distribute it on the Agar-Müller plate and then incubate the plates at 37°C (bacteria), for 24 hours.

VI. ILLUSTRATIONS AND ILLUSTRATIONS

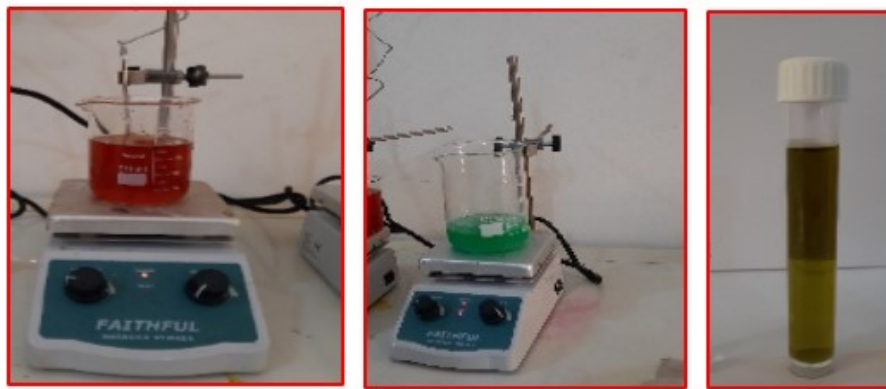


Fig. 1. Saffron extract on the left, nickel nitrate solution in the middle, and nickel oxide nanooxide on the right.



Fig. 2. Aloe vera plant solution on the left, cobalt nitrate solution in the middle, and cobalt oxide solution on the left

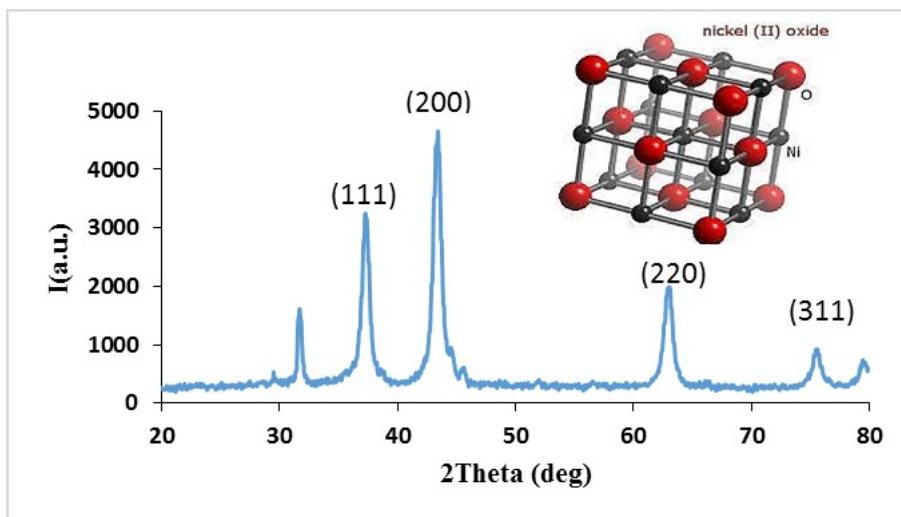


Fig. 3. NiO XRD Pattern for Nanostructure

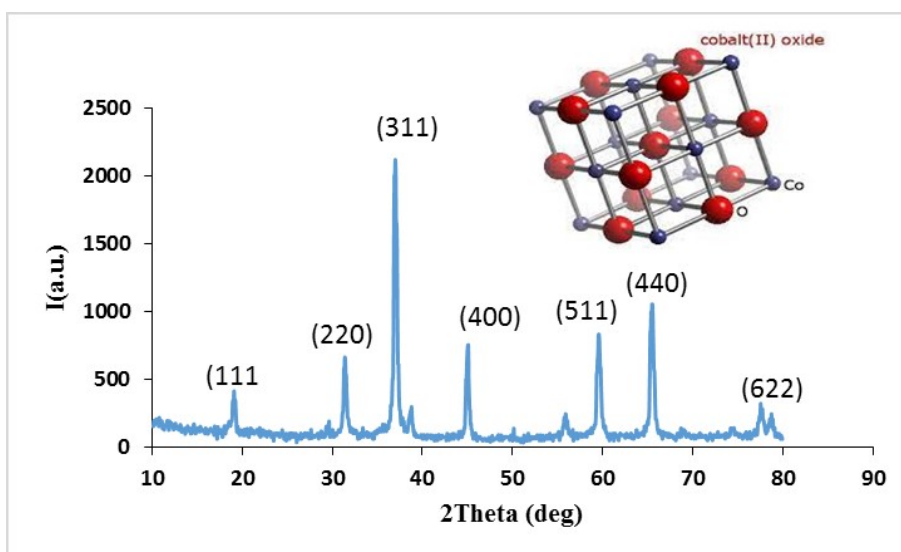


Fig. 4. XRD pattern of nanostructure of CoO membranes

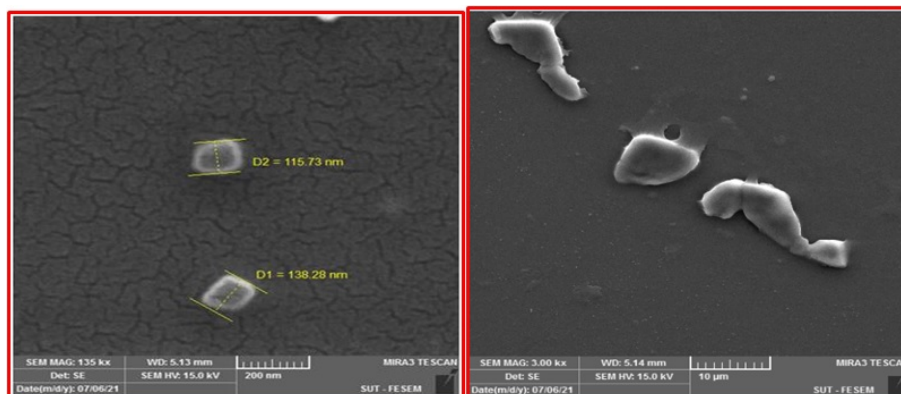


Fig. 5. XRD pattern of the nanostructure of the CoO compound with NiO.

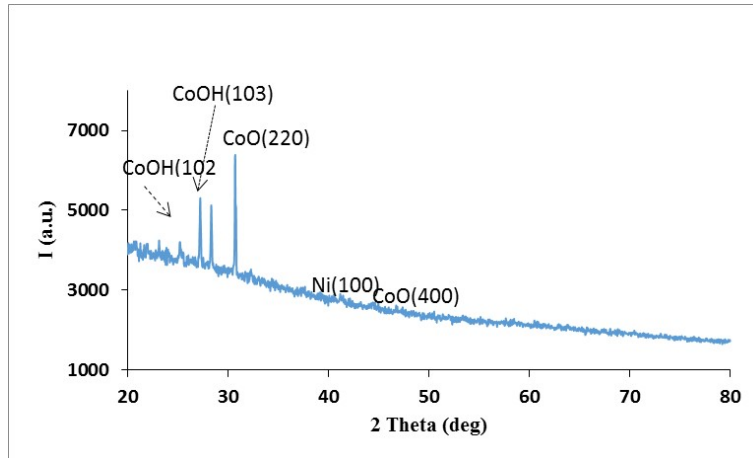


Fig. 6. SEM image of the membrane of the nanosolution (nickel oxide and cobalt oxide).

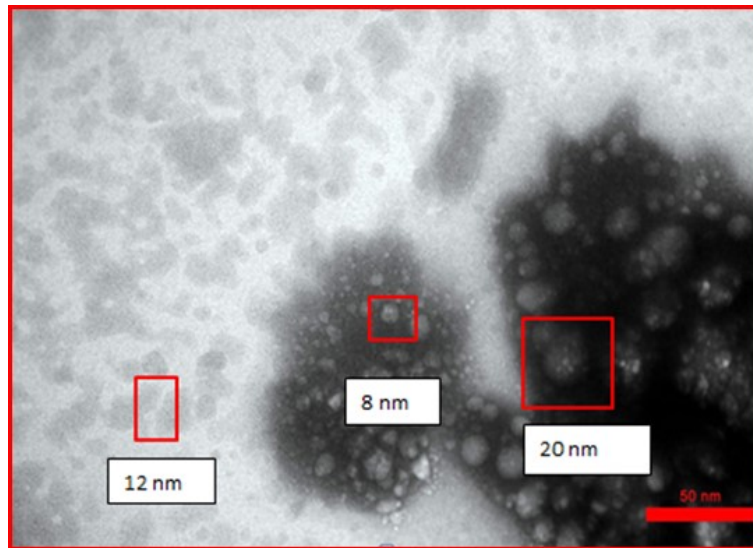


Fig. 7. TEM image of the solution of nanomaterials (nickel oxide and cobalt oxide)

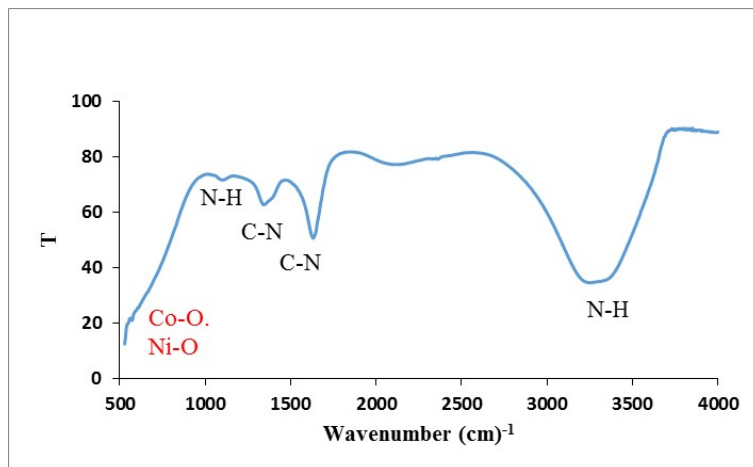


Fig. 8. FTIR examination of nanomaterial solution (nickel oxide and cobalt oxide)

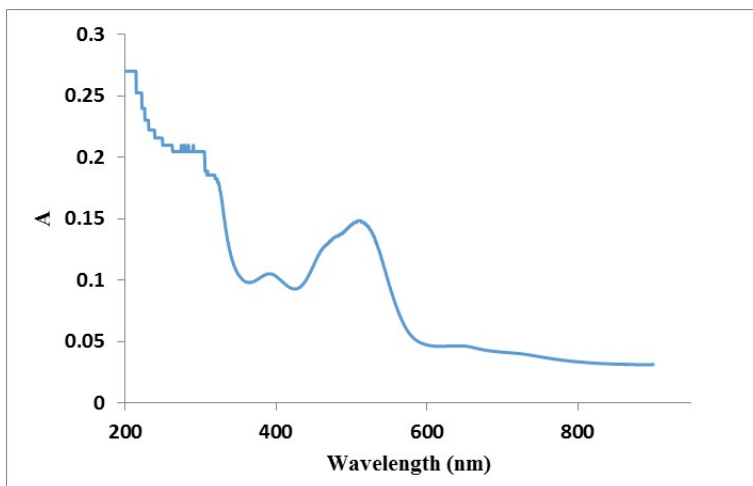


Fig. 9. Optical absorption spectrum of nanomaterial solution (nickel oxide and cobalt oxide).

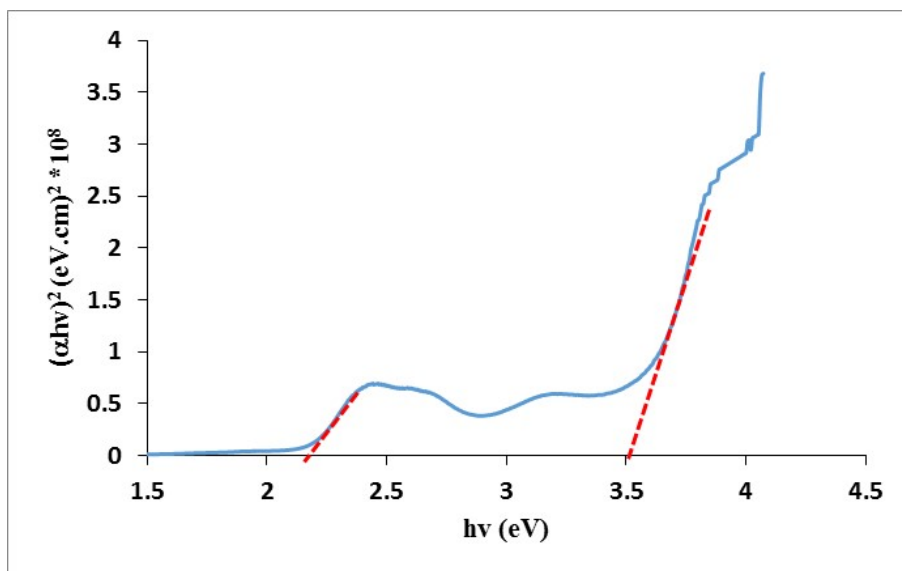


Fig. 10. The bandgap energy is estimated by $(\alpha hv)^2$ versus photon energy.

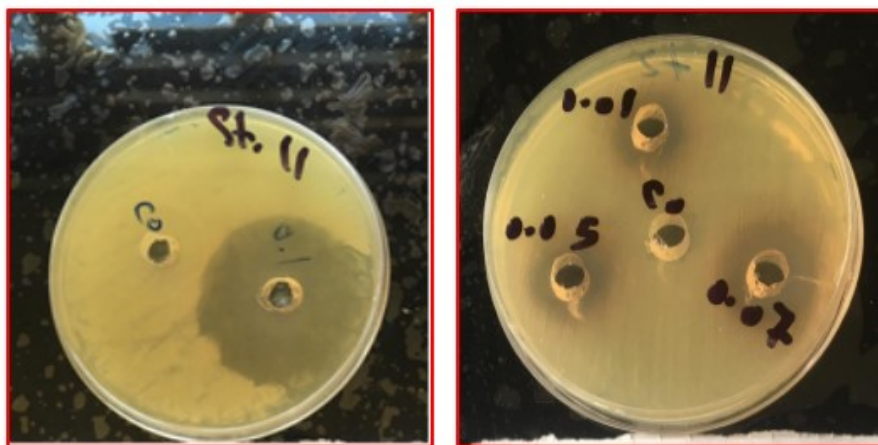


Fig. 11. Growth inhibition diameters of *Staph. aureus* at 0.1 M% on the left and diluted concentrations (0.01,0.05,0.07%) on the right.

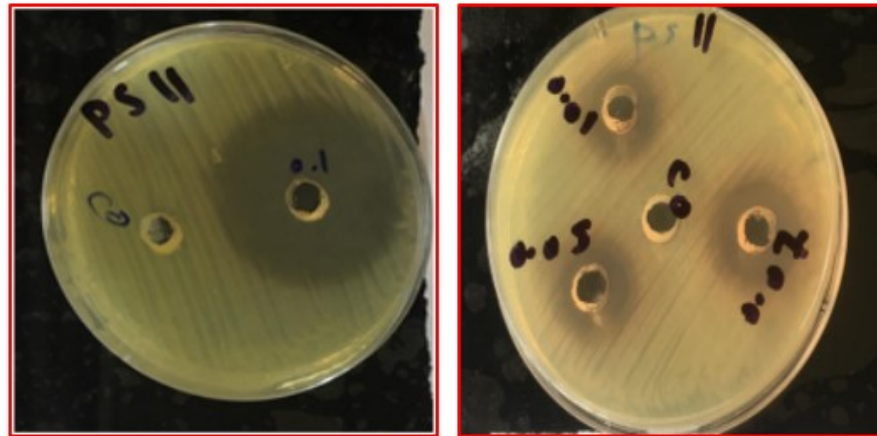


Fig. 12. Inhibition diameters for growth of *P. aerogenes* at 0.1 M% on the left and dilute concentrations (0.01,0.05,0.07 %) on the right.

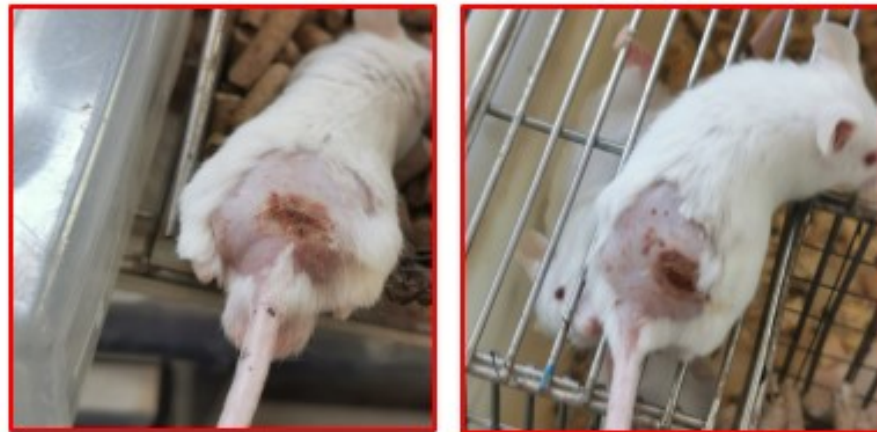


Fig. 13. Showing images of a mouse whose hair has been shaved in the dorsal and infected with the bacterium *Staph. aureus* on the left and a shaved mouse infected with *P. aeruginosa* on the right.

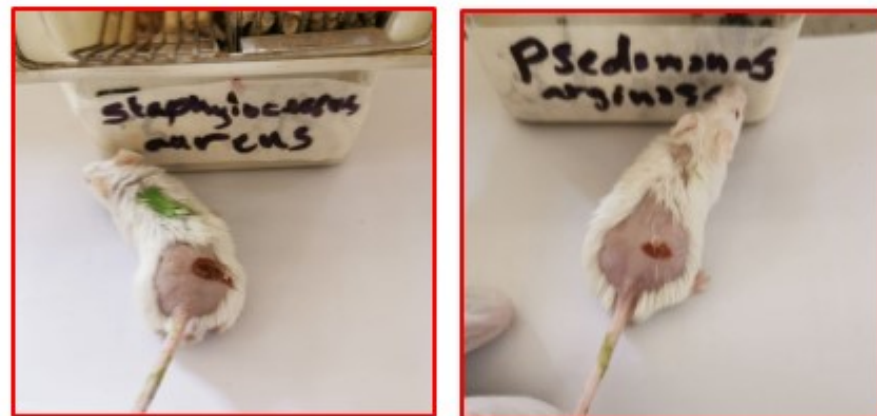


Fig. 14. Shows the condition of the mouse on the first day after taking the treatment and appears in response to treatment and the disappearance of red congestion of the skin and the disappearance of the tumor and swelling.

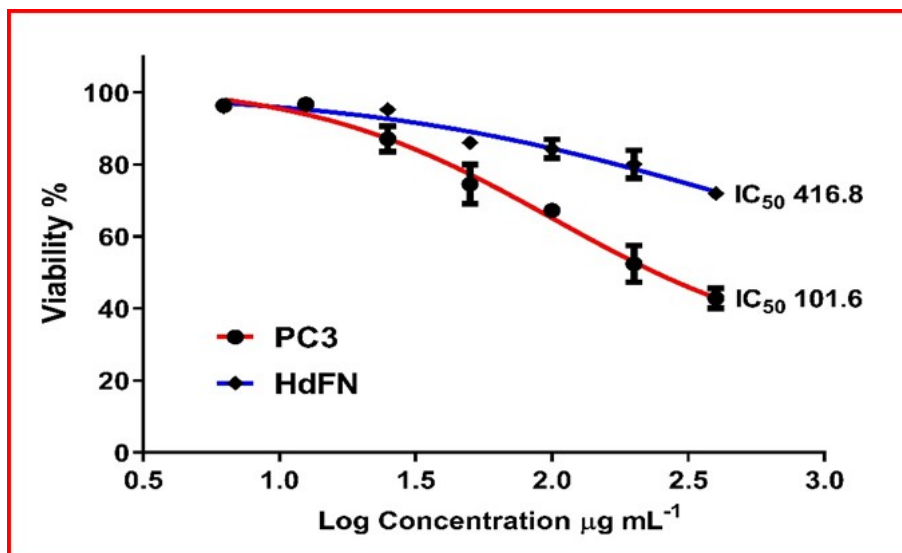


Fig. 15. The result of toxic MTT and normal cells in the body HdFN shows the amount of cancer cells denoted by PC3 for the embedded nanomaterial (nickel nitrate solution with saffron extract and cobalt nitrate with aloe vera extract).

VII. BENEFICIARY

Ministry of Health and Ministry of Higher Education and Scientific Research.

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