

# Studying the ability of olive leaves nanoparticles to reduce free radicals resulting from the effect of gamma rays on water

O. S. Ashour\* and A. H. Al-Mashhadani

*Department of Physics, College of Science, University of Baghdad, Baghdad, Iraq,*

*\*e-mail: omarashour217@gmail.com*

Received 1 September 2023; accepted 8 March 2024

This study aims to explore the potential of olive leaves nanoparticles (OLNPs) in scavenging free radicals, particularly 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), and their capability to mitigate the effects of ionizing radiation. The *in vitro* assessment of antioxidant activity revealed promising results for OLNPs, exhibiting effective DPPH scavenging within a concentration range of 0.00002 to 0.0001 g/l. Additionally, the natural nanoparticles demonstrated significant antioxidant capacity, suggesting their potential as radical scavengers against free radical-induced damages. Characterization of OLNPs was conducted using UV-Visible absorption spectroscopy, revealing surface Plasmon resonance absorption peaks around 287 nm. Furthermore, atomic force microscopy was employed to determine the morphology and size of the OLNPs, indicating an average diameter of 65 nm.

*Keywords:* Natural antioxidant; scavenging of free radicals; mitigation the effects of ionizing radiation.

DOI: <https://doi.org/10.31349/RevMexFis.70.041101>

## 1. Introduction

Radiation is the emission of energy or a transition over space or a material medium in the form of electromagnetic waves or particles (such as beta and alpha particles). It can be created artificially or through the radioactive decay of an unstable atom [1]. There are two types of radiation: ionizing and non-ionizing radiations. 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, neutrons etc) [2]. Radiation occurs from a variety of sources, like gamma rays from space, the atmosphere, and man-made causes like nuclear fuel and surgical practices. Since radiation has such a complex effect on the human body, it has been used in a variety of fields, including medical imaging and cancer treatment (such as radiation therapy) [3]. Gamma rays are electromagnetic rays of high energy, no mass, and travel very quickly and over long distances. Therefore, gamma rays have a high ability to penetrate materials more than other rays [4]. Human are exposed to nuclear radiation by different ways [5]. The human organism is affected by radiation in highly complex ways. Different grades of biological impacts, from grades to death of living tissues, include a number of pathological change in human cells [6].

Ionizing radiation causes oxidative stress (OS), an imbalance of free radicals, and antioxidant systems, leading to chronic diseases like cancer, Alzheimer and Parkinson [7]. The human body produces reactive oxygen species (ROS) and free radicals like hydroxyl radical and hydrogen peroxide during routine metabolic processes, and exposure to exogenous stressors like ionizing radiation and air pollution can negatively impact cells regular physiological activity [8]. Small amounts of ROS are emitted during metabolic processes. These highly reactive, oxygen-containing molecules

can harm complex biological components like lipids, proteins, or DNA [9].

Nanoparticles (NPs) are extremely small materials having size ranges from 0.1 to 100 nm, the top-down and bottom-up are the strategies by which nanomaterials are prepared [10]. NPs can be classified into various classes based on their size, properties, and shapes. NPs possess unique chemical and physical properties because of their nanoscale size and high surface area, these nanomaterials such as gold and silver were used to scavenge the free radicals, but these materials are not green and have some toxicity when used *in vivo* [11]. Due to their large surface area and tiny nanoscale dimensions, they have unprecedented electrical, optical, physical, chemical, and magnetic capabilities. This science integrates information from various fields, including biology, physics, chemical engineering, pharmacology, material science, and medicine [12]. The nano-materials have interesting properties depending basically upon one of the two features: surface area and quantum confinement effect [13]. There are four types of nanomaterials such as: zero- dimension (0D), one-dimension (1D), two- dimensions (2D), and three-dimensions (3D) [14]. Every area of medicine is becoming more and more interested in nanoparticles due to their capacity to deliver pharmaceuticals in the ideal dosage range. This frequently increases the therapeutic effectiveness of the drugs, lowers adverse effects, and improves patient compliance [15]. Free radical are unstable atoms that can damage cells, causing illness and aging [16]. The number of free radical is reduced in the presence of an antioxidant molecule while it is stable at chamber temperature, producing a colorless ethanol solution. Antioxidants may be quickly and easily assessed using spectrophotometry with the DPPH test, which makes it suitable for evaluating many goods at once [17,18]. The DPPH technique is a rapid, simple, accurate, and reasonably priced method for evaluat-

ing the antioxidant activity of foods and beverages as well as the ability of different compounds to act as hydrogen donors or free radical scavengers [19].

Olive Leaf Extract (OLE) is a natural wellness aid that possesses various beneficial properties. It is known for its anti-inflammatory effects, which help reduce the risk of inflammation in the body [20]. Additionally, OLE acts as an antioxidant, protecting cells from damage caused by oxidation. Its anticancer properties contribute to lowering the risk of cancer development. Furthermore, OLE aids in the elimination of free radicals, further enhancing its health-promoting effects. Notably, olive leaves contain significantly higher levels of antioxidants compared to other parts of the olive tree, with the content of compounds like oleuropein ranging from 1% to 14%, as opposed to the lower percentages found in olive oil [21].

The present study aims for the first time to utilize nanoparticles derived from olive leaves for the scavenging of free radicals in water samples, with a specific focus on mitigating radiation-induced risks. By targeting these free radicals, which are known to pose risks to water and contribute to harmful effects within the human body, the research aims to reduce the associated dangers of radiation exposure.

## 2. Experimental work

### 2.1. Olive leaves extract prepared

Plant extraction is one of the easiest ways to make nanoparticle solutions from olive leaves. This is out of all the known chemical, biological, and physical preparation methods for nanoparticles [22]. The roots of olive leaves were washed in cold water, dried and ground into a fine powder, 2 grams from olive oil leaves on 100 ml of deionized water and placed on a Stealer device for 40 minutes at 50°C, after that the color of the water begins to change from white to golden (the color of olive leaves extract), then the material is filtered by filter paper, adding a solution of extract with different concentrations gradually to the deionized irradiated water. The investigation of the scavenging of free radicals produced when water is subjected to various levels of ionizing radiation employed deionized water as a sample.

### 2.2. Free radical achievements

Irradiation was carried out using a  $^{137}\text{Cs}$  (a gamma source with a 6 mCi activity that generates gamma rays with a 662 keV energy) at a dose rate of 0.2 rad/hr for deionized water samples in an in vitro free radical scavenging (decontamination radiation) study. In this experiment, the radioactive isotope  $^{137}\text{Cs}$  was submerged directly under deionized water samples for the purpose irradiation.

Olive leaves nanoparticles were added with various concentration (0.00002, 0.00004, 0.00006, 0.00008, 0.0001) g/l to the deionized water (DIW) samples after irradiation, then mixed with fixed ratio of ethanoic DPPH solution to evaluate

the %inhibition of free radical. DPPH test was used to assess olive leaves nanoparticles ability to scavenge free radicals. Figure 1 illustrates the purple color and 517 nm absorption of a freshly prepared DPPH solution. Percentage inhibition of (DPPH) was calculated according to the following equation [1]:

$$\text{Inhib.}(I\%) = [(A_{\text{ref}} - A_s)/A_{\text{ref}}] \times 100\%, \quad (1)$$

where  $A_{\text{ref}}$  is the value absorbance of exposed to radiation deionized-water samples with DPPH as a control.  $A_s$  represents the absorbance of samples with various Honey nanoparticle concentrations,  $I\%$  is the % Inhibition.

## 3. Results and discussion

### 3.1. The characterization of olive leaves nanoparticles using Uv-visible spectrum

The absorption spectrum of prepared olive leaves was measured by Uv-visible method in the wavelength range of (250 – 800) nm. Figure 1 displays the absorption spectrum

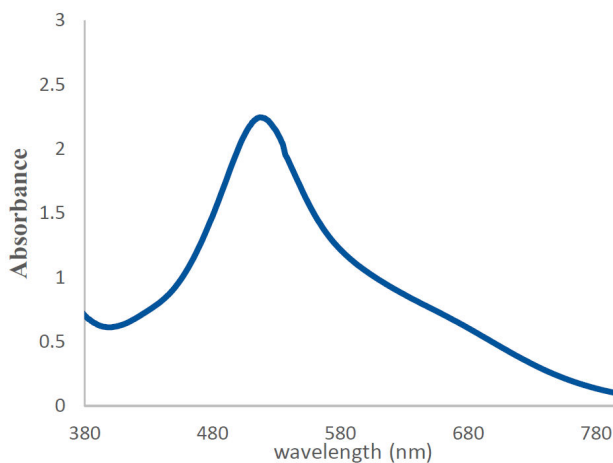


FIGURE 1. DPPH absorbance around 517 nm.

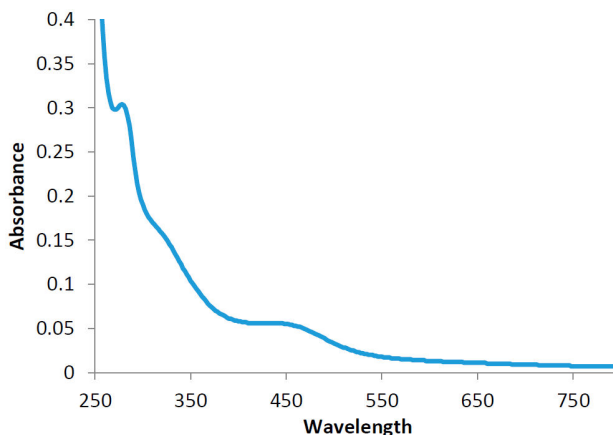


FIGURE 2. The absorbance of olive leaves nanoparticles only at 278 nm.

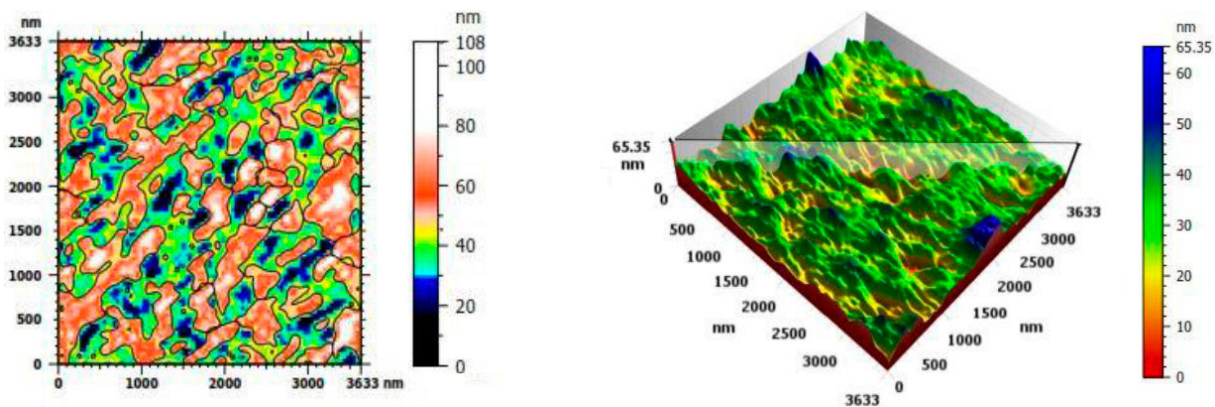


FIGURE 3. AFM images (2D and 3D) for olive leaves nanoparticles.

of DPPH, indicating peak absorption (2.246) at wavelengths of 224.6 nm and Fig. 2 shows the peak of absorption was around 278 nm for all olive leaves samples.

**3.2. Atomic force microscopy (AFM) micrographs**

In order to observe the surface roughness and topography of deposited thin films, Atomic Force Microscopy (AFM) micrographs were obtained using digital instruments, The morphology and the size of the prepared olive leaves nanoparticles was determined by atomic force microscopy. Average diameter of olive leaves nanoparticles was 65 nm. The AFM images together of the particles size distribution are shown in Fig. 3. It is observed that the particles are nanosized and approximately spherical in shape.

**3.3. The efficiency removing free radicals using olive leaves nanoparticles**

In this experiment, olive leaves nanoparticles (free radical scavenging or natural antioxidant) with various concentrations were added to the deionized water samples after exposed to gamma radiation were mixed with in varying concentration for the remove contamination of radiation (remove free radical). The free radical scavenging activity or natural antioxidant properties of olive leaves nanoparticles were estimated by reducing DPPH which leads to a reduction in

absorbance at 524 nm. Figure 4 shows DPPH absorbance at 524 nm for all deionized water samples with various concentrations of olive leaves nanoparticles. When Antioxidants (olive leaves nanoparticles) react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor or electron (free radical scavenging or natural antioxidant) and is reduced to the DPPH and as consequence the absorbance's decreased from the DPPH.

The color change from purple to yellow results the absorbance decreased when the free radical is scavenged by an antioxidant, through donation of electron to form stable

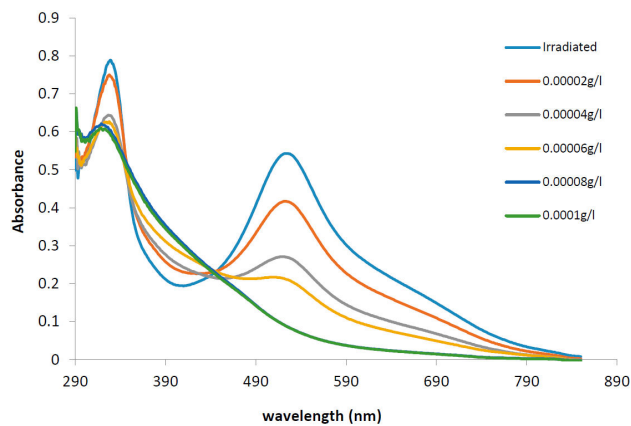


FIGURE 4. DPPH absorption for all water samples at 524 nm with different concentrations of olive leaves nanoparticles.

TABLE I. The value DPPH absorption, wavelength and % inhibition for various olive leaves nanoparticles concentrations added after deionized water is exposed to gamma rays.

Water Samples	DPPH absorption	Inhibition%	Wavelength (nm)
Irradiated	0.543		
OLNPs concentration gl			
0.2 × 10 <sup>-4</sup>	0.417	23%	524
0.4 × 10 <sup>-4</sup>	0.271	50%	524
0.6 × 10 <sup>-4</sup>	0.217	60%	524
0.8 × 10 <sup>-4</sup>	0.090	83%	524
1 × 10 <sup>-4</sup>	0.088	84%	524

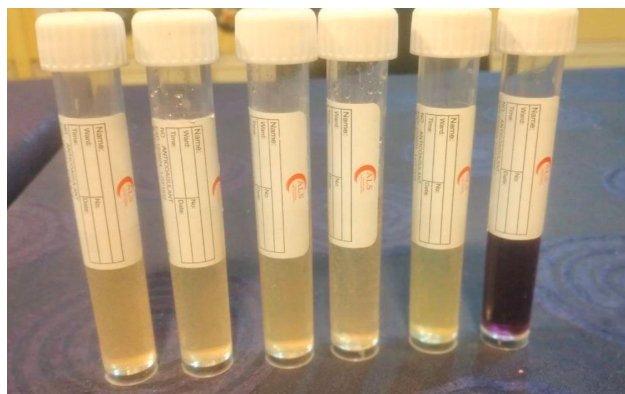


FIGURE 5. Tubes containing DPPH and deionized water exposed to radiation with a different concentration of olive leaves nanoparticles.

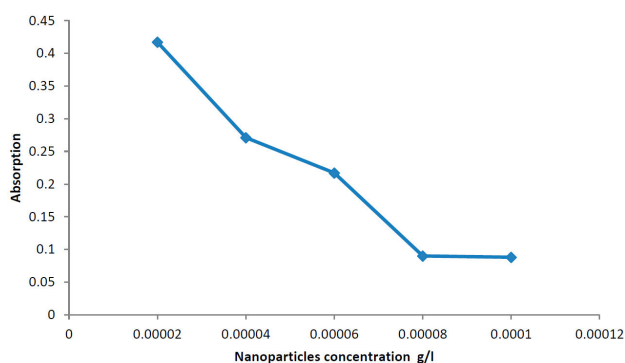


FIGURE 6. DPPH Absorption for All Samples with olive leaves nanoparticles added after Irradiation as a Function of Concentration.

DPPH molecule. The resulting depolarization is stoichiometric with respect to the number of electrons captures [23]. Figure 5 shows tubes containing DPPH and deionized water exposed to radiation with a different concentration of olive leaves nanoparticles. The first purple tube contains DPPH and deionized water only, while the other tubes contain DPPH and deionized water exposed to gamma rays with concentrations of olive leaves nanoparticles.

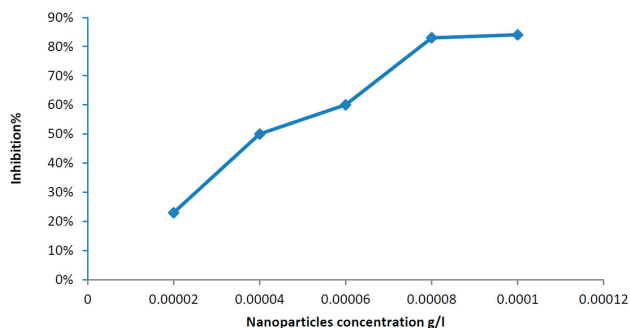


FIGURE 7. Inhibition % for all samples with olive leaves nanoparticles added after irradiation as a function of concentration.

Table I summarizes the values of DPPH absorption and free radical scavenging (inhibition %) for various concentrations olive leaves nanoparticles added after deionized water samples irradiation.

When olive leaves nanoparticles were added with various concentrations to the deionized water samples after the irradiation, the intensity of the DPPH absorbance decreases with the increasing of OLNPs concentration in finite range (0.00002-0.0001 g/l) as shown in Fig. 6. While free radical inhibition were increased with increasing OLNPs concentration in the same finite range as shown in Fig. 7. The absorption and inhibition behaviors were attributed to the interaction of olive leaves nanoparticles with free radicals, resulting in their conversion into neutral molecules.

## 4. Conclusions

This research is the first of its kind used to evaluate olive leaves nanoparticles as scavengers for free radicals formed in water samples irradiated with gamma rays by using the DPPH assay. The current results indicate that olive leaves nanoparticles have a high ability to act as natural antioxidants, as they work to remove free radicals produced after deionized water samples exposed to the gamma source ( $^{137}\text{Cs}$ ). The percentage of free radical removal reached 84%.

1. K. Apte and S. Bhide, Chapter 1 - Basics of radiation, *Advanced Radiation Shielding Materials* (Elsevier, 2024) 1-23. <https://doi.org/10.1016/B978-0-323-95387-0.00013-3>.
2. S. Chawla, N. Swarankar, and S. Singh, To Assess the Practice in Plain Radiography in Terms of Radiation Protection. *Indian Journal of Natural Sciences*, **14** (2023) 1.
3. A. B. Landau *et al.*, Exploring the Role of External Beam Radiation Therapy in Osteosarcoma Treatment: Impact of Diagnostic Imaging Delays and Innovative Techniques, *Cureus*, **15** (2023) e37442, <https://doi.org/10.7759/cureus.37442>.
4. A. Khalid, Asia H. Al-Mashhadani, The Danger Arising From Exposure to Natural Radionuclides as A Result of Working in Majnoon Oil Field in Southern Iraq, *AIP Conference Proceedings*, **2394** (2022) 090045.
5. A. Ghanim Muslim, A. H. Al-Mashhadani, Design and Creating a Specific Neutron Irradiation Instrument to Decrease the User's Radiation Exposure Time, *Iraqi Journal of Science*, **64** (2023) 166, <https://doi.org/10.24996/ijss.2023.64.1.17>.
6. N. Z. Habeeb, A. H. Al-Mashhadani, and A. M. Ali, Antioxidant Activity and Radical Scavenging Properties of Nano Natural Material, *AIP Conference Proceedings* **2414** (2023) 030017, <https://doi.org/10.1063/5.0116741>.

7. P. Janmeda *et al.*, Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases *Frontiers in chemistry*. **1** (2023) 1158198, <https://doi.org/10.3389/fchem.2023.1158198>.
8. B. L. Zaric, M. T. Macvanin, and E. R. Isenovic, Free radicals: relationship to human diseases and potential therapeutic applications, *The International Journal of Biochemistry and Cell Biology* **154** (2023) 106346. <https://doi.org/10.1016/j.biocel.2022.106346>.
9. S. Kishi *et al.*, Oxidative stress and the role of redox signalling in chronic kidney disease, *Nature Reviews Nephrology* **20** (2024) 101, <https://doi.org/10.1038/s41581-023-00775-0>.
10. K. Thummavichai *et al.*, Synthesis, Properties and Characterization of Metal Nanoparticles Nanoparticles Reinforced Metal Nanocomposites: Mechanical Performance and Durability (Springer Nature Singapore, 2023) 161-207. <https://doi.org/10.1007/978-981-19-9729-76>.
11. A. Marwa *et al.*, Green synthesis of nano binary oxide SiO<sub>2</sub>/V<sub>2</sub>O<sub>5</sub> NPs integrated ointment cream application on wound dressings and skin cancer cells. *Baghdad Science Journal* (University of Baghdad Publishing, 2023) 734-745. <https://dx.doi.org/10.21123/bsj.2022.7318>.
12. T. M. Joseph *et al.*, Nanoparticles: Taking a Unique Position in Medicine, *Nanomaterials* **13** (2023) 574. <https://doi.org/10.3390/nano13030574>.
13. J. Jayaprabakar *et al.*, Nano materials for green hydrogen production: Technical insights on nano material selection, properties, production routes and commercial applications. *International Journal of Hydrogen Energy* **52** (2024) 674. <https://doi.org/10.1016/j.ijhydene.2023.06.109>.
14. I. Khan *et al.*, 15 - Nanomaterials: fundamentals and applications, *Nanotechnology for Oil-Water Separation* (Elsevier, 2024) 403-436. <https://doi.org/10.1016/B978-0-323-95517-1.00016-0>.
15. J. Baranwal *et al.*, Nanoparticles in cancer diagnosis and treatment, *Materials* **16** (2023) 5354. <https://doi.org/10.3390/ma16155354>.
16. S. Kumar *et al.*, Free Radicals, Mitochondrial Dysfunction and Sepsis-induced Organ Dysfunction: A Mechanistic Insight, *Current Pharmaceutical Design* **30** (2024) 161. <https://doi.org/10.2174/0113816128279655231228055842>.
17. I. Gulcin, and S. H. Alwasel, DPPH radical scavenging assay, *Processes* **11** (2023) 2248. <https://doi.org/10.3390/pr11082248>.
18. R. Jessica, R. Burger, and M. Schulze, Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins. *International Journal of Biological Macromolecules* **233** (2023) 123470, <https://doi.org/10.1016/j.ijbiomac.2023.123470>.
19. A. Tolun, and Z. Altintas, Chemical sensing of food phenolics and antioxidant capacity, *Advanced Sensor Technology* (Elsevier, 2023) 593. <https://doi.org/10.1016/B978-0-323-90222-9.00004-2>.
20. O. S. Ashour, Asia H. Al-Mashhadani, Inhibition of Free Radicals in Water Using Nano Olive Leaf, *Iraqi Journal of Applied Physics* **20** (2024) 67.
21. B. Baccouri *et al.*, Tunisian wild olive leaves: Phenolic compounds and antioxidant activity as an important step toward their valorization. *Food Analytical Methods* **16** (2023) 436. <https://doi.org/10.1007/s12161-022-02430-z>.
22. H. M. M. Mansour *et al.*, Comparative analysis of silver nanoparticles and whey-encapsulated particles from olive leaf water extracts: Characteristics and biological activity. *Plos one* **18** (2023) e0296032, <https://doi.org/10.1371/journal.pone.0296032>.
23. I. Golchinand Saleh H. Alwasel, DPPH radical scavenging assay. *Processes* **11** (2023) 2248, <https://doi.org/10.3390/pr11082248>.