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## Studying the Difference between the Ability of Honey and Hibiscus Flower Nanoparticles to Protect Against Radiation Risks

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### Abstract

Honey and hibiscus flower nanoparticles have numerous unique characteristics, such as biocompatibility; they are excellent agents for biological applications. The aim of this study is to investigate honey and hibiscus flower nanoparticles for 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging activity and their ability to offer protection against ionizing radiation (gamma ray). In this research, natural nanoparticles prepared from honey and plants (hibiscus flower) were used for the purpose of studying their effectiveness as antioxidants in water by scavenging free radicals resulting from radiation. In vitro antioxidant activity study of honey and hibiscus flower nanoparticles has shown good free radical scavenging activity for DPPH radical assayed within a concentration range of  $(0.5 - 2.5) \times 10^{-4}$  g/l, for honey nanoparticles and range  $(0.1-0.5) \times 10^{-4}$  g/l for hibiscus flower nanoparticles. The absorbance and inhibition indicating this finite range of concentration is suitable for scavenging free radicals, also nanoparticles were found to have significant natural antioxidant capacity and thus can be used as potential radical scavenger against deleterious damages caused by the free radicals. Hibiscus flower nanoparticles have shown higher ability for free radical inhibition of 93.40% at  $0.5 \times 10^{-4}$  g/l, than honey nanoparticles of 49.80% at  $2.5 \times 10^{-4}$  g/l.

**Keywords:** Natural Antioxidant, Remove of Free Radicals.

### دراسة الفرق بين قدرة الجسيمات النانوية للعسل ولزهرة الكرديه في الوقاية من مخاطر الإشعاع

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### الخلاصة

أظهرت الجسيمات النانوية لزهرة الكرديه والعسل العديد من الخصائص الفريدة، مثل التوافق الحيوي وهي عوامل ممتازة للتطبيقات البيولوجية. الهدف من هذه الدراسة هو دراسة الجسيمات النانوية للعسل وزهرة الكرديه لنشاط 2، 2-ثنائي فينيل -1 بيكريل - هيدرازيل - هيدرات (DPPH) وقدرتها على توفير الحماية ضد الإشعاعات المؤينة (أشعة كاما). تم في هذا البحث استخدام الجسيمات النانوية الطبيعية المحضرة من العسل و النباتات (زهرة الكرديه ) لغرض دراسة مدى فعاليتها كمضادات للأكسدة في الماء عن طريق كسح

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الجذور الحرة الناتجة عن الإشعاع. أظهرت دراسة نشاط مضادات الأكسدة في المختبر للجسيمات النانوية من العسل وزهرة الكركديه لها نشاط كسح جيد للجذور الحرة DPPH ضمن مدى تركيز  $10^{-4} \times (2.5 - 0.5)$  غم / لتر، لجسيمات العسل النانوية ومد  $(0.1-0.5) \times 10^{-4}$  غم / لتر للجسيمات النانوية لزهرة الكركديه. إن الامتصاص والتثبيط الذي يشير إلى هذا النطاق المحدود من التركيز مناسب لكسح الجذور الحرة، كما وجد أن الجسيمات النانوية تتمتع بقدرة كبيرة كمضادات أكسدة طبيعية وبالتالي يمكن استخدامها ككاسح جذري محتمل ضد الأضرار الضارة التي تسببها الجذور الحرة. ووجد أن جسيمات زهرة الكركديه النانوية لها قدرة عالية على تثبيط الجذور الحرة تصل إلى 93.40% عند تركيز  $0.5 \times 10^{-4}$  غم / لتر ، في حين تصل جسيمات العسل النانوية إلى 49.80% عند تركيز  $2.5 \times 10^{-4}$  غم / لتر.

## 1. Introduction

Radiation is the emission or transfer of energy through a medium or space in the form of particles (such as beta and alpha particles) or waves (electromagnetic and sound waves) [1-3]. It is an inevitable part of life and can be produced from natural or man-made sources [4, 5].

Radiation is divided into: ionizing and non-ionizing radiation [6]. Ionizing radiation is a form of energy that can be transmitted as electromagnetic waves, photons (mostly gamma and X-rays), or charged particles (alpha, beta, etc) and has higher energy than non-ionizing radiation [7, 8]. Gamma rays are high-energy electromagnetic radiation that can penetrate materials deeper than alpha or beta radiation because they consist of photons that have no mass or electrical charge [9-11]. Gamma-rays interact with a material in three major processes: photoelectric, Compton and pair production [12]. The main cause of radiation damage is the aqueous free radicals, which are produced when radiation gamma interacts with water [13-15].

A free radical is a molecule possessing one or more unpaired electrons in its outer shell. These molecules are highly reactive and unstable. They are generated in the body during metabolic processes or through exposure to natural toxins or radiation sources like tobacco smoke or gamma rays [19, 20]. Free radicals in biological systems can arise from various sources [22]. Antioxidants are compounds that have a high ability to scavenge and neutralize free radicals [23]. These compounds, possessing unpaired electrons, can efficiently remove free radicals from biological systems.

Nanoparticles have been used for photon shielding through nanocomposite shields [24]. Nanoparticles are tiny particles ranging in size from 1-100nm ( $\text{nm}=10^{-9}\text{m}$ ) and are commonly synthesized using two strategies: top-down and bottom-up [25-27]. Nanoparticles (NPs) may be divided into several classes based on size, shape, and attribute [28]. The unique chemical and physical characteristics of nanoparticles (NPs) stem from their large surface area and nanoscale size [29, 30]. The materials at the nanoscale have interesting characteristics depending basically upon two features: surface area and quantum. There are four different kinds of nanomaterials: one-dimensional (1D), two-dimensional (2D), three-dimensional (3D), and zero-dimensional (0D) [31, 32].

Honey and hibiscus flower with their numerous unique characteristics, such as biocompatibility, are excellent agents for biological applications, and are known for their antioxidant properties. Hibiscus (H), known as "red sorrel," is a member of the Malvaceae family. It grows nearly worldwide. It is extensively grown as a decorative plant in the tropics and has red blossoms that resemble calyces. Hibiscus is also a medicinal plant [33-35] used

for its health benefits; it supports the health of the liver and heart and helps inhibit the growth of bacteria and cancer cells [36,37]. Hibiscus has antibacterial and anti-inflammatory properties. It is antioxidant-rich and is safe and non-toxic [38]. Honey is an essential natural product used as food and medicine [39]. Bees store the honey they make from the nectar from flowering plants in honeycomb cells [40]. There are different types of honey; differing in colour, content and taste in relation to the plants from which the nectar is obtained [41]. It is an important source of natural antioxidants [42]. The composition of the honey is 80% carbohydrates (35% glucose, 40% fructose, and 5% sucrose) and 20% water, along with a few additional active biological ingredients (such as flavonoids and phenolic compounds) [43,44]. In addition, trace levels of other substances like iron and copper, phenolic acids, vitamins, flavonoids, and enzymes are the primary constituents that give honey its antioxidant and reduction (or redox) qualities [45, 46]. Little is known about the antioxidant potential and the unique biochemical component of honey's impact in tropical regions, including its ability to reduce radical capacity or scavenge radicals [47, 48]. Various honey types range greatly in their basic properties and chemical makeup. The antioxidant ability of honey depends on its bioactive compounds [49].

The aim of the research is to study the effectiveness of hibiscus flowers and honey nanoparticles to scavenge free radicals caused by gamma rays, through donating electrons to free radicals to neutralise them and stop their harm.

## 2. The method of work

For the purpose of studying the abilities of honey and hibiscus flowers nanoparticles in scavenging free radicals, deionized water (DIW) (supplied from the medical laboratories at the University of Baghdad) was used as a substitute for the human body since water constitutes a large percentage of the human body (70%).

One gram of hibiscus flowers fine powder and one gram of natural honey were separately dissolved in 100 ml of deionized water in a 200 ml volumetric flask. The solution of each substance was placed on a Stirrer device for 40 minutes at 50C<sup>0</sup> then the solutions were filtered several times with filter paper; then placed in a container at 4C<sup>0</sup> for 24 hours. These solutions were used later as the nanoparticles. The morphology and size of the prepared hibiscus flower and honey nanoparticles were determined by atomic force microscopy.

Different concentrations of the prepared nanoparticles solutions were added to the deionized water (DIW) samples. After this, the samples were irradiated for 4 days with gamma radiation from a <sup>137</sup>Cs-radioactive source (of 6 μCi activity that generates gamma rays of 662 keV energy) at a dose rate of 0.2rad /hr. <sup>137</sup>Cs source was submerged in the deionized water samples for irradiation. Free radicals are formed when gamma rays interact with the deionized water. The samples were then mixed with a fixed ratio of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution to evaluate the % inhibition of free radicals. DPPH was used as a benchmark to assess how well an antioxidant works. The following formula was used to compute the percentage of inhibition (I%) [50]:

$$I\% = [(A_1 - A_2) / A_1] \times 100 \dots \dots \dots (1)$$

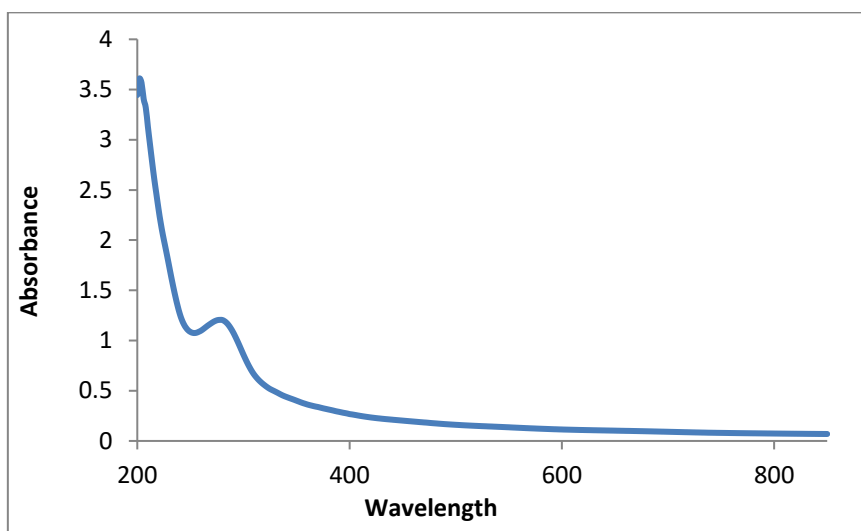
Where:

A<sub>1</sub>: peak absorbance of DPPH with the irradiated deionized-water samples as the control without the HNPS or HFNPs.

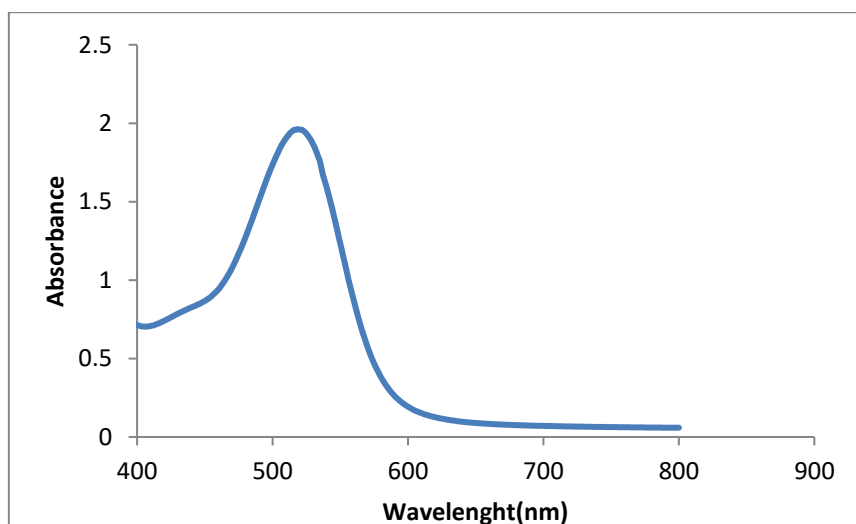
A<sub>2</sub>: peak absorbance of DPPH with the irradiated deionized-water samples and different concentrations of HNPS or HFNPs.

### 3. Results

Honey and hibiscus flower nanoparticles were used as antioxidants to remove free radicals formed in deionized water as a result to exposure to gamma rays. A UV-Vis spectrophotometer (ShimadzuUV-1800) was used to measure the absorbance of each sample. The UV-Vis spectra of the honey nanoparticles (HNPs) at 277 nm and hibiscus flower nanoparticles (HFNPs) at 518 nm are shown in Figure 1 and 2, respectively. From the figures, it can be noted that the honey nanoparticles (HNPs) and hibiscus flower nanoparticles (HFNPs) highest absorbance were 1.2059 and 1.962, respectively.

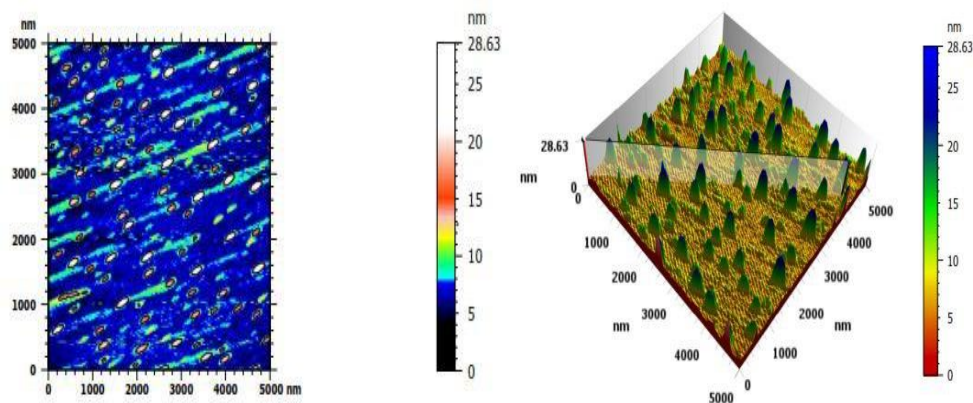


**Figure 1:** The UV-Vis spectrum of honey nanoparticles at 277nm.

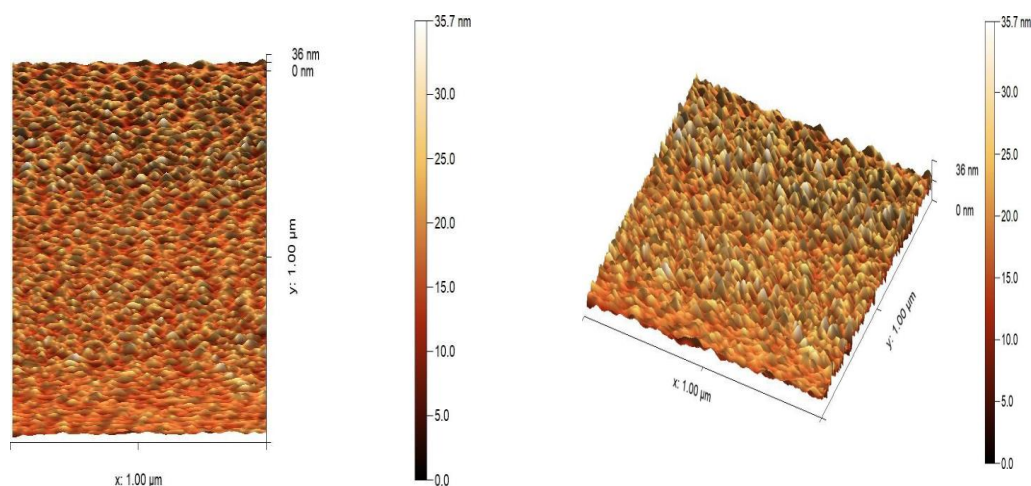


**Figure 2:** The UV-Vis spectrum of hibiscus flower nanoparticles at 518nm.

The atomic force microscope (AFM) images of HFNPs and HNPs are shown in Figures 3 and 4, respectively. The figures show the shape and size of HFNPs and HNPs. It is observed that the particles are nanosized and spherical in shape. The average diameter of HFNPs was 18 nm and HNPs was 17 nm.



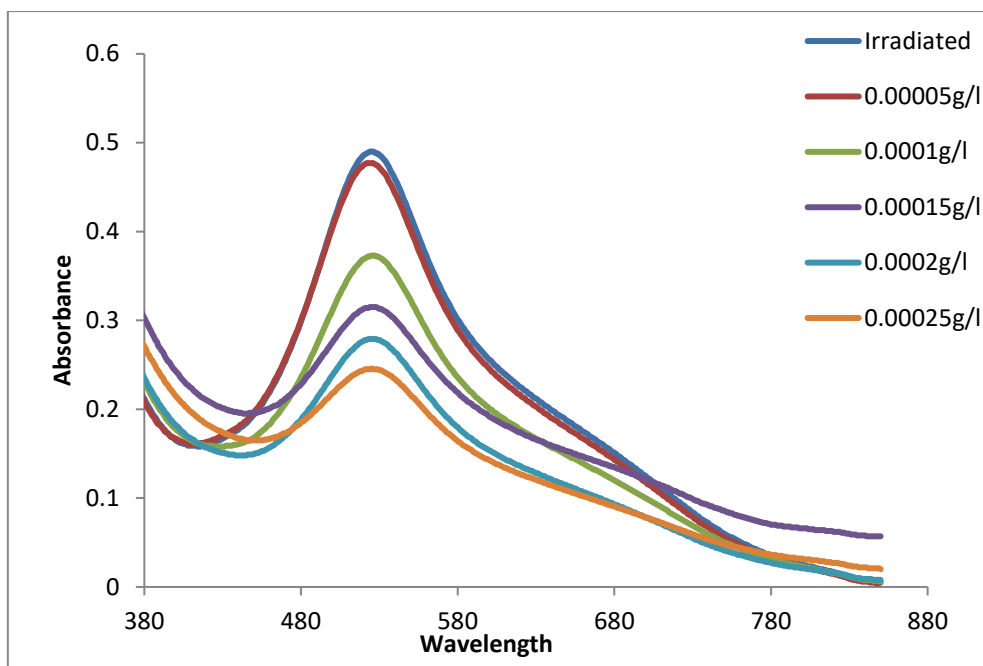
**Figure 3:** Atomic Force Microscope (AFM) images of HFNPs



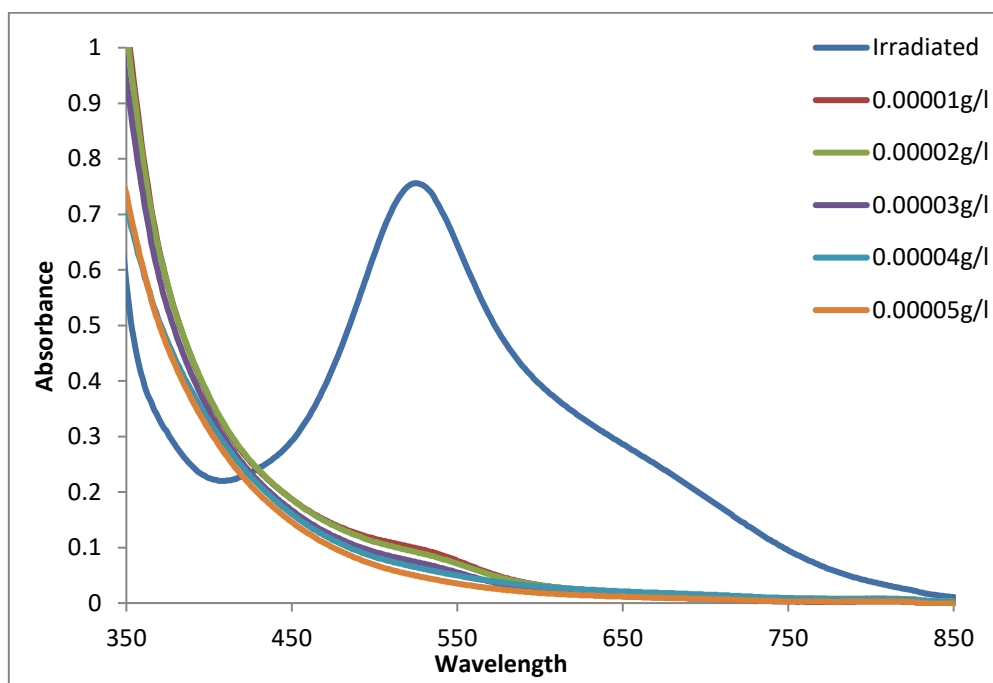
**Figure 4:** Atomic Force Microscope (AFM) images of HNPs

In this study, the DPPH test was used to evaluate the ability of honey and hibiscus flowers nanoparticles to scavenge the free radicals resulting from exposure to ionizing radiation (gamma rays).

Absorption spectra of the synthesised HENPs and HFENPs were investigated using UV-Visible spectrophotometry in the wavelength range of (350–850) nm. The absorption peak of the spectra appears at 524 nm, as shown in Figures 5 and 6. The figures also show the absorbance spectra with the different concentrations of the added HFNPs and HNPs. The absorption peak of DPPH remains at 524 nm, but the absorbance at 524 nm decreases with the increase of the HFNPs and HNPs concentrations. The hibiscus flower and honey nanoparticles donate electrons to the free radicals produced during exposure to radiation; then these free radicals become neutral and harmless.



**Figure 5:** UV-Vis Absorption spectra of the control DPPH and of that with honey nanoparticles added of different concentrations.



**Figure 6:** UV-Vis Absorption spectra of control DPPH and of that with hibiscus flower nanoparticles added of different concentrations.

Table 1 shows the absorbance value of the deionized water samples exposed to gamma rays with DPPH as a control to be 0.490, which decreased to 0.477, 0.373, 0.315, 0.279 and 0.246 after adding different honey nanoparticle concentrations of ( $0.5 \times 10^{-4}$ ,  $1.0 \times 10^{-4}$ ,  $1.5 \times 10^{-4}$ ,  $2.0 \times 10^{-4}$  and  $2.5 \times 10^{-4}$ ) g/l, respectively. The % inhibition (according to Equation 1) of all samples with the different concentrations of added honey nanoparticles is shown in Table 1. It can be noted that the absorbance decreased as the HFNPs concentration increased, leading to higher %inhibition, indicating higher free radicals scavenging.

**Table 1:** DPPH absorption and % Inhibition for all samples with different concentrations of honey nanoparticles

Samples	DPPH Absorption	Inhibition %
Irradiated (HNPs) Concentration	0.490	
$0.5 \times 10^{-4}$	0.477	2.56%
$1.0 \times 10^{-4}$	0.373	24.00%
$1.5 \times 10^{-4}$	0.315	36.00%
$2.0 \times 10^{-4}$	0.279	40.00%
$2.5 \times 10^{-4}$	0.246	49.80%

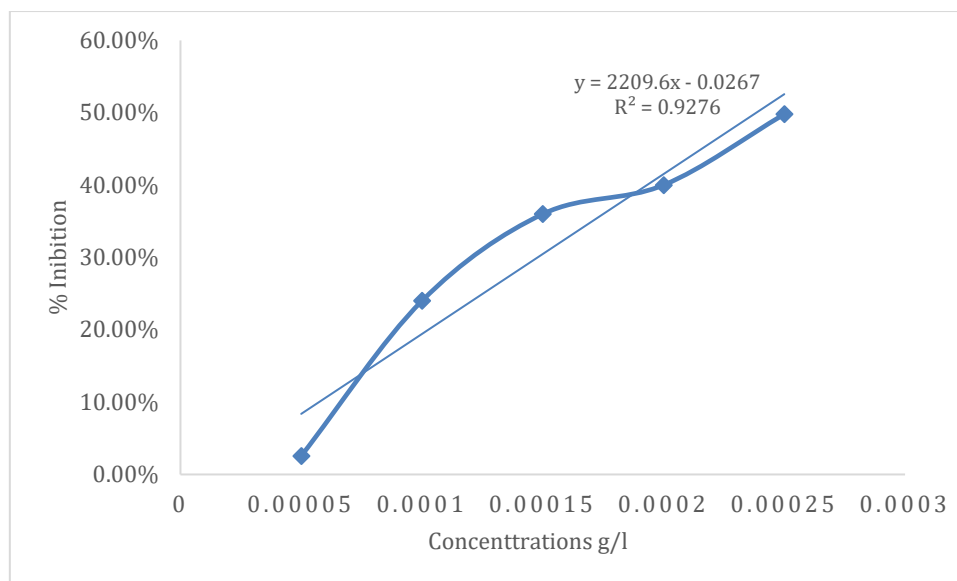
The absorbance value, presented in Table 2, of the gamma-radiation exposed deionized water samples with DPPH as a control is 0.756. While the absorbance of the samples with different hibiscus flower nanoparticle concentrations of ( $0.1 \times 10^{-4}$ ,  $0.2 \times 10^{-4}$ ,  $0.3 \times 10^{-4}$ ,  $0.4 \times 10^{-4}$  and  $0.5 \times 10^{-4}$ ) g/l, were 0.1, 0.093, 0.075, 0.065 and 0.05, respectively. The % inhibition of all samples increased with the increased concentrations of the added hibiscus flower nanoparticles, starting from 86.77% at  $0.1 \times 10^{-4}$  g/l concentration to 93.40% at  $0.5 \times 10^{-4}$  g/l concentration.

**Table 2:** DPPH absorption and % Inhibition for all samples with different concentrations of hibiscus flower nanoparticles

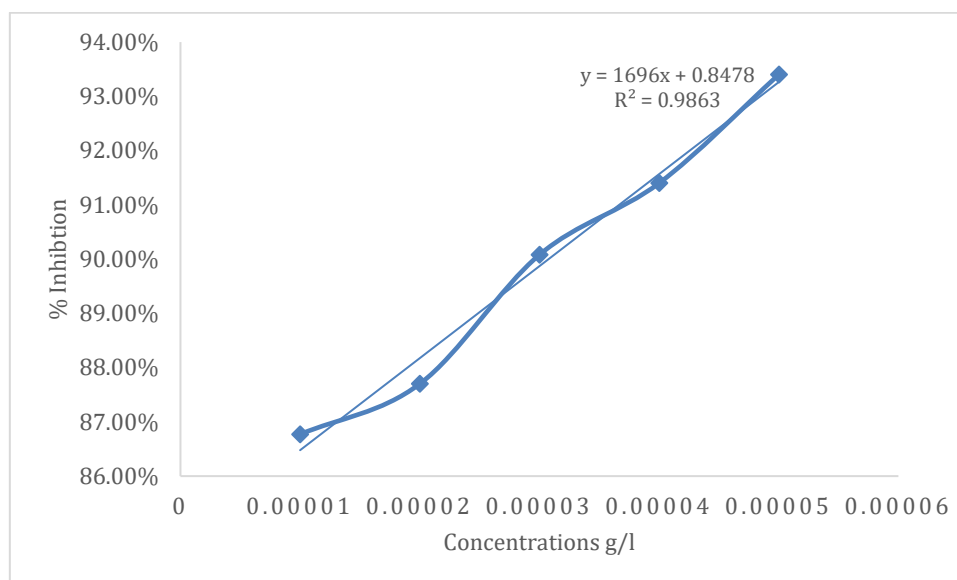
Samples	DPPH Absorption	Inhibition %
Irradiated (HFNPs) Concentration	0.756	
$0.1 \times 10^{-4}$	0.1	86.77%
$0.2 \times 10^{-4}$	0.093	87.70%
$0.3 \times 10^{-4}$	0.075	90.08%
$0.4 \times 10^{-4}$	0.065	91.40%
$0.5 \times 10^{-4}$	0.05	93.40%

Although the concentrations of HFNPs are much lower than HNPs, they proved to have a higher ability to remove free radicals. HFNPs act as good antioxidants much better than HNPs.

The effect of different concentrations of (HNPs and HFNPs) added to deionized water samples on increasing the efficiency of free radical inhibition is shown in Figures 7 and 8. The way the honey and hibiscus nanoparticles interacted with free radicals and converted them into neutral molecules caused the absorption and inhibitory behavior.



**Figure 7:** %Inhibition of free radical as function of honey nanoparticles concentrations



**Figure 8:** %Inhibition of free radical as function of hibiscus flower nanoparticles concentrations.

#### 4. Conclusions

The results in this research indicated that honey and hibiscus flower nanoparticles have the ability to scavenge free radicals resulting from the effect of gamma rays (from  $^{137}\text{Cs}$ ) in deionized water samples. Nanoparticles act as a natural antioxidant that can donate electrons to free radicals and scavenge them. However, hibiscus flower nanoparticles have shown higher ability for free radical inhibition of 93.40% at  $0.5 \times 10^{-4}$  g/l than honey nanoparticles of 49.80% at  $2.5 \times 10^{-4}$  g/l.



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