Green synthesis of gold NPs by using dragon fruit: Toxicity and wound healing

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Abstract. In this work, the study of *Hylocereus undatus* properties was done by studying quantitative phytochemical compounds and seeking for total phenolic compounds, synthesis of gold nanoparticles was created via reduction of aqueous gold ions with the aqueous fruit extract of *Hylocereus undatus* (dragon). The synthesized AuNPs were asserted by using (Uv-Vis) spectrophotometer; Fourier transforms infrared (FT-IR) spectroscopy, Atomic force microscope (AFM), Scanning Electron Microscopy (SEM) Zetasizer. The absorbance for SPR is noticed in 546 nm by using Uv-Visible spectroscopy The SEM and AFM analysis evidenced the particle size between 35-100nm, and spherical in structure. Mechanisms of AuNPs synthesis had been suggested and free radical scavenging activity was examined quantitatively by thin-layer chromatography and quantitatively by DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay. The biosynthesized AuNPs showed much higher antioxidant activity compared to *Hylocereus Undatus* fruit extract alone. The toxicity of the nanoparticles and extract was examined by giving all of them at dose 50mg/ b.w orally to mice and the diagnosis of the result of pathological changes, which showed that both extracts are simple toxicity. So, the results confirmed that the fruit extract was a good bio reductant for the synthesis of AuNPs. Which can be applied as good agents for antibacterial applications and Anti-inflammatory and it could be helpful for the preparation of pharmacologically useful drugs.

Keywords: Gold nanoparticles, dragon fruit, wound healing, green synthesis.

1. Introduction

Nanotechnology is could be formulated as the handling of matter through specific chemical or physical processes to synthesis materials with customized properties that can be used in different applications. It is the probability to measure, manipulate, and produce things on a Nano level occasionally between 1-100 nm. They have a great surface area to volume ratio which is their most important advantage responsible for the popular use of these Nanomaterials [1].

Green nanotechnology has been described as the development of clean technologies, "to minimize potential environmental and human health risks associated with the manufacture and use of nanotechnology products, and to encourage replacement of existing products with new nano-products that are more environmentally friendly throughout their lifecycle. It uses existing principles of green chemistry and green engineering to make nanomaterials and nano-products without toxic ingredients, at low temperatures using less energy and renewable inputs wherever possible and using lifecycle thinking in all design and engineering stages.

Due to it is very special properties [2]. Gold nanoparticles (GNPs) with controlled geometrical, optical, and surface chemical properties are the subject of intensive studies and applications in biology and medicine. To date, the ever-increasing diversity of published examples has included genomics and biosensors, immunoassays and clinical chemistry, photo thermolysis of cancer cells and tumors, targeted
delivery of drugs and antigens, and optical bioimaging of cells and tissues with state-of-the-art nanophotonic detection systems [3].

Cactaea and of genus Hylocereus The *Hylocereus* Pitaya fruit is a perennial, epiphytic, climbing cactus with a triangular beefy, jointed stalk which belongs to the family Cactaeaceae and of genus *Hylocereus* [4]. Phytochemicals and antioxidants revealed that Pitaya fruit is also known to possess medicinal and pharmaceutical properties that could prevent diabetes, cancer, and neutralizes toxins in the body. It is even helpful in reducing blood sugar levels in people those carrying type 2 diabetics [5].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), act as important turn in pathological processes [6], such as senility, cancers, and coronary cordial diseases, neurodegenerative disorders, Alzheimer’s disease, atherosclerosis, inflammations and cataracts [7]. Living organisms have antioxidant protection systems that defend against oxidative damage by repair or even removal of damaged molecules [8]. The word ‘antioxidant’ indicates the activity of a huge number of vitamins, phytochemical, and minerals which supply security against the damage caused by RNS & ROS. Antioxidants overlap with the oxidative procedure by scavenging free radicals, acting as electron donors, and chelating free catalytic metals [9]. The natural antioxidant mechanisms may be scant in a set of conditions and hence dietary intake of the antioxidant component are important. The therapeutic effects of many medicinal plants are commonly referring to their antioxidant phytochemicals. It has been proposed that there is an opposite relationship between the dietary intake of antioxidant-rich foods and the happening of human diseases [10].

The study was conducted to check up the phytochemical contains *Hylocereus undatus* fruit extract, determine the total phenolic compounds, the work on the preparing of nanoparticles by usage of gold, characterization of nanoparticles by different methods them determination and comparison between anti-oxidant activity of fruit and nanoparticles, other steps contain the study of the toxicity of fruit and nano by trying that on animal models, then; the extract and AuNPs used to prepare a cream used for wound healing on the mice back and study the histological effects for the two creams.

2. MATERIALS AND METHODS

2.1. Collection of the Fruit Samples

The *Hylocereus undatus* fruit (pitaya) was collected from a local market for vegetables. The fruit was transported to the laboratory of biochemistry /College of Science /Al-Mustansiriyah University, washed in tap water, cleaned well to remove all kinds of pollution. after that, the fruit turns into juice by using a blinder, centrifuge and filtered by a filter paper then put in the freezer at 2-4°C to use in the next experiments.

2.2. Chemical detection of the plant components

2.2.1 Quantitative Phytochemical analysis:

The Phytochemical checking up the test of the dragon juice for qualitative analysis metabolites contains in the fruit extract worked as a standard method used by Romero *et al* [10].

2.2.2 Ash determination:

Five gm of the sample were weighed and put in a dry weighting Ceramic vase. The sample was burned in a muffle furnace for (500 C) and for (24hour) than the ceramic vase from the furnace to be cooled .the vase with the sample then weighted and the difference between the weights is the weight of the ash.

2.2.3 Moisture determination:
Hylocereus undatus fruit was weighted accurately (2-10 gm) and put in a Thermal oven for (100-120 C) for (2-3 hours) and cooled to room temperature then weighted and pack it in the oven for half an hour and replayed that for (3/4 times) until the different of weights rate were stable or (+2mg).

2.2.4 Kjeldhal method for determination of protein:

Hylocereus undatus were cut and weighted, (0.5-1 gm) from the sample were put in a sensitive balance then put in a digestion tube, after that we added (10-15ml) of concentrated H2SO4 to the digestion tube, next move was to Add (0.2 gm CuSO4 + 0.2 gm selenium + 7 gm Na2SO4) to the tube and Cover it with the evacuation pipe then turning on the heater to the boiling degree and let it until the changing in color to the green, lift the pipes from the evacuation part and leaving covered to being cool to the room temperature. The pipes were transported to the distillation part and adding (50 ml) of water and 50 ml of 0.3% NaOH) to the tube. The distillation started, the collection completed in a can containing (25ml) of boric acid, the distillation continued until the volume was being 150 ml. Titrate the distilled part with (0.1N) HCL and the total protein measured by using the equation:

\[ M \times V \text{ (for distilled part)} = M \times V \text{ (for 0.1 N HCL)} \]

2.2.5 Soxhlet method for fats determination:

Ten to fifteen gm of Hylocereus undatus were weighted in a sensitive balance the weighted sample was moved into the extraction thimble, then put a ball of cotton wool wetted by hexane or ether extraction solvent. then the thimble put in Soxhlet instrument, the solvent was added with enough amount to extraction, then the extract starts on a water bath for more than 6 hours. The solvent was evaporated by a nitrogen air current and dried in a furnace, then left in a room temperature to cool and then accurately weighed.

2.2.6 Determination of total phenolic content:

Total phenolic content was specified according to the method of Begum et al [11] with modulation. Samples (0.5 mL) from (Gallic acid and The Hylocereus undatus fruit) were measured into test tubes followed by 1.0 mL of Folin Ciocalteu’s reagent (diluted 5 times with water) and 1.0 mL of sodium carbonate (7.5% w/v) were added into test tubes, the final volume was 2.5 ml. The tubes were vortexed, covered with Parafilm, and allowed to stand for 30 min. Absorbance at 765 nm was measured against a reagent blank. The total content of phenol compounds in plant extracts in Gallic acid equivalents (GAE) was calculated by the following formula equation:

\[ C = \frac{(c \times V)}{m} \]

Where: C = total content of phenolic compounds, mg/g plant extract in GAE, c = concentration of Gallic acid obtained from calibration curve (mg/ml), V = the volume of the sample solution (ml), m = weight of the sample (g).

2.3. Bio synthesis of gold nanoparticles

The nanoparticles were synthesized by mixing the gold nanoparticles with a natural plant fruit and reducing Au^{3+} to Au^{0}, after fixed all the conditions by use different (temperature, time, pH, AuHCl4 concentration, and plant concentration, and determine the optimum conditions.

2.4. Characterization of Au-NPs

2.4.1 UV-Vis spectra analysis:

UV–Vis spectral analysis was done by using (PG Instruments Limited, T80, Germany) spectrophotometer. The reduction of Au-NPs was made sure by using UV-vis spectroscopy at uniformed intervals in the domain of 400 to 1000 nm.3 milliliters of the sample was pipetted into a
test tube and posteriorly analyzed at room temperature. The deionized water is used as a blank. The NPS solution showed the farthest absorbance near 517 nm [11].

2.4.2 FTIR analysis:

After the accommodation of Au-NPs, nanoparticles were centrifuged at 6000 rpm for 12 minutes. The treating was repeated 3-5 times. Then take the precipitate and dry it in the oven at 40°C for 5 hours. The fruit extracts powder of Hylocereus undatus fruit was washed with distilled water a lot of times to get rid of dust and polluters, then dry it with 45°C in the oven. For comparison, the dried NPs and fruit extract powder of Hylocereus undatus were analyzed by FTIR-Shimadzu-8400S spectrophotometer, the spectrum was listed in the range of 500-4000 cm⁻¹ [12].

2.4.3 AFM analysis:

Atomic Force Microscope (AFM) (Model AA3000, Angstrom Advance Inc., USA) were used to inspect the size and size distributions of the metals NPs. The evaporation method of the dropper was used to prepare AFM samples of liquid suspension. Drain a drop of liquid on a glass cover slide (2x2 cm²). To dry the sample before wiping, either leave it overnight in a dust-protected environment or use the/ heater (in Low temperature) to speed up the drying operation [12].

2.4.4 Zeta potential analysis:

Zeta potential measurement has been used to describe composite nanomaterial. Zeta potential was a possibility measured by light dispersion using the Zeta Plus instrument (Brookhaven Instruments Corp., USA). The data were averaged with five measurements [13].

2.4.5 SEM analysis:

Scanning electron microscopy (SEM) has been utilized to characterize the shape and morphologies of created biogenic synthesized of AuNPs, using (SEM-Angstrom Advanced Inc.-AIS2300C) [12].

2.5. Qualitative determination of free radical scavenging activity (TLC method)

According to Kannan et al [14], antioxidant ingredients were analyzed by thin-layer chromatography (TLC) followed by DPPH (2, 2- Diphenyl-1-picrylhydrazyl). About 100 µg of the extract of Standard Gallic acid Hylocereus undatus fruit and pAuNP was loaded on TLC plates (Merck, 10 x 10 cm²). The plates were air-dried and observed under visible and UV light (240 and 300 nm). Different separated spots were founded as their Rf values. After this examination, 0.05% of DPPH solution in methanol was splashed on the face of TLC plates and incubated for 30 min at room temperature. The active antioxidant pitaya and pAuNP ingredients were detected as yellowish spots the strength of activity for compounds selected by studying the color change.

2.6. Quantitative determination of Free radical scavenging activity assay (DPPH method)

Fruit extract (0.5 mL) was added to 1 mL DPPH solution (2mL of 0.013g/L DPPH) in methanol. The reduction of DPPH was measured at 517 nm versus a blank assay at 30 min. The percentage of residual radical in the medium is calculated as the absorbance of the sample split by that of DPPH control at the same time multiplied by 100. The amount of sample necessary to decrease the initial DPPH concentration by 50%, IC₅₀, was calculated graphically [14].

2.7. LD₅₀ Examination
In the study, we recorded the clinical signs for 24hr. till 14 days ten adult albino mice (male) were divided at random into five groups containing two mice (25-30g) in every group, all cured groups treated orally by gastric Gavage once daily with different doses of each extract, and mice were kept under continuous observation for 24 hours after the administration; as follows:

Group (1): control group was given distilled water.
Group (2): given extract at dose 50 mg/kg b.w orally by gastric lavage one time.
Group (3): given Nano-extract at dose 8.6 ppm/ml orally by gastric lavage one time.
Group (4): given Nano extract at dose 4 ppm/ml orally by gastric lavage one time.
Group (5): given Nano-extract at dose 2 ppm/ml orally by gastric lavage one time.
At the end of LD<sub>50</sub> analysis, all the lab animal (mice) were sacrificed and vital organs (brain and heart) were used for histopathological analysis [15].

2.8. Histological Study

All mice were sacrificed after 24 hours of the last treatment. The vital organ (heart and brain) were dissected out and keep in a tube contain formalin 10% until sent for Histological examination. The vital organ (Liver and brain) were dehydrated in progressively more concentrated alcohols, then embedded in paraffin and cut into sections of 4-5 µm thickness and stained with hematoxylin and eosin (H&E) for microscopical examination. The slides were examined with fewer than 40 X magnifications using an optical microscope.

2.9. Cream preparing

The dragon fruit was cut by the blender to get the fruit juice and after that, the juice was dried and thoroughly ground to get a homogeneous powder, then (1) gm of fruit powder were dissolved in the paraffin in 70 c<sup>0</sup> for 2 hr with stirring to have a good mix.

2.10. Wound healing for mice

The mice were placed in cages with appropriate conditions and to ensure that there was no injury or symptom is prevented the examination process, The dorsal area of the mice circled and the area was localized with lidocaine sprayer at a concentration of 10%. To create the wound in a radius of (1.0) cm<sup>2</sup> by surgical scalpel, and the wound left open until redness is indicative of acute inflammation, the mice were treated daily and record the observations that occur during the treatment process Monitor the behavioral changes that the animal exhibits during it [16].

3. Result and discussion

3.1. Quantitative Phytochemical analysis

Quantitative determination for <i>Hylocereus undatus</i> fruit bioactive compound measured by different methods to show the effective compounds, the study appears that fruit juice contain a different concentration of (moisture, protein, fat, ash, fiber and C.H.O).

### Table 1: the quantitative phytochemical compounds in <i>Hylocereus undatus</i>.

| Compound name       | %moisture | Protein% | Fat% | Ash%  | Fiber% | C.H.O   |
|---------------------|-----------|----------|------|-------|--------|---------|
| Hylocereus undatus  | 86.219    | 0.329    | 0.81 | 0.769 | 0.93   | 10.943  |

3.2. Determination of total phenolic content
The total phenolic compound has been studied in the fruit to show the ability of *Hylocereus undatus* to work as an antioxidant compound and show it is anti-bacterial activity: the work appeared that *Hylocereus undatus* have a good amount of phenolic compounds. The result was higher than Choo et al [17].

Table 2: Absorbance of total phenolic compound in *Hylocereus undatus* with different concentrations.

| Sample absorbance | Concentration (µg/ml) | Best Fit Equation | R² Value | The total content of phenolic compounds in mg/g plant |
|------------------|-----------------------|-------------------|----------|-----------------------------------------------------|
| 1.123            | 250                   |                   |          | 141.6                                               |
| 0.93             | 200                   |                   |          | 88.05                                               |
| 0.86             | 150                   | y = 0.0036x + 0.613 | 0.84     | 78.6                                                |
| 0.723            | 100                   |                   |          | 33.55                                               |
| 0.639            | 50                    |                   |          | 7.22                                                |

![Figure 1](image1.png)  
**Figure 1.** Standard curve of Gallic acid.

3.3. Biosynthesis of gold nanoparticles
The biosynthesis of AuNPs in this study was done by reducing the gold aqueous solution (AuHCl4) using Dragons fruit extract, it was observed that the color of the solution turned from yellow to reddish-purple, which indicated the formation of gold NPs, the change in color shown in Figure 2.
This result obtained had agreed with several other studies [18, 19]. The equation that describes the reduction of the gold solution to AuNPs is:

![Figure 2](image2.png)  
**Figure 2.** the change in color indicating the synthesis of AuNPs.
AuCl₄·xH₂O + Hylocereus undatus → [Au\ Hylocereus undatus]

**Figure 3.** synthesis of AuNPs by using Dragon fruit juice as a reducing and stabilizing agent (M⁺-metal ion).

3.4. Characterization of Au-NPs

3.4.1 UV-Vis spectra analysis

In the present study, the top of the peak was in 544 nm, and it is referred to synthesize AuNPs by the indication of suitable surface Plasmon resonance (SPR) with high band intensities and peaks under the visible spectrum., the surface Plasmon resonance (SPR) behavior of nanoparticle synthesized by plant extract, were shown by absorption at various wavelength, all of the studies containing AuNPs synthesis are in agreement with the current study [20, 21] Reduction and capping of newly formed AuNPs were achieved with the Dragon fruit extract.

3.4.2 FTIR analysis
The FTIR analysis was used to identify the possible bio-reducing biomolecules in the fruit extract that were restricted specifically on the synthesized AuNPs. The spectra of fruit extract of *Hylocereus undatus* and synthesized AuNPs (after reaction with AuHCl₄) shown in the Figures below.

![Figure 5. The FTIR for Dragon fruit extract (Up) and for AuNPs capped by Dragon fruit (Down).](image)

The peaks near 686 and 669 cm⁻¹ are Designation to CH out of plane bending vibrations of replaced ethylene systems – CH=CH, the peak at 1058 to 1174 cm⁻¹ represents C-O stretching vibration. The peak of 1435 to 1484 cm⁻¹ corresponds to the aromatic group contained C-C stretching. The peak at 1637 cm⁻¹ corresponds to the C=O stretching vibrations which represented the carbonyl group for ketone structure. The peak at 2852 and 2926 cm⁻¹ corresponds to the O-H of the carboxyl group [12]. The peak at 3031 cm⁻¹ also contains O-H group which is the hydroxyl group in dragon fruit dye, Broad peaks between 3288-3417 and 3230-3416 cm⁻¹ corresponds to -NH stretching in amide, most of the active components such as C=O (which are attributed to carbonyl) and O-H (corresponds to the hydroxyl group) usually in a carboxylic acid, a large shift in the absorbance peak with decreased band intensity was observed from 3288-3417 to 3230-3416 and 1450 to 1454 cm⁻¹, with the increase in the peak at 1745 cm⁻¹, implying the binding of gold with hydroxyl and carboxylate groups of the extract [22]. The weaker band at 2926 cm⁻¹ corresponds to asymmetric stretching of C-H groups [23].

### 3.4.3 AFM analysis
The Au-NPs were characterized by AFM for their size and morphology of AuNPs. The origin of the surface morphology of the irregularly shaped particle sizes and the size distribution broaden of Au-NPs synthesized by natural plant extract are shown in Figure 6.

Figure 6. the AFM analysis 2D, 3D and the size distribution chart for characterization of AuNPs.

The images confirmed the uniform distribution of AuNPs as most of the particles were approximately 40–100 nm in diameter with a sphere topology, the highest number of NPS between 60–80 nm, the AFM sample measured after 1 month of AuNPs synthesis, no aggregation or agglomeration seen in the slide sample, that indicates the high stability of AuNPs created. The size obtained in this study almost agreed with other studies [24, 25]. While other studies synthesis AuNPs with different sizes like [26, 27], this difference in size back to the methods of synthesis, the change in the type of plant and the difference in the condition choice for synthesis.

3.4.4 SEM analysis

The SEM was employed to analyze the structure and morphology of the NPS to give further insight into the features of the AuNPs obtained from the proposed biogenic synthesis method, the SEM analysis results are shown in Figure 7.
The result of this study confirmed the results obtained from AFM analysis, the figures measured in 50µm, 20 µm, and 10 µm respectively show the average size of the synthesized NPs in the range of 40_100 nm, the SEM analysis also showed that the AuNPs synthesized had aspherical shape and crystalline morphology, this result agreed with the results of Shao et al [28].

3.4.5 Zeta potential analysis
Zeta potential analysis was important to study the net charge on the surface of extract and NPs and calculate the difference in charge between before and after AuNPs synthesis to understand the stability of AuNPs, the result of zeta analysis is shown in Figure 8a and b.
The particle superficies characteristics and charge doing an important role in the particle’s agglomeration tendencies, physical state, interaction with biological systems, and stability in different media. Zeta potential measurement supplies an indirect measure of the net charge and as a device to test batch-to-batch consistency. Also, to learn some insight into the mechanism of NPs size stabilization. A widely cited experiential rule holds that electrostatic stabilization demands zeta potentials of at least ±30 mV. Stability at low zeta potential more commonly reveals some degree of steric stabilization. So, the results show that the particles in a solution are less stable than the standard degree because the zeta potential little is less than ±30 mV even for fruit and NPs, but as a Comparison [12]. Zeta potential for NPs has a more negative charge (-23.6) than the zeta potential for fruit (-18.98) it is an indicator for more stability to the NPs than fruit [29], AuNPs were found to be stable for one month with little or no agglomeration. Because AuNPs start to agglomerate with each other due to low surface charge on particles and getting a more stable energy state with time [30].

3.5. Determination of anti-oxidant activity by DPPH method

3.5.1 Qualitative determination of free radical scavenging activity (TLC method)

In TLC method the spots on the layer determine the activity of fruit and AuNPs as compared with standard Gallic acid, the figure shows that the fruit and AuNPs have good activity against DPPH compound.

Figure 9. TLC picture for 1-standard Gallic acid. 2- Hylocereus undatus fruit. 3- AuNPs

3.5.2 Quantitative determination of Free radical scavenging activity assay (DPPH method)

The DPPH assay was used to study the quantitative scavenging activity for fruit extract and AuNPs, the result of this study is shown in Table 3.
Table 3. The scavenging activity for 1- standard Gallic acid 2-AuNPs 3- Dragon’s extract.

| Concentration compound (µg/ml) | Standard Gallic acid Scavenging activity % | Hylocereus undatus fru Scavenging activity % | AuNPs Scavenging activity % |
|-------------------------------|------------------------------------------|---------------------------------------------|-----------------------------|
| 60                            | 82.9                                     | 65.7                                        | 73.31                       |
| 50                            | 69.3                                     | 60.8                                        | 67.1                        |
| 25                            | 53.3                                     | 50.4                                        | 52.1                        |
| 15                            | 46.1                                     | 44.6                                        | 45.1                        |
| 10                            | 43.98                                    | 34.39                                       | 44.2                        |

The present results show the scavenging activity for extract and AuNPs compared with Gallic acid as a standard. It is obvious from the data that radical scavenging activity of compounds under study increased with increasing concentration exhibiting, its dose-dependent nature [31]. So the highest scavenging percentage presented with 60 ppm of AuNPs with 73.31%, where the extract had a percentage of 65.7% compared with Gallic acid with 82.9%. This result ensures the result in the figure above that made sure the AuNPs have a better ability of scavenging than the extract itself. The IC$_{50}$ for each compound were determined and demonstrated in Figures (10 a, b and c).

Figure 10a. The IC$_{50}$ for standard Gallic acid.

Figure 10b. The IC$_{50}$ for AuNPs.
The IC$_{50}$ for extract was 29 µg/ml and for AuNPs it was 22 µg/ml, compared with Gallic acid which had 20 µg/ml scavenging activity, this study shows that the dragon fruit is an important source for anti-oxidant [32]. This study is in agreement with another study [33] that showed a lower scavenging activity for Dragon fruit.

The fact that each fruit extract and AuNPs capped with extract has a good scavenging activity will help to give a great base for compounds that work as anti-bacterial and anti-inflammatory.

3.6. LD$_{50}$ Examination

The acute toxicity test of the dragon fruit extract and AuNPs were examined by giving mice the extract and different concentrations of AuNPs, the result of the study is shown in Table 4.

| No. | Dose of extract | No. of mice/group | No. of dead No. of animal | Sign of animal treated with extract |
|-----|-----------------|-------------------|--------------------------|----------------------------------|
| control | | | | No Sign of animal treated with D.W |
| 1 | Extract | 50mg/kg b.w | 2 | 0/2 | Irregular heartbeat (tachycardia), Simple tremor, stiffness or stiffened hair which take few minute then disappeared. |
| 2 | Nano extract | 13.6 ppm/ml | 2 | 0/2 | slight and superficial of scoliorachitic for few minute |
| 3 | 8ppm/ml | 2 | 0/2 | Tacky cardiac, accelerate breathing. |
| 4 | 2ppm/ml | 2 | 0/2 | Tacky cardiac, increase breathing, slight and superficial of scoliorachitic for few minute which turn finally to ballooning like |

The present study obtained that The acute toxicity test of the dragon fruit extract compared with the control group shows that after the plant is given to the laboratory animal, many clinical signs like Tacky cardiac, internal respiration, sedation, have been shown which continue for 1-2 hours, but after 24 hours all sign
disappeared and all animal return to normal state this may be due to the nature of the plant extract and its abundance with active ingredients that may show their effects immediately after giving.

In the case of the NPs extract, the animal (white albino mice) showed clinical signs relative to the concentration used. In high concentration, the sign was more severe like Tacky cardiac, increase breathing, arching back. while the signs became slight within low concentration as in 2ppm/ ml show minor signs that may explain the extract when mixed with NPs did not change the natural structure of extract in another way both extracts have no mortality or morbidity of all doses but the appearance of different signs and that revealed the extract or Nano extract were not toxic in that dose or concentration used.

3.7. Histological Study
Histological examination of the collected groups with the plant extract observed in the section of the tissue of the heart that there is degeneration in some heart cells with emergence the case of apoptotic for others, while the brain section shows the emergence of edema, degenerative changes in the brain tissue with the advent of apoptotic cells. For some brain cells and this indicates that the plant extract contains some active substances that have a simple toxic action effect on the animal immediately after administration, this is confirmed by its concentration in the cardiac and cerebral tissues and by the resulting pathological changes [34].

![Figure 11. Heart section of group treated with fruit dragon extract: Cardiac muscle structure shows few apoptotic cells, degenerative changes. (H&E X40).](image1)

![Figure 12. Brain section of group treated with dragon fruit extract: Showing Oedema, degenerative changes, apoptotic cells .H&E(x10) (X40).](image2)
In the histological examination of the heart and brain at high concentration groups of the nano extract, the appearance of simple signs of disease observed do not exceed the occurrence of an expansion in the heart tissue and this is fully linked with clinical signs where the animal notes the speed of breathing and speed Pulse so, any increase in the amount of blood flowing which may lead to the condition of muscle relaxation and the emergence of the case of apoptosis perhaps is due to the nature of the extract and its association with NPs this is not considered evidence of the fact that NPs have negative effects Apoptosis. The evidence enables the body to get rid of any cell changing to rebuild tissue as it observes that most brain tissue has not shown any histological changes [35].

**Figure 13.** Brain section of the group treated with AuNPs at concentration 8.6ppm/ml: the tissue show certain cells of purkinji fibers showing apoptotic changes with glial cells look normal. (H&E x10).

**Figure 14.** Heart section of group treated with AuNPs at concentration 8.6ppm/ml: show dilated space of helmi with apoptosis of cardiac muscle fibers cells. (H&E x40)
In the histological examination of the heart and brain in the high concentration groups of the nano extract, the appearance of simple signs of disease observed does not exceed the occurrence of an expansion in the heart tissue, and this is fully linked with clinical signs where the animal notes the speed of breathing and speed Pulse Any increase in the amount of blood flowing and may lead to the condition of muscle relaxation and the emergence of the case of apoptosis perhaps Is due to the nature of the extract and its association with NPs and this is not considered evidence of the fact that nanoparticles have negative effects apoptosis The evidence enables the body to get rid of any cell changing to rebuild tissue as it observes that most brain tissue has not shown any histological changes.

3.8. Treatment of developed wounds in the white mice

The treatment of wounds by using creams of dragon fruit, AuNPs and aqueous extract of dragon fruit were studied and results were shown in Table 5.

By examining cream in the treatment of wounds developed in white mice the ability of cream was observed by making the healing faster than the water extract as seen in the figures, where the cream speeds up the formation process of the connective tissue in the outer skin areas [36]. This is because the plant contains a lot of Active substances that interact with the components of cream give the cream this effective therapeutic role, cream works on the composition of the outer envelope surrounds the open wound so that it can be removed from external influences The ability of the cream to penetrates the skin tissue which increases the tensile strength in the skin tissue and increases the production height the epithelial layer and collagen composition around the wound area thus enhance the healing process and return the mouse skin
to its normal state [37]. In the Comparison with the fucidin used as a standard, the appearance of hair is near to be equal to the cream of the dragon, while the cream shows better ability than a control group in the healing ability and hair appearance. No side effects or behavioral changes were observed by the laboratory animal (mice) length treatment period [38].

**Table 5.** the effect of treatment groups (control group, standard group, cream of AuNPs1% w/w group, cream of Dragon fruit 1% w/w group and group treated with Dragons fruit extract) on wound healing.

| type | 1st day | 3rd | 6th | 9th | 12th |
|------|---------|-----|-----|-----|------|
| Control group | ![Image](image1) | ![Image](image2) | ![Image](image3) | ![Image](image4) | ![Image](image5) |
| Stander (fucidin) group | ![Image](image6) | ![Image](image7) | ![Image](image8) | ![Image](image9) | ![Image](image10) |
| Cream of AuNP (1%w/w) group | ![Image](image11) | ![Image](image12) | ![Image](image13) | ![Image](image14) | ![Image](image15) |
| Cream of Dragon fruit (1%w/w) group | ![Image](image16) | ![Image](image17) | ![Image](image18) | ![Image](image19) | ![Image](image20) |

3.9. Histopathological Changes

**Figure 17.** (A) Skin section of control group (×100) (H&E); skin tissue showing non-healing and presence of granulation with chronic inflammatory cells infiltration. (B) Skin section of standard fucidin group(×100) (H&E). (C) Skin section of group treated with cream of AuNPs 1% (×100) (H&E); Section showing beginning re-epithelization forming combined with infiltration of few number of inflammatory cells (mainly neutrophil) and presence of layer of granulation tissue. (D) Skin
section of group treated with cream of Dragon fruit extract 1% (x100) (H&E); the section showing presence of large number of inflammatory cells with thin layer of granulation tissue.

Histopathological changes in treated tissues of the skin of mice treated with AuNPs cream group at 12 days post-injury were shown re-epithelialization was complete and the epithelium was thicker than in other group and control one. Other, the treated tissue show minor inflammatory cells as compared to other groups.

The presence of neutrophils in the treated lesions with many fibroblasts and loops of new growing blood vessels were seen in the repaired were present at the site of injury. The rate of healing in this group was much faster than the other groups.

In another way, compared treated mice with AuNPs cream with other groups show the injured area decrease in thickness may be due to a decrease in the inflammatory cells at the site with better maturation of the repair cells, enhanced the fibroblasts, collagen fibers, and blood vessels content.

Treated mice with AuNPs cream show the area of wound healing greater than others may be due to few neutrophils which means the extract has anti-inflammatory effects that can accelerate healing.

The accelerated healing in the skin section may be due to active ingredients that play a significant role in the wound repair process and they showed that this process will accelerate fibroblast production [39].

4. Conclusions

The present work explained the containing compounds, phytochemicals, and antioxidant activity of *Hylocereus undatus* fruit, then study the extracellular biogenic synthesis of green AuNPs using an aqueous extract of *Hylocereus undatus* and study of their bioactivity versus pathogenic bacteria and study the toxicity effect for each of fruit and NPs. visibly, *Hylocereus undatus* showed that it is a good source for dietary antioxidant as calculated in the chemical DPPH method, the synthesis of NPs in fruit extracts (plant biomasses), despite clear limitations, has a significant chance and many essential advantages relative to traditional methods of NPs synthesis. The results proved that the extract of *Hylocereus undatus* plays an important role in the reduction and stabilization of gold. The data implied that the average formation of the AuNPs increased significantly in acidic medium with increasing temperature. The formation of AuNPs was specified and characterized by UV-Vis, AAS, AFM, SEM, and Zitasizer. The AFM and SEM analysis cleared that the particle size between 35-100 nm, and spherical in structure. The fruit contents erewere rich in phenolic compounds, antioxidants, unsaturated fatty acid, terpenes, and others, likewise, for technical view, the successfully biogenic These properties can be assigned to their total surface area, as a larger surface to volume ratio of NPs provides more functional means for enhanced its activity against pathogenic bacteria, and with surgical and histological study show that both of fruit extract and coated AuNPs have a very little toxic effect, with an amazing ability to heal the wounds and have a higher activity and this may be useful in a wide variety of applications in pharmaceutical, biomedical fields, industrial appliances like a bandage, food, water storage and wastewater treatment in a low price.

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5. References

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