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Synthesis of novel Iron Nanoparticles using the aqueous extract of the Pumpkin plant, and used it in the treatment of burns

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Abstract

This aimed to study of the use of pumpkin aqueous extract in the preparation of iron nanoparticles, the use of ultraviolet (UV) spectroscopy, atomic force microscopy (AFM), scanning electron microscopy (SEM), infrared spectroscopy (FTIR) and X-ray diffraction (XRD) in characterizing the prepared nanoparticles and determining the active groups, and study of the effect of nanoparticles prepared using pumpkin aqueous extract in treating burns. The nanoparticles were prepared using aqueous extract of pumpkin and iron salts, which are 100% local materials for treating burns and pathogenic microorganisms. As the study showed that iron nanoparticles showed high inhibitory efficacy for the microorganisms under study, which included: Ps. aeruginosa, Pr. Mirabilis, Staph. aureus, E. coli, K. pneumonia. These bacterial types are among the most dangerous bacterial types in the contamination of burns and wounds. As it showed the highest percentage of Ps. Aeruginos, it indicates 42.8%, followed by Staph isolates. Aureus 28.73%, E. coli 12.1%, P. mirabilis, 8.2%, K. pneumoniae8.2%, E. coli 3.37%, respectively. A peak also appeared at the wavelength of 479.2 nm for colloidal solution of nanoparticles prepared using pumpkin aqueous extract and iron salts using a spectrophotometer. The nanoparticles were characterized using SEM, AFM and FTIR. The prepared nanoparticles showed high efficacy in inhibiting the micro-organisms under study when performing MIC and MBC assays. Milliole concentration of nanoparticles also showed high inhibition of all types of microorganisms. When studying the effect of nanoparticles on the burns that may have caused in laboratory mice, a high efficiency in burn restoration and hair growth compared to control groups, treatment with burn ointment and aqueous extract of pumpkin.

Keywords: Iron nanoparticles, pumpkin, SEM, AFM, FTIR, Iraq

1. Introduction

The pathogenic bacteria that cause pollution in hospital halls, and that cause infections to burns as well as to wounds, the most important are Staphylococcus Aureus, Pseudomonas, and Klebseilla Sp, these bacteria are considered as opportunistic pathogens, ,these bacteria are transmitted from one patient to another through the hand and contaminated medical supplies [as explained by 1]. All of Ps. Aruginosa and Staphylococcus aureus possess exogenous enzymes or toxins that act selectively on various host tissues such as Alkaline protease, Elastase, Coagulase, hemolysin, Lipase, gelatinase, DNase, Alkaline phosphatase, Lecithenase, Leukocidin, as well as iron carriers (Siderphore) [2].

The percentage of fungi pathogenic to humans is less than that caused by bacteria and viruses, but its importance increases, especially in cases of exposure of the host to immune deficiency resulting from malignant tumors and the use of chemical drugs that suppress the immune system, in addition to the ability of fungi to produce mycotoxins that cause allergies and directly attack tissues [as comes in 3].

The infection-causing fungi are a group of dermatophytes that inflict specific lesions on the layers of the skin below the stratum corneum [as concluded by 2, 3]. Green nanotechnology has attracted a lot of attention as it includes a wide range of processes that reduce or eliminate toxic substances and thus work on the safety of the environment, so the green synthesis of nanoparticles makes the use environmentally friendly [as depicted by 4].

The cubic structure of iron oxide (Fe_3O_4) has unique electrical and magnetic properties on the basis of

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electron transfer between Fe^{+2} and Fe^{+3} at octahedral sites (Bharde et al., 2005). As well as having suitable surface properties, it offers great facilities in many biomedical applications, such as cellular therapy and tissue repair, drug delivery and MRI. In addition, the accumulation of Magnetic Iron Nanoparticles (MINP) in tumor tissues makes them play an important role in their detection by magnetic resonance imaging or electron microscopy. There are a large number of physical, chemical, biological, and hybrid methods available to produce different types of nanoparticles [5].

2. Materials and Methods

Prepare the aqueous extract of the pumpkin fruit, weigh 20 g of the pumpkin fruit and add to 400 ml of deionize water (DI) sterilized with an Autoclave device. The mixture was heated with a magnetic stirrer and allowed to boil for 10-15 minutes. The mixture was cooled and filtered with sterile gauze, then filtered with Whitman (No 1) filter paper, the pH was measured 6.1 with a pH meter and the extract was kept in opaque glass bottles in freezing at -20 ° C until use [as explained by 6].

Chemical detection of some of the plant's active ingredients

Detection of Alkaloids

It was followed the method [as comes in 7], which includes boiling 15 grams of plant powder with (50) ml of distilled water acidified with 4% HCL acid. Filtered after cooling and put (0.5) ml of the filtrate in a watch glass bottle and add a few drops of the prepared Dragendroff reagent and the orange coloration of the precipitate indicates the presence of alkaloids.

Detection of Tannins

In the detection of tannins, the method [8, 9] was followed, as 15 gm of vegetable powder was boiled in 50 ml of distilled water, then the solution was filtered and left to cool, the filtrate was divided into two parts and a lead acetate solution (1%) was added to the first section, as the appearance of a white, gelatinous precipitate was evidence of the presence of tannins, and for the second part a ferric chloride solution (1%) was added, indicating the appearance of color.

Detection of glycosides

The method of (11) was followed in which the examination was done by adding (2) ml of Benedict's reagent to (1) ml of plant extract placed in a test tube and shaken the solution well, and put in a boiling water bath for (5) minutes, then left to cool until the appearance of a precipitate the red color indicate the presence of glycosides.

Detection of saponins

The test was performed [as stated by 10, 11, 12] by following the following two methods: -

A- The aqueous solution of the vegetable powder was prepared in a test tube and shaken vigorously that the appearance of dense foam and its persistence for a long time indicates the presence of saponins.

B- Then 1-3 ml of mercuric chloride solution was added to 5 ml of aqueous extract of the plant, and the appearance of a white precipitate is evidence of the presence of saponins.

Detection of phenols

15 grams of dry vegetable powder were boiled with 50 ml distilled water in a water bath, then the solution was filtered using filter papers and the filtrate was left to cool, and (1)% of ferric chloride was added to it and that the appearance of a bluish green color is evidence of the positive test [as explained by 13].

Detection of Flavonoids

Two equal parts of each of the following solutions were mixed:

- Solution A: Prepare by dissolving (15) g of dry vegetable powder in (50) ml of ethyl alcohol at a concentration of 5%, then filter the solution using filter paper.
- Solution B: prepare by dissolving 15 ml of ethyl alcohol at a concentration of 50%. When equal quantities of both solutions are mixed, the appearance of yellow indicates the presence of Flavonoids [14].

Determination of the lowest inhibitory concentration MIC, and the lowest lethal concentration is MBC of the plant extract and the nanoparticles

A series of double concentrations of plant extracts (4, 8, 16, 32, 64, and 128) mg / ml were prepared using nutrient broth medium. The tubes were inoculated with (0.1) ml from the bacterial culture at (24) hours old and containing (1.5 x 108) cells / ml, then the tubes were incubated at (37) m for a period of (48) hours. The results were compared with the two controls (1, 2) represented by a culture medium and with plant extract only, respectively. The experiment was repeated in the event that no turbidity appeared in the control (1) and it appeared in the control (2).

The MIC value of the Minimum inhibitory concentration was determined to be less concentrated than the extract which prevents the occurrence of visible turbidity to the naked eye in the culture medium. The Minimum bactericidal concentration (MBC) was determined by transferring (0.1) mL from

all tubes in which no turbidity appeared to dishes containing a nutrient agar media. The dishes were incubated for 24 hours at a temperature of (37 ° C). The value of MBC was determined to be less concentrated than the extract inhibiting bacterial growth [15, 16].

Preparation of iron nanoparticles for pumpkin fruit extract

The nanoparticles were prepared using the aqueous extract of the pumpkin fruit with iron salts according to the method [as explained by 17], 25 ml of the previously prepared extract were added to 250 ml of DI and the mixture was heated at 80 °C with constant stirring.

0.01 g of ferrous sulphate $Fe_2(SO_4)_3$ was added to the extract with stirring and the color change was observed immediately to black, and the solution was allowed to cool with a recalculation of the pH of 3.74. The colloidal solution for the nanoparticles was stored in the refrigerator at 4 °C until further checks are carried out.

Characterization Nanoparticles

The properties of iron nanoparticles were identified at the Nanoscale Research Center (University of Technology, Baghdad, Iraq) using the following devices:

UV-Visible spectrometry

Ultraviolet radiation was used to study the optical properties with a UV-Visible spectrometry (meterteeh sp 8001) in the range of 300-800 nm [as comes in 18].

Scanning Electron Microscope (SEM)

A scanning electron microscope (TESCAN-VEGA) with a wavelength of 3 nm and an electrical voltage of 30 KV) was used [19].

Atomic Force Microscopy (AFM)

Atomic force microscopy (SPA-AA3000) was used with side wavelength 0.26 nm, vertical 0.1 nm to obtain a three-dimensional image of the blocked nanoparticles [as explained by 20].

X- Ray Diffraction Analysis (XRD)

X-ray (Shimadza maxima-a) X-Ray Diffract (XRD-700) was used to determine the crystal phases and estimate the size of the crystals, with an electric voltage of 40KV and a current of 30 MA, with a scanning range between (100.000-20.000) degrees. As the XRD patterns were recorded within 0.12 seconds of the scan speed, using Cu tubes with a copper wavelength of 1.54 A [21].

Infrared spectrometer

The infrared ray was analyzed for iron nanoparticles with FTIR spectroscopy (FTIR - 8400S, SHIMAZU-FTIR)) with a wavelength ranging between (500 - 4000) cm^{-1} [22].

Preserving and perpetuating isolates

The bacterial isolates were preserved in slanted culture media ((Slants) from the clays fed at 4 °C temperature and the perpetuation process continued on a monthly basis through the renewal of cultivation in new media to ensure their survival throughout the study period.

To preserve the bacterial isolates for a long period of time, use the nutrient broth medium with glycerol at a ratio of (15)%, then distribute the medium in small bottles at a rate of (5) ml each and sterilize it with an autoclave, then the medium was left to cool down at room temperature, then the medium was inoculated with pure colonies of bacteria growing on the clogs fed by the conveyor (Loop) and the bottles were kept at (-20 °C) until use [as 23].

Bacterial Antibiotics Sensitivity Test

This examination was performed to test the sensitivity of bacteria to antibiotics using the method [as in 24].

A- Preparation of the bacterial suspension

A bacterial suspension was prepared from the isolation to be tested on the degree of the standard turbidity constant (108 × 1.5) cells / ml and the standard turbidity comparison of (McFarland) solution by means of a sterilized wicker (Swab), a portion of the bacterial suspension is spread on the surface of container dishes on the Muller-Hinton agar medium, and the dishes are left for (20) minutes to complete the absorption of the culture.

B- The test

Take a sterile micropipette (0.1 ml) of the prepared bacterial suspension in the vertebra and spread it on the surface of the culture medium using a sterile culture diffuser (Spreader) for the purpose of distributing it homogeneously. The dishes were left in the atmosphere of the room for (20-30) minutes in order to absorb the culture and dry the medium, then antibiotic tablets were placed on the surface of the culture media by means of sterile forceps with light pressure on them, with (6) tablets per dish. The dishes were air incubated at a degree of (37) m for a period of (24) hours, and the results were read by measuring the areas of inhibition of antibiotics, and the bacteria were considered sensitive (Sensitive) or resistant (resistant) according to the specifications mentioned in [as described by 25].

The agar-well diffusion method

Inoculation of the surface of the blisters fed with a swab from the culture of bacteria containing (1.5 × 108) cells / ml for comparison with a solution of constant turbidity, then the dishes were left to dry at

room temperature. A pit with a diameter of (5) mm was made in the culture media by the cork punch, then different concentrations of the original concentration were prepared using sterile distilled water as follows (8, 16, 32, 64, 128 and 256) mg / ml. Then an amount (0.1) of the above concentrations was added to each pit in sequence, and the control pit was created by adding sterile distilled water. The plates were incubated at (37 °C) for a period of (24) hours. The efficacy of each concentration of extracts was determined by measuring the diameter of the inhibition zone around each pit.

Preparation of the bacterial suspension

The bacterial suspension was prepared by transferring a colony of Ps. aeruginosa, Pr. mirabilis, Staph. aureus, E. coli, K. pneumonia, that was previously grown in a pure form on the media of the clays fed to tubes containing a solution (physiological salt), with shaking well to prepare a culture medium containing (10^{-2}).

Study of the effect of nanoparticles and pumpkin plant extract on burns in laboratory animals

Laboratory animals: Swiss white mice of the BALB / C strain of the male sex were used, their ages ranged between (12-16) weeks, and their weights between (26-28) gm. They were raised in special cages with the appropriate conditions of temperature, light and nourishment. The animals were prepared for the experiment by removing hair from the back area at a rate of (15%) of the total body area. The area from which the hair was removed was sterilized with ethyl alcohol (70%) and then burned with (37%) HCl acid.

3. Results and Discussion

Figure 1 shows the percentages of bacterial isolates that have been isolated and diagnosed. The study showed that most of the samples collected from burn cases were from hospitalized patients in the following hospitals: Medical City, Yarmouk, Al-Kindi, Al-Sadr General Hospital and the number of swabs that collected 181 samples, as it was found that the percentage of bacteria Ps. Aeruginosa the highest percentage, reaching 33.7, while the percentages of pathogenic bacteria species were as follows: Staph. Aureus 28.73%, E. coli 12.1%, P. mirabilis, 8.2%, K. pneumoniae8.2%, E. coli 3.37%, respectively. These bacterial types are among the most dangerous for burns and wounds contamination. The results obtained are consistent with [26] as it was determined that it investigated Ps bacteria. Aeruginosa with the highest percentage was 42.8%. These bacteria are the most common in burn contamination and they are opportunity-developing bacteria that are widely distributed in the environment [26]. Ps. Aeruginosa has a distinctive smell when planted on the culture medium, it is the smell of rotting fish. This bacterium is widely spread in hospitals [as mentioned by 27]. The virulence of the bacteria is due to the presence of lysosome enzymes, B-lactamase and hemolysin. Due to the lack of necessary supplies for good sterilization of the hospital environment and the widespread use of antibiotics without a doctor's prescription, this led to contamination of wounds and burns in hospitals.

As for staphylococcus, its percentage was 28.73% and it is consistent with what was found [as concluded by 28, 29]. It is the bacterium that causes burns to become infected if it is contaminated with these bacteria, as these bacteria act to suppress the immunity as these bacteria remain alive inside the white blood cells of burn sufferers. Since the virulence of the bacteria is due to the presence of the capsule and to the presence of the coagulase enzyme for the blood and the anti-beta-lactamase (B-lactamase), [as found by 30] that the streptococcus bacteria are the most prevalent in festering burns. The bacteria E. coli 12.1% is of importance when burns and wounds are contaminated, as it is a bacterium that optimizes for opportunities, as it produces Colicin toxin and possesses virulence factors and the most important virulence factors are (LPS) (LipopolySaccharide) and the presence of surface antigens, including preservative (K), flagellum (H) and somatic (O) [as comes with 31]. Burn contamination with pneumoniae.K is evidence of wound contamination from patient supplies [as found by 32]. Contamination of wounds and burns with P. mirabilis is an antibiotic-resistant bacteria that has the ability to produce the enzyme urease

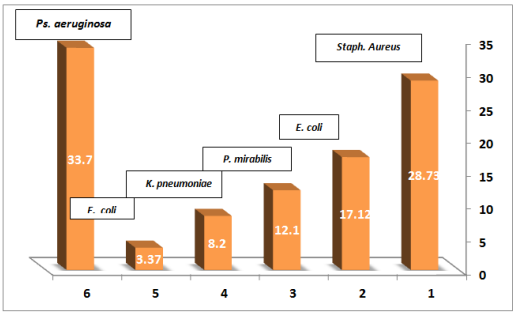

Figure 1: shows the percentages of bacterial isolates

Qualitative detection of some active compounds of the aqueous and alcoholic extract of the pumpkin plant

Table (1) shows the results of the qualitative detection of the active compounds in the alcohol and aqueous extracts. It was found that the phenolic

compounds, flavonoids, saponins and glycosides appeared at the highest concentration compared to the active ingredients of the alcoholic extract, and this is consistent with the findings [33, 34], upon detection of the active ingredients in a number of medicinal herbs, the above mentioned active ingredients are present in the alcoholic extract at a higher concentration than in the aqueous extracts because most of the active compounds dissolve in organic solvents at a higher rate than in their solubility in water [as explained by 33].

Figure 2 shows the change in the color of the aqueous extract from orange to black, evidence of the formation of nanoparticles, and figure 3 also indicates the ultraviolet absorption spectroscopy of the prepared nanoparticles using the pumpkin aqueous extract, where a peak appeared at the wavelength of 479.2 nm.

Table 1: The qualitative detection of the active compounds in the alcoholic and aqueous extracts of the pumpkin fruit

Type of Extract	Detection type					
	Flavonoids	Alkalis	Phenols	Tannins	Saponins	Glycosides
Aqueous extract	+++	++	+++	++	++++	++++
Alcoholic extract	+++++	+++	++++	++++	+++	+++

Figure 2: A- Pumpkin extracts aqueous powder, B- Silver nanoparticles

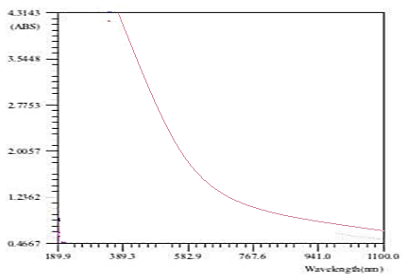

Figure 3: Absorbance Peak for nanoparticles prepared using aqueous pumpkin extract with iron salts using UV-Vis rays

Scanning Electron Microscope (SEM)

Figure 4 shows the shape of iron nanoparticles prepared by using aqueous extract of pumpkin plants with iron salts of the shape at a size of 34.5 nm.

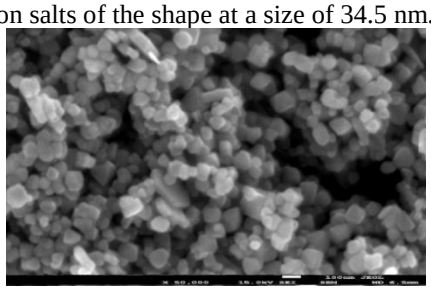

Figure 4: SEM analysis of nanoparticles prepared using pumpkin aqueous extract with iron salts

Atomic Force Microscope (AFM)

Figure 5 indicates the results of the analysis of the results of the AFM analysis, the shape and arrangement of the prepared minutes, where the results indicated, as the minutes have an irregular distribution of shape and sizes (70.23,46.4, (80.34 nm) respectively.

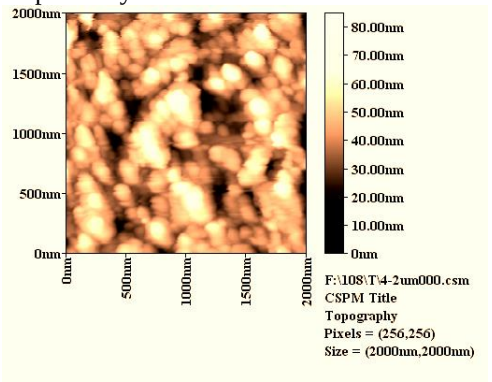

Figure 5: AFM analysis of nanoparticles prepared using pumpkin fruit aqueous extract with iron salts

FTIR spectroscopy

Figure (6) shows the results of FTIR spectroscopy to determine the functional groups of the active components of the nanoparticles. As the results show that the strongest absorption of the beam is at the wavelength of 520-620 cm-1, which refers to the frequencies of the iron nanoparticles, Fe-O, the results also showed that the peaks in the wide range (3000-3934cm-1) are due to the active hydroxyl group, otherwise a major role in forming the structures of the nanoparticles.

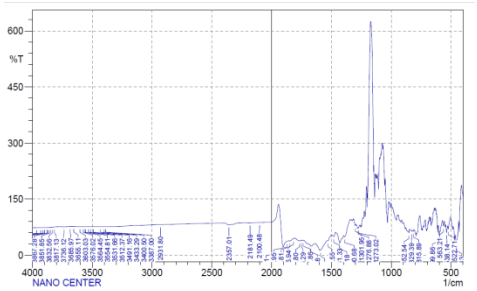

Figure 6: FTIR absorption for prepared nanoparticles

The inhibitory activity of nanoparticles under study on in vitro microorganisms

The effect of different concentrations of nanoparticles and pumpkin extract on the six types of pathogenic bacteria under study was investigated, by using the Agar well diffusion method. Minimum inhibitory concentration (MIC), minimum lethal concentration (MBC), and the Tow fold dilution method were determined, as, well as determining the minimum inhibitory concentration (MIC) and Minimal Biocidal Concentration (MBC) of the extracts that were given anti-bacterial activity. It was found from this study that these plants have a different anti-bacterial effect, and that the severity of the effect depends on the type of extract, its concentration and the type of bacteria. Table 2 shows the results of the tests to determine the minimum inhibitory concentration (MIC) and minimum biocidal concentration (MBC) of the extracts and nanoparticles.

The results showed that the lowest inhibitory concentration for all bacterial species and for all types of aqueous and alcoholic extracts of the pumpkin plant under study was 6.25, while the lowest lethal concentration of bacteria was in the following way, as the nanoparticles were the most effective and the least concentrated on all types of bacteria. It was found that the concentration of 12.5 mg / ml was the lowest lethal concentration of all types of bacteria under study except for Ps bacteria. Aeruginosa and K. pneumonae. The lowest lethal concentration was 12.6 mg / ml, while for the aqueous extract the lowest lethal concentration was 25 mg / ml for all bacteria under study except for Ps. Aeruginosa was 50 mg / mL and K. pneumonae was 12.5 µg / mL.

As for the alcoholic extract, the lowest lethal concentration is 25 g / ml for all types of bacteria, except for E.coli and K. pneumonae, 12.5 mg / ml. The results show that the nanoparticles were the most effective in killing and inhibiting pathogenic bacteria and at the lowest concentration. It is possible that the effect of the nanoparticles is due to the small size of the nanoparticles and their high concentration on the bacterial cells and this is due to the presence of attraction in the charges as the bacterial cells carry a negative charge, while the nanoparticles carry positive charges, an attraction takes place, which prevents the permeability of the plasma membrane of the microorganism and leads to its death. Table (2) shows the results of the tests to determine the minimum inhibitory concentration (MIC) and minimum biocidal concentration (MBC) of the extracts and nanoparticles.

Table 2: MIC, and MBC for aqueous NPs extract

Type of Bacteria	Concentration mg / mL of aqueous extract					
	6.25	12.5	25	50	100	200
Ps. aeruginosa	+	+	+	+	-	-
Staph. Aureus	+	+	+	+	-	-
P. mirabilis	+	+	+	-	-	-
E.coli	+	+	+	+	-	-
K. pneumonae	+	+	-	-	-	-
C.albicans	+	+	+	+	-	-

Table 3: MIC, and MBC for alcoholic NPs extract

Type of Bacteria	Concentration mg / mL of aqueous extract					
	6.25	12.5	25	50	100	200
Ps. aeruginosa	+	-	-	-	-	-
Staph. Aureus	+	-	-	-	-	-
P. mirabilis	+	-	-	-	-	-
E.coli	+	-	-	-	-	-
K. pneumonae	+	-	-	-	-	-
C.albicans	+	-	-	-	-	-

The concentration of 100% of the nanoparticles has an effective effect in inhibiting the growth of the microorganisms under study, as it was found that the Ps bacteria. Aeruginosa showed the highest effect in nanoparticles compared to other isolates, so the inhibition diameter was 48 mm, followed by Staph. Aureus 47 mm, E. coli 40 mm, P. mirabilis 38 mm, K. pneumoniae 33 mm, and E. cloacae 30 mm.

The effect was shown, while the concentration of 50% was shown, the effect of Ps. Aeruginosa and Staph. K. pneumoniae, E. coli, P. mirabilis, and. E. cloacae, the concentration of 50% of the nanoparticles were (28, 21, 16, 16, 21, 15) mm, respectively.

As for the concentration of 25% Ps. Aeruginosa 11 mm, E. coli, K. pneumoniae 6 mm, E. cloacae, S. Aureus 4 mm, and L. mirabilis 0 mm, it was found that 100% concentration was the most effective (1 mmol) showed the highest effect compared to the rest of the concentrations.

This is evidence that by increasing the concentration, the inhibition of the bacteria under study increases,

and this is likely due to an interference in the osmosis of the plasma membrane of bacteria due to the accumulation of nanoparticles on the plasma membrane of bacteria, that due to its possibility that the nanoparticles can interfere in the process of replication and division Nuclear matter in microorganisms figures (7-12).

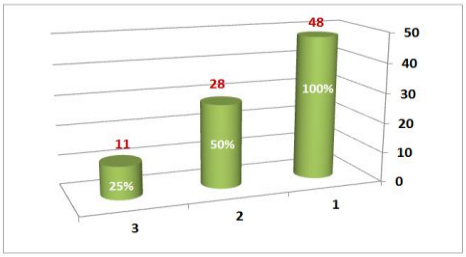

Figure 7: The effect of different concentrations of nanoparticles on Ps. Aeruginosa bacteria.

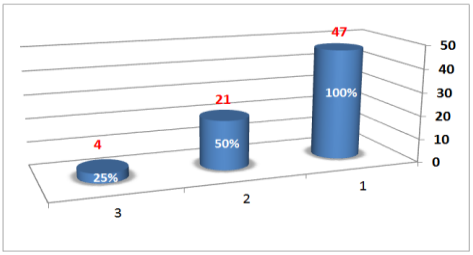

Figure 8: The effect of different concentrations of nanoparticles on Staph. Aureu bacteria

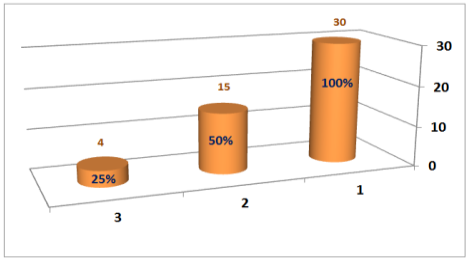

Figure 9: The effect of different concentrations of nanoparticles on *E. cloacae* bacteria

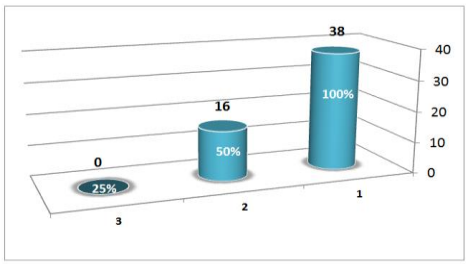

Figure 10: The effect of different concentrations of nanoparticles on P. mirabilis bacteria

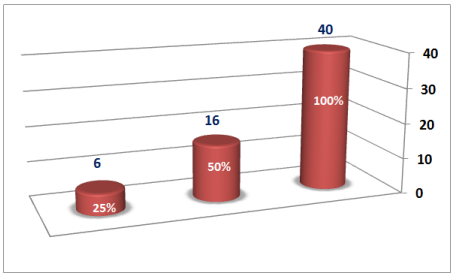

Figure 11: The effect of different concentrations of nanoparticles on E. coli bacteria

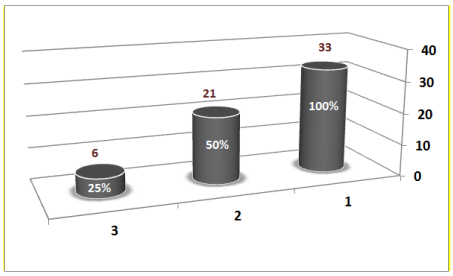

Figure 12: The effect of different concentrations of nanoparticles on K. pneumonae bacteria

Figure 13 shows the pictures of the animals on the first day after burning and refer to the pictures of Figure No. 1, where all the animals were burned. White Swiss mice of the BALBIC strain of the male sex between (16-12) weeks and their weights from (28-26) gm. These animals were attended by removing hair in the back area with 15% of the body area, the removed hair area was sterilized with 70% ethyl alcohol, then burned with 37% HCl acid, after the samples were prepared, a control group was used (control) and a group treated with burn ointment of the type (Flamazien).

Then the burning was done using, by placing drops of acid on the hair-removed area in the back and as shown in the pictures where we notice that the burn area is the same in all mice with approximately the same area. The treatment was done on the first day (that is, immediately after burning) with the use of aqueous extract of pumpkin () as well as the use of nanoparticles treated and the last group was treated with burn cream (Flamazien), and we did not notice on the first day on the animals any change except for redness and swelling of the skin and this is due to the sensitivity of the skin to hydrochloric acid.

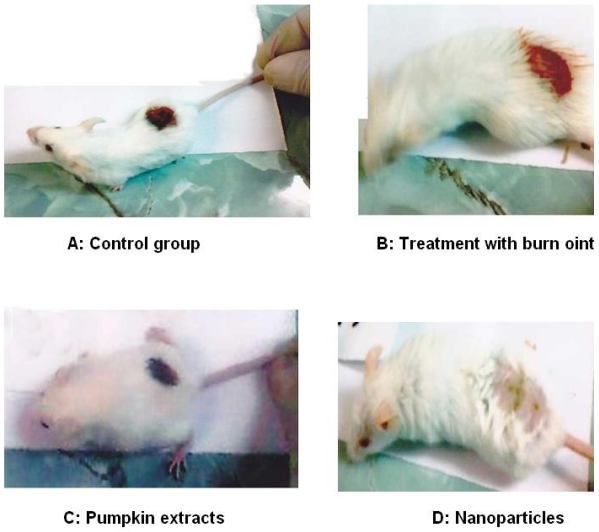

Figure 13: the first day, groups of laboratory mice

Figure 14 shows photos of treated animals after 14 days of burning, and it was notice through the treatment pictures after (14) days as the animals have not recovered and there are still some infections, redness and pus, especially in the control group and the group that is treated with Flamazien, which indicates the treatments used, whether burn ointment or aqueous extract, do not significantly affect the bacterial growth for each of the pathogens (bacterial or fungal). But the treatment with nanoparticles, there is a clear effect that is illustrated by the rate of inhibition diameter in burns and their similarity to healing compared with the control group and the treatment group with burn ointment, and this indicates the efficiency of the nanoparticles and their effect, therefore, on the pathogens (bacterial or fungal) that help burn inflammation.

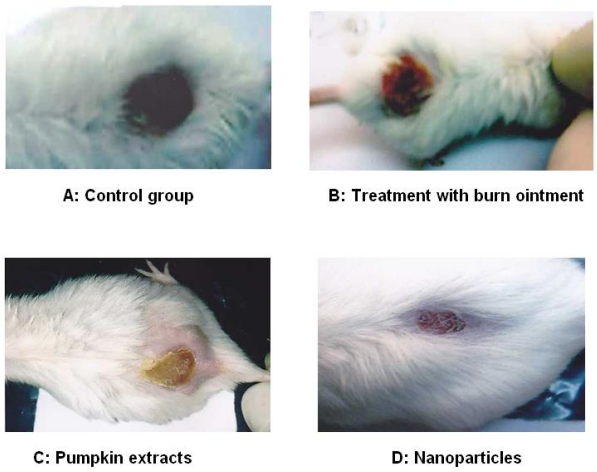

Figure 14: Groups of laboratory mice after 14 days

Figure 15 represents pictures of treated animals after 21 days of burning, it was found that the animals in the control group (control group) that were not treated with any extract or any ointment, as well as the group treated with burn ointment, did not recover. Rather, keratinization of the skin at the edges of the burn area and the center of the burn, the skin tissue is still soft and reddish, and the two groups treated with the aqueous extract recovered to a lesser degree as the diameter of the burn became smaller and the burned skin began to keratin up and hair began to grow at the edges of the burn area.

As for the group treated with minutes, they recovered with a high rate, and as the pictures show, the site of the burn grew hair that covered a high proportion of the burn area, which indicates that the nanoparticles had a significant role in healing burns significantly after (21) days of treatment compared with the control group, and treatment group.

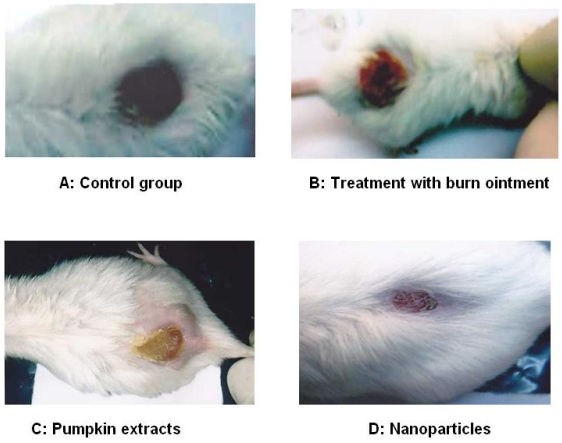

Figure 15: Groups of laboratory mice after 21 days

Applications

First - The idea can be used in Ministry of Health and the Ministry of Industry to prepare a drug for the treatment and prevention of Cryptosporidium parvum with iron nanoparticles prepared using walnut shells and without side effects.

Second - The use of prepared nanoparticles can control other parasite types that are resistant to antibiotics.

Advantages

1- Preparing environmentally friendly nanoparticles using the aqueous extract of walnut shells and iron salts.
2- The prepared nanoparticles are fixed and unchanging, that is, when the nanoparticles are formed, the color of the extract changes from orange to black.
3- The raw materials used in preparing nanoparticles are inexpensive.

4- Nanoparticles do not generate generations of parasites resistant to antibiotics as is the case with antibiotics.

4. Conclusions

- The first protection element is to produce an environmentally, and friendly antibiotic using nanoparticles
- An indication of the first protection component that the method of preparing nanoparticles was accomplished by using the concentration of aqueous extract of pumpkin, for the first time in Iraq and the world, in preparing nanoparticles of iron.
- Referring to the first protective element, nanoparticles were used in MBC and MIC tests, and they showed high efficiency in inhibiting microorganisms under study under study.
- Referring to the first protection element, the method of designing an experiment was used to study the efficiency of the nanoparticles proposed under the study, in the treatment of burns.

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