RESEARCH PAPER

A Study of the Effect of Using a Water-Alcoholic and Nano-Based Onion Extract Against Some Pathogenic Microbes That Cause Food Spoilage

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ARTICLE INFO

Article History:
Received 11 June 2024
Accepted 28 September 2024
Published 01 October 2024

Keywords:
Biological activity
Biosynthesized FeNPs
Food spoilage
Onion (AlliumCepa L)

ABSTRACT

As a result of the strong tendency that has emerged recently to use plants and their active components in medicine and as a result of the abundance of these plants (as most of them are used in the kitchen) and their use in traditional medicine, therefore, nanoparticles of iron oxides were manufactured by the reduction reaction of iron ions due to the presence of phenolic compounds in the onion plant. Which works as a biological carrier agent, as the diagnostic techniques used have proven the correct formation of iron oxide nanoparticles, as the scanning electron microscope (SEM) technique confirmed that the size of the particles ranged between 5-50 nanometers, while the study showed the descriptions of the prepared nanostructure using X-ray diffraction. The appearance of four diffraction peaks when creating onion peels with embedded iron particles compared to the iron nanoparticles alone, which showed the presence of three diffraction peaks when diagnosed with the same technique, while FTIR infrared spectroscopy showed the presence of different and distinct peaks for the nano-onion peels compared to the iron nanoparticles. The biological effectiveness of nano-onion peels was studied on two types of harmful bacteria, one of which was *E. coli* positive and the other *Staphylococcus aureus* negative. The results showed that there was a difference between the biological effectiveness of onion nano-peels compared to iron nanoparticles and onion peels in affecting these two strains.

How to cite this article
Abdul- Razaq A. A Study of the Effect of Using a Water-Alcoholic and Nano-Based Onion Extract Against Some Pathogenic Microbes That Cause Food Spoilage. J Nanostruct, 2024; 14(4):1280-1286. DOI: 10.22052/JNS.2024.04.027

INTRODUCTION

There is an urgent and continuous need to discover new antimicrobials with diverse chemical structures and valuable mechanisms of action because there is an increase in the incidence of recurrent and new infectious diseases, and another major reason is the increase in resistance to the antibiotics that are constantly used.[1,2] At the present time, scientists have resorted to conducting new research on plants to overcome microbial resistance to antibiotics and obtain natural treatments to strengthen immunity. Therefore, countries have begun to find alternatives to them and move towards medicinal plants as a natural source of medicines. Human use of medicinal plants in prevention, treatment and treatment goes back to the beginning of human civilizations. Cuneiform texts indicate that the inhabitants of Iraq, including the Sumerians, Akkadians, Babylonians, and Assyrians, and thousands of years before Christ, used plants to treat diseases. These tablets may be considered

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the oldest pharmacopoeia in the world. [3,4] These plants have proven effective in influencing pathogenic microorganisms isolated from some disease cases. One of these alternatives is onion (*Allium Cepa L*), one of the oldest and most important vegetable crops since ancient times. Its production has increased over the past ten years by more than 25% of the total production. In most countries, approximately 10% of the total annual crop is discarded or is inappropriately treated in the market as worthless onions because it fails to meet the quality standards required for marketing to customers. Some consider onions to be a plant with long medicinal folklore, as onion bulbs perform culinary functions such as flavoring food and medicinal use .[5,6] Likewise, the leaves, stems and roots have medicinal uses and can be used in preparing dishes. It belongs to the Alliaceae family, which contains more than 250 genera and about 4,200 species. It is considered one of the winter vegetable crops that must be available throughout the year in most countries of the world, including Iraq. It grows from through rhizomes, bulbs, swollen fleshy roots, or tubers; it is characterized by its high nutritional value, as it contains carbohydrates, proteins, and a number of vitamins and elements, and a rich source of phenols and flavonoids such as quercetin and kaempferol, more than any other crop.[7,8] Many studies have confirmed that onions contain a higher amount of quercetin, kaempferol, luteolin, and other quercetin derivatives that have been examined as antifungal and antibacterial agents, as well as the presence of the chemical compound thiosulfate, which has proven effective in killing many diseases. Common bacteria including *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*. [9] It can also be used as a natural preservative to replace artificial ingredients in food products on the one hand, and on the other hand it can be used in treating arthritis and rheumatism, treating skin diseases, treating cancerous diseases, protecting the heart and being an antioxidant due to the presence of volatile organic sulfur molecules, and it can also be used to lower blood pressure. Blood and harmful cholesterol Low Density Lipoprotein and its extracts also play an inhibitory effect on the types of bacteria that cause tonsillitis, in addition to its effect on pathogenic bacteria present in the mouth, and its alcoholic extracts are effective in treating skin allergies and bronchial obstruction, as it has been proven to have an effect Similar to histamine, while its concentrated aqueous extracts have been proven to treat people with diabetes, and its oil has anti-growth effects on a number of fungi, especially Trichophyton spp. [10]. Interest has increased in recent years in the production of nano-metallic materials due to their uses in various fields, such as biomedical, agricultural, environmental, and industrial journals. The importance of nano-materials is primarily due to their high surface-to-volume ratio due to their extreme smallness. This feature increases their contact surface with other bodies. Biosynthesis of nanoparticles using the metabolites of microorganisms (viruses, bacteria, actinomycetes, and true fungi including yeasts and algae) or plant extracts. The advantages of this method are that it is environmentally friendly, does not require energy, is cheap, and fast.[11] As a result of the above, our current study aimed to use onions in the form of aqueous and alcoholic extracts to produce iron nanoparticles (FeNPs) , evaluate their inhibitory effectiveness against some pathogenic microbes that cause food spoilage.

MATERILAS AND METHODS

This study used the local red onion variety obtained from the local market as an experimental material for the synthesis of iron nanoparticles. After it was brought to the laboratory, it was cut with a weight of 100 grams, immersed in 200 ml of distilled water and left for 16 hours, and then the filtrate was extracted using Whatmann No-1.

Preparation of Onion (Allium cepa) Extract

Allium cepa (onion) was used experimental plant material for the synthesis of Iron nanoparticles. The onion bulbs were collected from the local market, minced into small pieces 100 g, immersed in distilled water 200 ml for 16 h. Then they were filtered using Whatmann No-1 filter paper and filtrate was used [12].

Preparations of Iron nitrate solutions

Iron nitrate was dissolved in double distilled water in dark place to avoid photo-oxidation of Iron nitrate. Solution was used as a substrate for Iron nanoparticles [13].

Combination of Allium cepa extract with FeNPs Assays used in biosynthesized FeNPs

The combination between *Allium cepa* (onion) extract and FeNPs biologically synthesized ,Its

antibacterial effectiveness was also studied, as two strains of harmful bacteria were used, one of which was gram positive *staphylococcus aureus* and other gram negative *E. coli*m[14].

Biosynthesis of Iron nanoparticles

For biosynthesis of FeNPs was used in the present study , 10% (v/v) Allium cepa (onion) extract was added to 3 mM iron nitrate solution and incubated at 27°C at 100 rpm in dark for 5 days. nanoparticles FeNPs (3 mM) and *Allium cepa* (onion) extract incubated one side, supernatant *Allium cepa* (onion) extract without iron nitrate served as controls and kept. After the incubation period, the mixtures 2 were observed for the presence of dark brown color, which indicated the positive formation of FeNPs [15,16] .

Characterization of biosynthesized FeNPs
SEM analysis

Biosynthesized FeNPs were also subjected to SEM analysis to evaluate their size and morphological characteristics [17].

XRD analysis

The freeze-dried FeNPs that were biosynthesized were put to use for X-ray diffraction observations. The powder diffractometer was hired to use to scan the Bragg angles 2θ at a rate of 0.377/min.

Fourier Transform Infra-Red spectrometer analysis

FTIR used to determine the functional group present on the surface of Iron nanoparticles. These measurements were carried out using range at the 1000-3500 at resolution of 4 cm^{-1} [18].

Antagonistic activity of onion extract against some pathogenic microbes that cause food spoilage

It was followed the method of work mentioned before [19] by mixing the onion extract, the nano-onion and the nano-material separately with the melted Mueller-Hinton culture medium after it was sterilized and cooled to a temperature of 45°C at a concentration of 1 mmol/100 ml of the culture medium, in three repetitions, and after solidifying the culture medium, the volume was transferred. An amount of 0.1 ml of bacterial suspension with a cell count of 107 cells/ml, then the dishes were left at a temperature of 15°C in order to absorb the inoculum. After that, Wells holes were made in the food medium inoculated with the Gram-positive bacteria *Staphylococcus aureus* and the Gram-negative *E.coli* bacteria using a sterile cork auger with a diameter of 7 mm. Transfer 50 microliters of onion extract, nano-onion, and nanomaterial were administered using a micropipette and placed inside the hole. At the same time, control dishes were made, and the dishes were incubated at a temperature of 37°C for 24 hours. After that, the results were read by measuring the diameter of the inhibition zone, which represents the area of no bacterial growth surrounding the hole.

Statistical Analysis

It was accomplished using the Minitab 16 program. The experimental outcome, including derived from the technical and biological triplicates, were all expressed as mean ± standard deviation. The "t" test for pairings was used to assess the statistical significance of the data. P-values below 0.05 were seen as significant. [20].

RESULTS AND DISCUSSION

Biosynthesis of FeNPs

Allium cepa (onion) was used for biosynthesis of FeNPs .The filtrate was initially yellow in color. When filtrate mixed with FeNPs , the color of the mixture was turned to yellowish increased with long period of incubation at 37°C, so color was changed to dark gray after 24-72 hrs. (Fig. 1).

Characterization of biosynthesized FeNPs
SEM analysis

Electron microscopy has been employed to

Fig. 1. Showed (A) FeNPs alone (B) biosynthesis FeNPs after 24hrs. (C) Biosynthesis FeNPs after 72 hrs.

determine the shape, size and morphology of biosynthesized FeNPs. (Fig. 2) reveals a typical EM micrograph of FeNPs obtained by the reduction of FeNPs solution. The morphology of FeNPs was rounded in shape, without significant aggregation. The particle size was ranged from 5-50 nm.

X-ray spectroscopy (XRD)

X-ray Diffraction analysis of synthesized FeNPs showed three diffraction peaks at 1H = 34.10, 45.03 and 58.06 ,the structure of metallic Iron, (204), (212) and (293) respectively (Fig. 3). synthesized FeNPs showed four diffraction peaks at 1H = 39.05, 47.03 , 60.02 and 73.07 , structure of metallic Iron, (209), (214), (295) and (307) respectively (Fig. 4). The optical absorption peak was noticed at approximately (36.02) KeV.

Result this study was agreement with [16-17], results indicate the formation of diffraction peaks (209), (214), (295) and (307), and the obtained angles of 39.05, 47.03, 60.02 and 73.07 are better than the first analysis, with an increase in some peaks.

Fourier Transform Infrared Spectroscopy (FTIR)

The measurement of the dried sample of the FeNPs was performed to provide information about chemical and molecular structures of a possible material that could have a role in the reduction of Fe ions for FeNPs. FTIR spectrum of FeNPs revealed the presence of different distinct peaks located at 2310, 1520, 1030, cm^{-1} (Fig. 5). biosynthesized FeNPs. Revealed the presence of different distinct peaks located at 3325, 2601, 1770, 1255 cm^{-1} (Fig. 6).

At field 2310, it indicates the OH group found in biomolecules. Likewise, the aliphatic group was designated in fields 1520 , while at field 1030, which represents a peak indicating the C=O group. This study was agreement with [18], where different

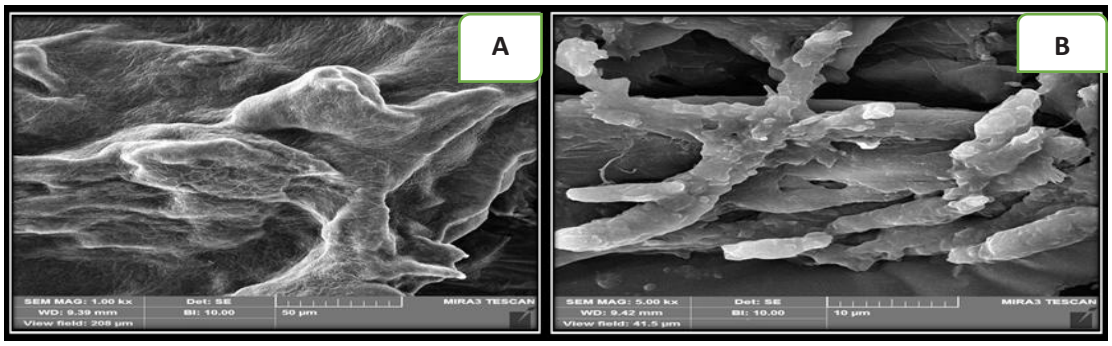

Fig. 2. SEM analysis of biosynthesized FeNPs. The image shows size and star shape of FeNPs. (A): SEM: FeNPs alone (B): SEM: biosynthesized FeNPs.

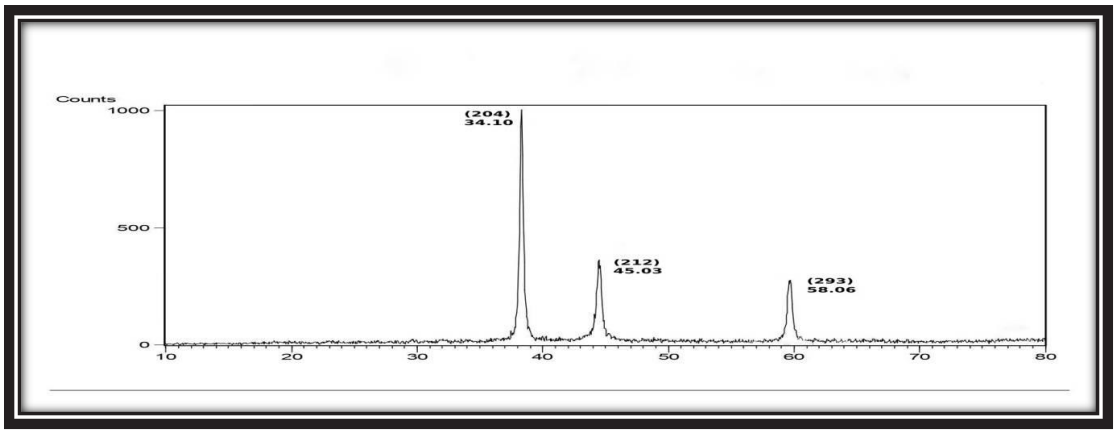

Fig. 3. XRD analysis of FeNPs.

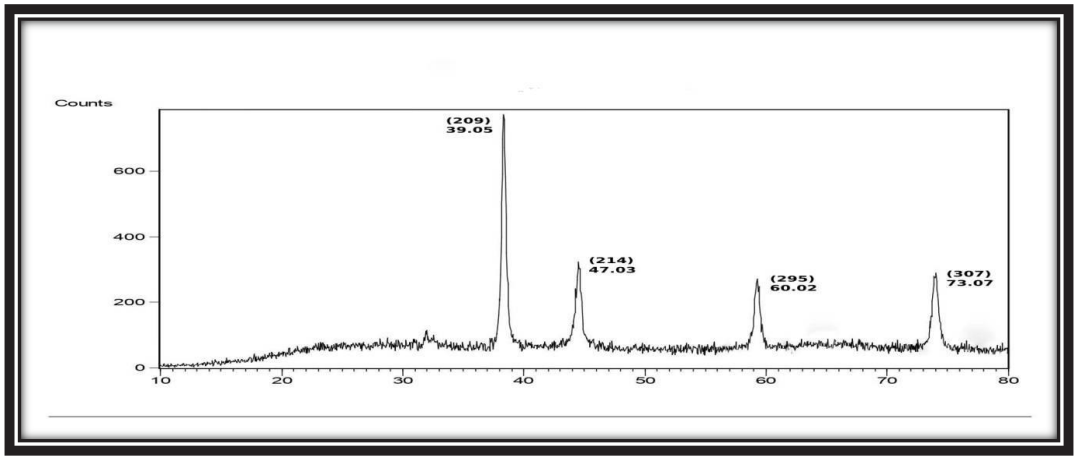

Fig. 4. XRD analysis of biosynthesized FeNPs.

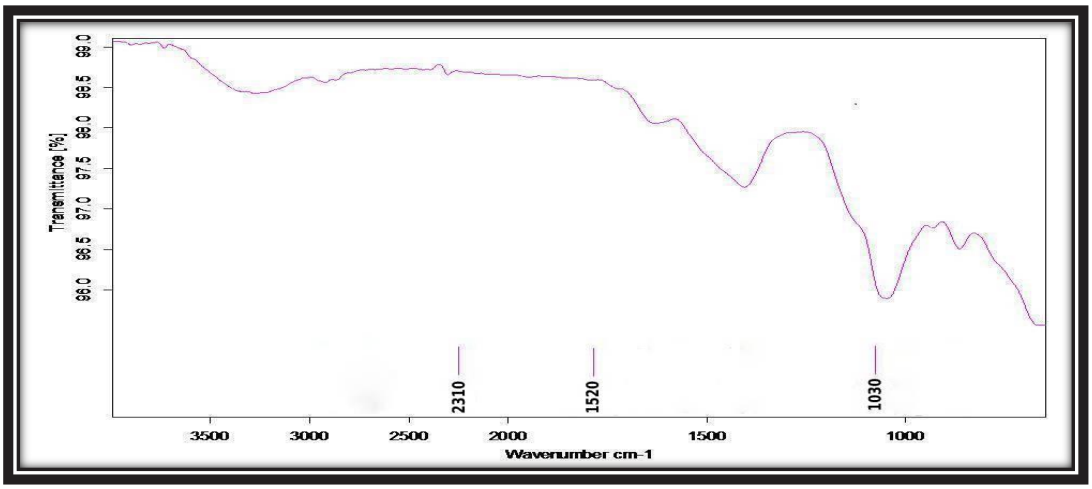

Fig. 5. FTIR analysis of FeNPs with distinct peaks.

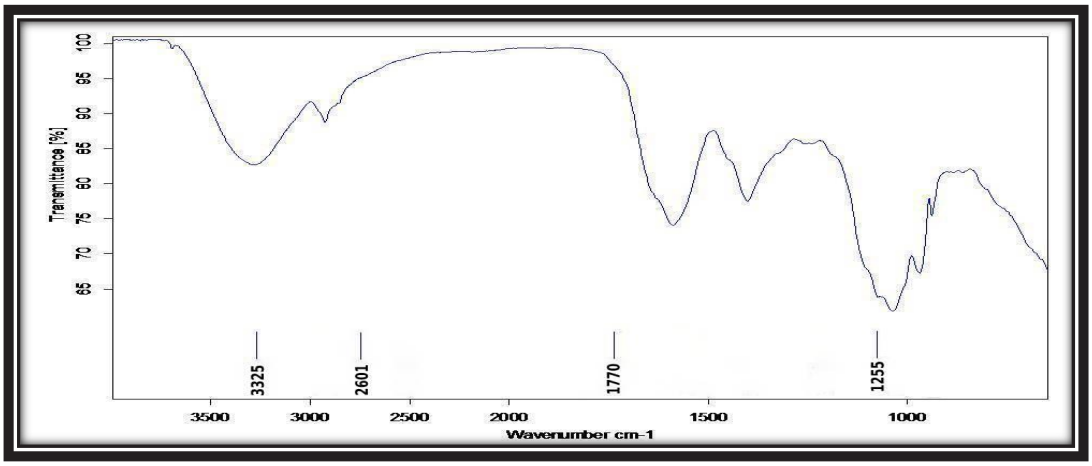

Fig. 6. FTIR analysis of synthesized FeNPs with distinct peaks.

peaks of bonds appeared, as in the 3325 field an N–H group appears, and in the 2601 field the C=O group appears, while the 1770 field corresponds to the C-N stretch of the amine vibration, and the 1255 field corresponds to a primary amide.

Combination of biosynthesis FeNPs against Resistance bacteria E.coli and staphylococcus aureus

Food spoilage is a group of apparent and sensory changes in food or changes to make it unhealthy due to contamination with toxic chemicals or microorganisms to make it unfit for human consumption.

The effect of FeNPs against *E.coli* was resistant to FeNPs showed inhibitory zone of diameter 5.5 mm while, *E.coli* showed sensitive to biosynthesized FeNPs , inhibitory zone diameter was 9.1 mm. (Fig. 7).

FeNPs alone against *staphylococcus aurous*, showed an inhibitory zone diameter of 7.03 mm while The effect of biosynthesized FeNPs showed enhance zone of inhibition of 12.05 mm against *staphylococcus aurous* (Fig. 8). (Table 1)

The results was agreement with [19-20] showed that the inhibitory effectiveness was that the nanomaterial achieved a positive response against gram-negative bacteria. This may be due to the

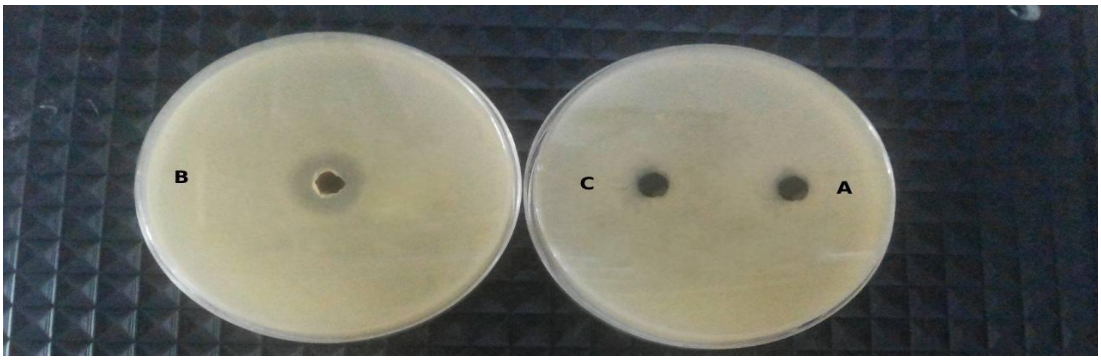

Fig. 7. Showed FeNPs against E.coli: (A) AgNPs (B): biosynthesis FeNPs (C) control.

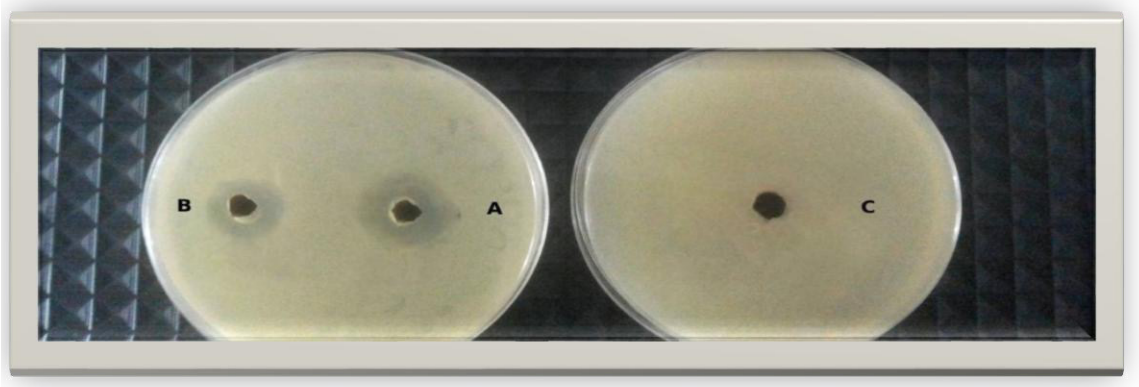

Fig. 8. Showed AgNPs against staphylococcus *aureus*, (A) biosynthesis AgNPs, (B) AgNPs, (C) control.

Table 1. Effect of onion extract, nano-onion, and nanomaterial (mM) at a concentration of 3 mM on the bacterial isolates under study in Mueller-Hinton agar medium.

FeNPs		biosynthesized FeNPs	
E.coli	*staph aureus*	*E.coli*	*staph aureus*
5.5	7.03	9.1	12.05

difference in the cellular structure of the bacteria, as its wall is thinner than that of gram-positive bacteria. While some previous studies showed the effect of nanomaterial's on bacteria due to the invasion of Nanoparticles affect the DNA of bacterial cells by inhibiting the synthesis of their enzymes, which leads to their death. Studies have shown the possibility of using iron nanoparticles to inhibit the growth of the bacteria under study and their ability to affect bacterial cell membranes through their interaction with the sulfur present in the bacterial cell wall, which leads to an increase in membrane permeability and ultimately causes the death of the bacterial cell. (Table 1)

CONCLUSION

Success in obtaining iron nanoparticles with dimensions ranging from 5-50 nanometers after confirmation of microscopic examinations represented by SEM, XRD, and FTIR. The possibility of preparing hybrid nanoantigens from onion peels as an inhibitor against some pathogenic microorganisms.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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