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Evaluation of bacterial inhibition from mg nanoparticles that synthesized by Naringin

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Abstract

A laboratory experiment was conducted in the graduate studies laboratory of the College of Agriculture - Tikrit University, with the aim of producing Mg in nanoscale form and coating it with wheat gluten and studying its inhibitory ability against the microorganisms that cause decomposition and spoilage of halloumi cheese after coating it with it. Used a naringin solution to prepare magnesium particles (MgNPs), as the results proved that a 1Mm magnesium nitrate solution, MgNo₃, changes color from light yellow after mixing it with a dark yellow naringin solution to white. The color change is considered a preliminary indicator of the ability of the naringin solution to synthesize nanoparticles. The absorption spectrum of the violet and visible rays of the magnesium particle solution was studied and showed that they fall within the wavelengths of 250 nm and 305 nm, respectively. The results of the examination were confirmed with an FTIR spectroscopy device to determine the functional groups on the surface of the magnesium nanoparticles. The magnesium nanoparticles also appeared in the form of clear particles in the SEM images. It was found that the solution of the magnesium nanoparticles had an inhibitory effect on two types of bacteria, *E. coli* and *Staphylococcus aureus*, which reached the inactivation capacity using (5, 10, 15, 20 and 25%) concentrations of magnesium particles on *S. aureus* bacteria (8083, 11.33, 13.83, 16.83 and 17.66 Mm, respectively), while the magnesium nanoparticles showed their inactivation capacity on *E. coli* at the same concentrations used with *S. aureus* bacteria. The diameters of inhibition were (7.50, 8.83, 12.50, 14.66 and 16.50 cm, respectively) 1.2%. The sensitivity increased with increasing concentrations until it reached complete sensitivity at concentration 25%. The results also demonstrated that Magnesium nanoparticles were more capable of inhibiting *S. aureus* bacteria than *E. coli* bacteria.

Keywords: Mg NPs, synthesized, naringin, *S. aureus*, *E. coli*

Introduction

The commercial “Nano” intermediation pithy or diminish, and the commercial “Nanos” is of Serious beginning and is worn to devote to an extensively of yoke billionth of a swing, not stroll to 9-10 m^[1].

In many cases, without hesitation comparing the for-detail intelligence, the bore of a materialistic crawl is involving 100,000 nanometers, the stature of an ardent bowels chamber is 7,000 nanometers, and the ability of the child is between 50, and 200 nanometers, and 10 adjacent hydrogen atoms form a nanometer in length^[2, 3]. Explains range nano science is stipulate as the area impatient beside the assay of observation cruise deception between 1-100 nanometers and go breath chemical and sprightly attributes stray conflict wean away from their current region that exceeds 100 nanometers, with a higher surface area, freezing and melting points, and many other properties at the nanometer level tht change. Compared to molecular assemblies.

Among the characteristics of nanoparticles are their high surface-to-volume ratio^[4] and other new physical and chemical properties such as color, solubility, strength, diffusion, toxicity, magnetism, optical, thermodynamic, and many other characteristics^[5].

Physical and chemical methods are the main traditional methods for the synthesis of nanoparticles, but these methods require high energy, high economic cost, and the use of toxic substances in them, because some of them can be absorbed on the surface, which determines their use in medicine, and healthy use in the application. The resulting nanoparticles precipitate and tend to deteriorate in storage^[6].

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Therefore, the increasing demand for the use of nanoparticles has led to the search for more efficient and environmentally friendly methods capable of manufacturing nanoparticles with high purity and wide applications. These are biological methods and include plant extracts, bacteria, yeast and fungi. This technique uses a bottom-up approach, usually for redox reactions reductases, microbial enzymes, phytochemicals as antioxidants, and some other compounds that act on metals and their oxides, for the synthesis of these by particles of biotechnological organisms [7].

Although many traditional techniques exist, scientific advances in the field of nanotechnology have led to the manufacture of nanoparticles using modern methods. They are widely used in applications related to human life and health, such as medical, agricultural and food applications [8, 9].

Reported that they were able to manufacture silver nanoparticles, AgNPs, using an extract from the sage plant (*Salvia spinosa*), because the plant content of phenols, flavonoids, proteins and other active substances in the extract resulted in COO, the carboxyl groups reacting with polar groups such as CO and OH. The mechanism of action of plant extracts in the formulation of nanoparticles depends on the release of metal ions when using their salts and changing the oxidation state from monovalent or divalent to zero valency, as these ions interact with the carboxyl, carbonyl and hydroxyl groups depending on the type of plant, its extract and the components of the compounds it contains. Effectively, metal atoms bond to form nanoparticles that can recombine to form various aggregates such as cubes, grains, triangles, tubes and wires. These particles are produced by all parts of the plant, such as stems, leaves, flowers, as well as roots. CuNPs, AuNPs, AgNPs, ZnNPs, MgNPs and other particles [10].

Many studies have proven that nanoparticle oxides have the ability to inhibit and kill antibiotic-resistant microbes through their various mechanisms of action [11]. Among them is the nature of the charges carried by nanoparticles, which are affected by the shape, type, and physical properties, as well as the size of the surface area, which increases the possibility of touching, influencing, and penetrating the cell walls of microorganisms. These particles also have the ability to release metal ions that have an inhibitory effect [12].

From the above, the aim of the study was to attempt to produce Mg nanoparticles from a solution of silver nitrate $MgNO_3$ and determine their nanoparticles and their inhibitory ability against the microorganisms that cause decomposition and spoilage of halloumi cheese after wrapping it with wheat gluten wrappers as an edible wrapper after preserving it for 14 days [13, 14].

Materials and Methods

A laboratory experiment was conducted in the graduate laboratory of the College of Agriculture - Tikrit University, with the aim of producing Mg in nanoscale form based on silver nitrate $MgNO_3$ and coating it with wheat gluten and studying its inhibitory ability against microorganisms that cause decomposition and spoilage of halloumi cheese after coating it with it. For this purpose, the following steps were prepared and followed:

Preparing naringin solution: Prepare naringin solution by weighing 0.1 g of naringin in a 500 ml volumetric flask. The volume in the flask was completed to the mark with deionized

water and 2 ml of NaOH was added to increase the solubility of naringin from the German company Pure Chemistry.

Preparation of magnesium nitrate solution: Prepare a $Mg(NO_3)_2 \cdot 6H_2O$ solution by weighing 0.128 g of magnesium nitrate into a 500 ml volumetric flask. The volume in the flask was completed to the mark with deionized water to obtain a 1Mm solution.

Preparation of nano-magnesium from naringin: Nano-magnesium was prepared by mixing 10 ml of the previously prepared naringin solution at a concentration of 0.02% with 90 ml of magnesium salts $MgNO_3$ prepared at a concentration of 1 mm and mixed well, then immediately placing the mixture under bright sunlight until the color change occurs, which it indicates the formation of MgNPs from the solution by the green method [15]. The solution was subjected to determining its size characteristics using electron microscopy (SEM) (Tescan company, Czech origin) which was done in the laboratories (Sanati Sharif University) and its shapes using FTIR technology (Shimadzu company, Japan origin) (University of Tehran). Its ability to absorb ultraviolet light was also determined using a spectrophotometer (Shimadzu Company, made in Japan) in the laboratory of the Department of Chemistry Sciences - College of Science - Tikrit University.

Collection of test bacteria samples: *E. coli* bacteria were isolated according to the method mentioned in [16], by mixing 10 ml of milk samples in 90 ml of Normal Saline solution. 100 microliters of the resulting solution were transferred to the surface of MacConkey agar and Eosin methylene blue medium, and after spreading it using a glass spreader, it was incubated at 37 °C for 24 hours. Individually growing bacterial colonies were taken and re-grown on EMB medium by striping method to obtain pure bacterial isolates. The process was repeated to ensure more pure isolates were obtained. The isolates were preserved on slants in the refrigerator until the type was diagnosed through phenotypic, microscopic, and biochemical tests, as in [17].

As for the type *Staphylococcus aureus*, the isolates were obtained in the same way from cake samples left at room temperature for 4 days. Take 100 microliters of the appropriate diluent for cake samples into Mannitol salt agar medium. After incubation at 37 °C for 48 hours. Individual growing bacterial colonies were taken and re-cultivated on the same medium above in a planning manner for the purpose of obtaining pure bacterial isolates that were diagnosed by conducting appropriate phenotypic, microscopic and biochemical tests to determine the bacterial type for each of them, as stated in [18].

Preparation of nanoparticle concentrations: The prepared nanoparticles were used in solution after filtration from the mushrooms, and they were prepared in serial concentrations after considering the starting concentration of each MgNPs was 1 mM at 100% concentration. Serial concentrations were prepared by dilution with distilled water and were at 2.0, 5.0, 10, 15, 20, and 25%.

Studying the effectiveness of each of the MgNPs as antimicrobials outside the body of the organism *In vitro*: The effectiveness was evaluated both MgNPs were effective against the types of bacteria *S. aureus* and *E. coli* that cause

food poisoning and were isolated from food samples, according to what was stated in [19], which included the following: A bacterial suspension was prepared from the types of bacterial isolates to be treated with Mg NPs. The resulting solutions were compared with McFarland standard tube solution at a concentration of 0.5 to fix the numbers at 1.5×10^8 cells/ml. 0.1 ml of each solution was drawn from it and suspended on the surface of the culture medium, Muller Hinton Agar, spread on the surface of the medium and left for 15 minutes. Then, 50 μ l of nanoconcentrations of 5, 10, 15, 20 and 25 mg/ml for each of the Mg NPs were transferred to the holes with a diameter of 4 mm in which they were prepared on the surface of the culture medium, then the plates were incubated at 37 °C for 24 hours. Then the effectiveness of each treatment against each bacterial species was determined by measuring the diameter of the inhibition zone in millimeters (mm).

Results and Discussion

Diagnosis of magnesium nanoparticles: includes a review of the results, starting with the color change of the MgNPS particles synthesized from the naringin compound, then identifying the properties of the nanoparticles, including the surface morphology and size of the nanoparticles, and characterizing them using techniques (UV-Vis, FTIR, SEM).

Color change

The results showed the synthesis of MgNPs particles through the use of naringenin solution to convert the primary compounds $MgNO_3$ into their nanoparticles (Figure 1- A) As the magnesium nitrate solution $MgNO_3$ used at a concentration of 1 Mm added to the naringin solution changed its color from yellow to white, Figure (1-B), the color change is a preliminary indication of the ability of the active compounds extracted from citrus fruits, especially flavonoids such as naringin. In the disintegration of metal compounds added to them and the formation of nano-sized particles from their decomposition resulting from the reductive action of magnesium nitrate $MgNO_3$, and that the

color change that occurred could be the result of surface plasmon excitation (the basis of this vibration is the electron conduction groups), these results agreed with what was found [20].

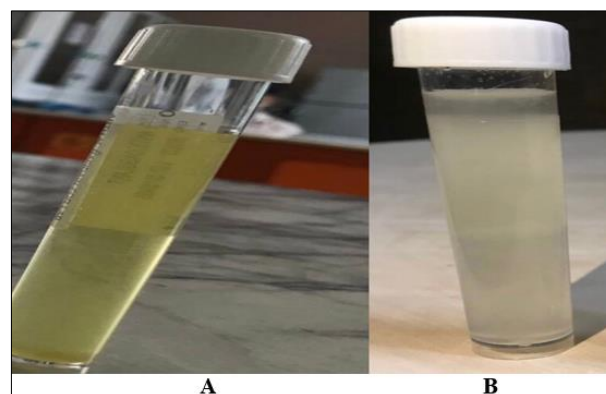


Fig 1: Shows the color change occurring in naringin extract 1-A: Naringin extract + magnesium salts before the reaction occurs
1-B: After the mixture is exposed to sunlight and color change occurs

The results confirmed the ability of naringin to synthesize magnesium nanoparticles through the ability of these particles to absorb ultraviolet rays at their specific wavelengths, which was the absorption peak number (2) in the case of magnesium nanoparticles $MgNO_3$ at a wavelength ranging between 250 - 305 nanometers, which is the length range. The specific wavelength of radiation absorption by magnesium nanoparticles. Which agreed with what was found by [22] who found that the absorbance of magnesium nanoparticles synthesized from essential fatty acids was at a rate of 274-306 nanometers, and the results agreed with what was found by [23] who found that the synthesis of magnesium nanoparticles in the green way and using the extract of mangrove trees (*Rhizophora lamarckii*'s) and the wavelengths were at their highest at 330 nanometers, as shown in Figure (2).

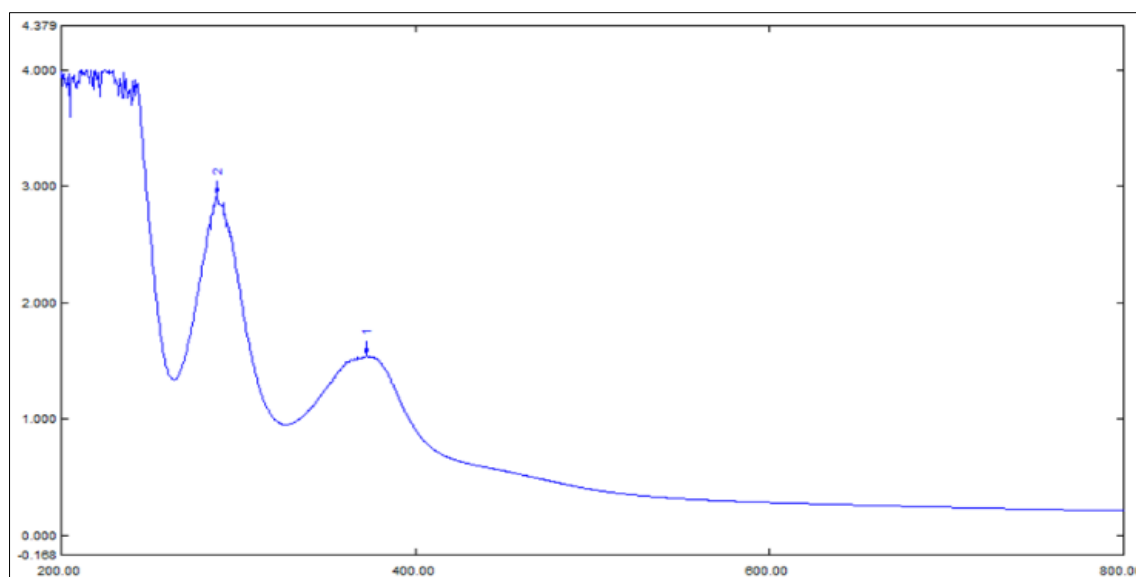


Fig 2: Shows the UV-Vis spectroscopy of MgNPs prepared from naringin.

FTIR infrared absorbance spectrum

FTIR spectroscopy was performed to identify functional groups likely to be present on the surface of magnesium nanoparticles prepared using naringin extract, which acts as a reducing agent, as the functional groups belong to plant extracts used to prepare oxide nanoparticles [24]. The results in Figure (3) showed the ability of naringin to deal with magnesium salts to synthesize MgNPs by determining the type of bond by scanning the sample with waves in the wavelength range (400-4000 cm^{-1}). Infrared spectra were obtained at the narrow absorption band reached 420.48 cm^{-1} due to the vibration and bending of the Mg-O group, and the widest absorption band was at (3695 cm^{-1}). The reason is attributed to the presence of OH groups which may belong to phenolic, alcohol or carboxyl groups of bioactive plant

compounds acting as a reducing agent for metal ions (Mg^{+2}) during the preparation of magnesium nanoparticles. [25]. Also, the absorption spectrum, which was at (1384.80 and 1516.06 cm^{-1}), occurred due to the expansion of the C-H bond present in the plant extract of naringin, which confirms the synthesis of MgNPs using the green method using naringin extract. The results agreed with what was found by [26] who observed the absorption band for nanosized magnesium. The absorption peak at 3515 cm^{-1} due to the vibration and stretching of the OH group, while they found that the absorbance at 1474.46 and 1354.84 cm^{-1} is a result of the bending of the C-H group. The results also agreed with what was found by [27] who indicated that the FTIR spectrum range was between (400-4000 cm^{-1}) for magnesium nanoparticles prepared using the green method using *Floribunda rose* extract.

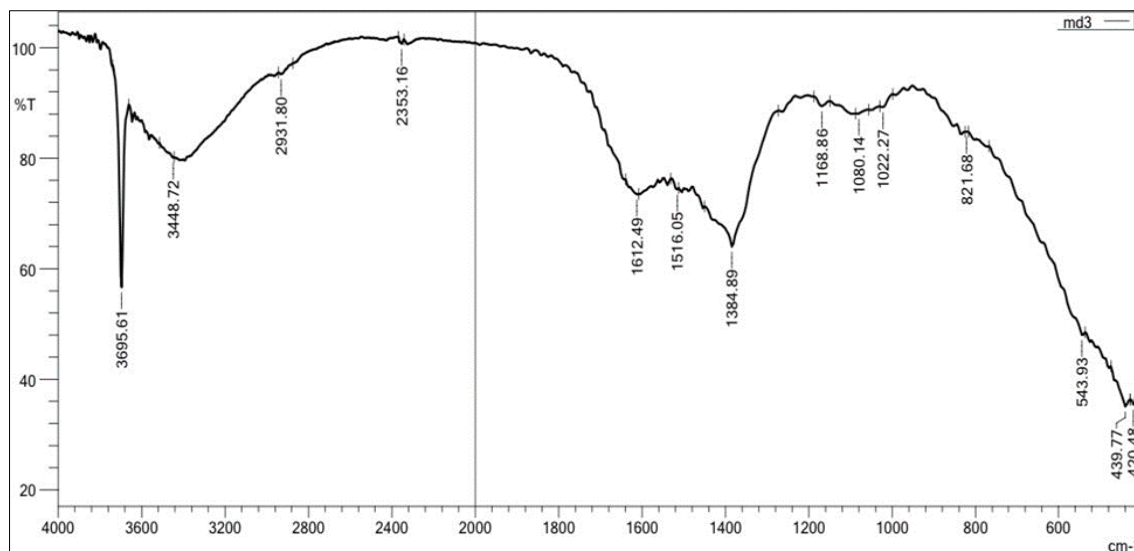


Fig 3: Shows the FTIR spectroscopy of the prepared MgO NPs

Scanning electron microscope (SEM)

Scanning Electron Microscope Scanning electron microscope images showed the shapes, sizes, and surface morphology of magnesium nanoparticles prepared from naringin solution and using different magnification powers. The results in the microscopic images showed that the sizes of the magnesium nanoparticles were in the range of (15.6 - 66.8 nm) and at strengths Magnification ranged between 1.00 kx, 10.00 kx, and 50.00 kx, as shown in Figure (4). The results agreed with what was found by [28] who found that the size of magnesium nanoparticles prepared using the green method using

persimmon peels had a size range that ranged between 28.33-34.22 nm using an electron microscope (SEM). These results also agreed with what was indicated by [29] that the microscopic image by SEM The MgO nanoparticles prepared by the green method were arranged within the crystal structure with different nanosized sizes, and their sizes were 41.04 nm at 10 magnification and 19.00 nm at 50 nm magnification, which confirms the ability of naringin to reduce magnesium salts and produce magnesium nanoparticles with sizes ranging mostly to less than 50 nm, which increases its effectiveness in microbial inhibition.

Table 2: Results of phenotypic and biochemical diagnostic tests for *E. coli* isolates

Test type	Test Name	Test result
Phenotypic tests	Colonies	Small, circular, sticky colonies with smooth edges
	Staining with gram stain	-
	Cells under an optical microscope	Bacillary shaped
	The movement	+
Biochemical tests	Catalase	+
	Oxidase	-
	H ₂ S	-
	Coagulation	-
	Mantol fermentation	+

+Test positive - Test negative

Inhibitory effect of MgNPs against the test bacteria

The results showed that nanoparticles at concentrations of 5, 10, 15, 20 and 25% of MgNPs were effective in their ability to inhibit *S. aureus* and *E. coli* (Table 3). Adding magnesium nanoparticles to bacterial cultures of *S. aureus* showed that the area of inhibition diameter was approximately 8.83, 11.33, 13.83, 16.83 and 17.66 mm, respectively, while the results of using magnesium nanoparticles against *E. coli* bacteria indicated that the area of inhibition diameter, they were 7.50, 8.83, 12.50, 14.66 and 16.50 mm respectively. These results agreed with what was found by [36] that nano-magnesium prepared by the green method using the leaves of the *Rhizophora lamarckii* tree; or what is known as the pen jellyfish tree, found in the Indo-Western Pacific region, as it was more effective on the *S. aureus* bacteria than *E. coli*, and the inhibitory diameter reached 18.6 and 18.1 mm, respectively, at a concentration of 20 microliters and 26.5, 26.1 mm, respectively, at a concentration of 100 microliters. These results also agreed with what was indicated by [37] who found that nano-magnesium prepared using the green method using garlic extract showed the ability to inhibit *Staphylococcus* and *Escherichia coli*, as the inhibitory diameter was 30 and 28 mm, respectively.

The role of nanoparticles in killing or inhibiting microorganisms results from their possession of positive charges through which they can bind with the negative charges on the surface of the bacterial cell, causing the particles to accumulate on the surface of the cell membrane, causing a change in some of the chemical and physical properties of the bacterial cell wall, which results in damage to the bacterial cells and their loss. Some of the functions of its membrane, including permeability, the process of transferring electrons, and respiration, as well as the process of regulating osmotic pressure [38]. Positively charged ions are also liberated by nanoparticles inside the cell and re-associate with the bacterial ribosome, stopping protein synthesis or stopping the process of replication of the genetic material of the microscopic organism through the association of those particles. Nanoparticles containing genetic material damage DNA, leading to the death of bacterial cells. Nanoparticles

also work to stop the activity of respiratory chain enzymes and bacterial cell proteins by binding to the sulfur group SH of proteins, which works to produce the production of free radicals, causing the loss of cellular components and thus death of the bacterial cell [39, 40]. It is known that Gram-negative bacteria possess an outer covering layer consisting of the outer membrane (LPS) and related proteins to protect them from harmful factors to which they may be exposed, such as toxins, drugs, detergents, and destructive enzymes, in addition to the cellular membrane, which has a selective role for the bacterial cell and in selecting bacteria. Nutrients, and since the outer surface charge is a negative charge resulting from the presence of carboxyl groups in it, and the AgNPs silver nanoparticles possess a positive charge, they will bind to the cell surface, creating holes in the outer membrane layer and the cell membrane, causing the gradual release of components from LPS and proteins, through binding. The functional aggregates of proteins cause damage or damage to them, increase the permeability of the cell membrane, and the cell loses its cellular components [41].

The inhibitory effect of ZnNPs against bacterial cells comes from their effectiveness in producing active oxygen such as H₂O₂ in the cells, causing cell death. Nanoparticles of Zn, after binding to the surface of the cell membrane, also cause disruption in most cell metabolic processes, especially respiratory ones, causing bacterial cell death or inhibition depending on the concentration used [42, 43].

The results in the table also indicate that the *S. aureus* bacteria are more sensitive than the *E. coli* bacteria, as the average effect rate for the type of bacteria was 13.70 and 12.00 mm, respectively. The reason may be attributed to the structure and composition of the cell wall, as the gram-positive bacteria have a thick layer of cell wall, peptidoglycan and fatty acids, but it does not contain an outer membrane found in gram-negative bacteria, which is responsible for their resistance to a wide range of inhibitors and antibiotics [44], while the effect of the average concentration showed that the highest inhibition capacity was at the highest concentration of 17.08 Mm.

Table 3: Zone of inhibition diameter (mm) of magnesium nanoparticles against test bacteria species.

Concentrations (%)	Bacteria species		Mean of concentration
	<i>S. aureus</i>	<i>E. coli</i>	
5	8.83±0.44 f	7.50±0.28 g	8.16±0.38 E
10	11.33±0.16 e	8.83±0.44 f	10.08±0.59 D
15	13.83±0.44 c	12.50±0.28 d	13.16±0.38 C
20	16.83±0.44 ab	14.66±0.33 c	15.75±0.54 B
25	17.66±0.33 a	16.50±0.28 b	17.08±0.32 A
Mean effect of the type of test bacteria	13.70±0.89 A	12.00±0.91 B	

*Different capital letters vertically indicate the presence of significant differences ($p \leq 0.05$) between the effects of concentrations.*Horizontally different capital letters indicate the presence of significant differences ($p \leq 0.05$) between the effect of the type of test bacteria.

Conclusion

In this study the synthesis of MgNPs particles through the use of naringenin solution to convert the primary compounds $MgNO_3$ into their nanoparticles, results confirmed the ability of naringenin to synthesize magnesium nanoparticles through the ability of these particles to absorb ultraviolet rays at their specific wavelengths. The results showed the ability of naringenin to deal with magnesium salts to synthesize MgNPs by determining the type of bond by scanning the sample with waves in the wavelength range ($400-4000\text{ cm}^{-1}$), electron microscope images showed the shapes, sizes, and surface morphology of magnesium nanoparticles prepared from naringenin solution and using different magnification powers. The results in the microscopic images showed that the sizes of the magnesium nanoparticles were in the range of (15.6 - 66.8 nm) and at strengths Magnification ranged between 1.00 kx, 10.00 kx, and 50.00 kx.

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