Iraqi Journal of Market Research and Consumer Protection
(2026) 18(1): 69-86.
DOI: http://dx.doi.org/10.28936/jmracpc18.1.2026.(7)
Zahraa & Wedad

المجلة العراقية لبحوث السوق
وحماية المستهلك

STUDYING THE EFFECT OF PUMPKIN PEEL NANO EXTRACT ON INHIBITING THE ACTIVITY OF SOME MICROORGANISMS

***Zahraa A. M.*[1] * , *Wedad F. A.*[2]**
[1]Department of Home Economics, College of Education for Women, University of Baghdad, Baghdad Iraq, zahraa.ali1210a@coeduw.uobaghdad.edu.iq
[2]Assistant Professor, Department of Home Economics, College of Education for Women, University of Baghdad, Baghdad Iraq, wid.nut82@coeduw.uobaghdad.edu.iq

Received 2/ 4/ 2024, Accepted 28/ 5/ 2024, Published 31/ 3/ 2026

ABSTRACT

The study aimed to evaluate the anti-alcoholic activity of pumpkin and nanoscale extract against some types of bacteria. The results showed that the Cu/Cs-NPs was more effective than the methanolic extract of pumpkin peel on all types of Used becteria in the study, despite the use of the plant extract at higher concentrations. Concentrations, the were 100 and 200 mg/ml, while the nano composite was used in lower concentrations, which were 16 and 32 mg/ml. The Cu/Cs-NPs gave the highest inhibition zones of (20.33, 17.33, 12.67, 16.33) mm at a concentration of 32 mg/ml for each. Of the For each bacteria *Staphylococcus aureus, Salmonella Typhi, Pseudomonas aeruginosa, and Escherichia coli* , respectively, while the methanolic extract of Pumpkin peels gave the highest zones of inhibition of (19.67, 16.67, 10.33, 67.15) mm at a concentration of 200 mg/ml for each of the bacteria. *Staphylococcus aureus, Salmonella Typhi, Pseudomonas aeruginosa, and Escherichia coli,* respectively. The minimum inhibitory concentration (MIC) values for the Cu/Cs-NPs on all isolates of *Escherichia coli and Pseudomonas aeruginosa* bacteria were 0.5 and 1 mg/ml, respectively, and 2 mg/ml,. On all isolates of *Salmonella Typhi* bacteria, 0.5 mg/ml for strains No. 1 and 4, and 0.25 mg/ml on isolates 2 and 3 for *Staphylococcus aureus* bacteria, while the MIC values for the methanolic extract of Pumpkin peels reached 8 mg/ml on all isolates of *Escherichia coli* bacteria, with the exception of Isolate No. 2 had a minimum inhibitory concentration (MIC) of 16 mg/ml and 64 mg/ml on all isolates of *Pseudomonas aeruginosa* bacteria, and the MIC values of the methanolic extract of Pumpkin peels reached 8 and 4 mg/ml on all isolates of *Salmonella* bacteria. Enterococcus and *Staphylococcus aureus*, respectively. The nanocomposite also showed high inhibitory activity compared to the methanolic extract against the tested fungi.

Keywords: Pumpkin peels, chitosan, Nanotechnology, Methanol extract

***The article is taken from a master's thesis by the first researcher.**

المجلة العراقية لبحوث السوق وحماية المستهلك

Iraqi Journal of Market Research and Consumer Protection

Zahraa & Wedad

(2026) 18(1): 69-86.

دراسة تأثير مستخلص قشور القرع النانوي في تثبيط فعالية بعض الأحياء المجهرية

زهراء علي محسن[1] ، وداد فاضل عباس[2]

[1]قسم الاقتصاد المنزلي، كلية التربية للبنات، جامعة بغداد، بغداد، العراق، zahraa.ali1210a@coeduw.uobaghdad.edu.iq

2 الاستاذ المساعد، قسم الاقتصاد المنزلي، كلية التربية للبنات، جامعة بغداد، بغداد، العراق، wid.nut82@coeduw.uobaghdad.edu.iq

الخلاصة

هدفت الدراسة الحالية تقييم النشاط المضاد للمستخلص الكحولي لقشور نبات القرع و المستخلص النانوي،اتجاه بعض انواع البكتريا، حضرالمستخلص النانوي من المستخلص الميثانولي لقشور القرع والمحمل بجسيمات الكايتوسان النانوية copper/ chitosan nanoparticles **باستخدام طريقة الهلام الايوني في دراسة سابقة لنا. أظهرت النتائج أن المركب النانوي** Cu/Cs-NPs **كان أكثر فاعلية من المستخلص الميثانولي لقشور نبات القرع على جميع انواع البكتريا المستخدمة في الدراسة على الرغم من استخدام المستخلص النباتي بتراكيز اعلى حيث كانت 100 و 200ملغم/مل اما المركب النانوي فقد استخدم بتراكيز اقل كانت 16 و 32 ملغم/ملم، حيث أعطى المركب النانوي** Cu/Cs-NPs **أعلى مناطق تثبيط بلغ (20.33، 17.33، 16.33،12.67)مم بتركيز 32 ملغم/مل لكل من بكتريا** *Staphylococcus aureus* **و** *Salmonella Typhi* **و***Pseudomonas aeruginosa* **و** *Escherichia coli* **على التوالي، بينما اعطى المستخلص الميثانولي لقشور نبات القرع اعلى قطر تثبيط بلغ (19.67 ، 16,67 ، 10,33،67.15)ملم بتركيز 200 ملغم/مل لكل من بكتريا** *Staphylococcus aureus* **و** *Salmonella Typhi* **و***Pseudomonas aeruginosa* **و***Escherichia coli* **على التوالي، كما بلغ قيم التركيز المثبط الادنى** MIC **للمركب النانوي** Cu/Cs-NPs **على جميع عزلات بكتريا** *Escherichia coli* **و** *Pseudomonas aeruginosa* **0.5 و 1ملغم/مل على التوالي، و 2 ملغم/مل على جميع عزلات بكتريا***Salmonella Typhi* **و 0.5 ملغم/مل للسلالتين رقم 1 و4 و 0.25 ملغم/مل على العزلتين 2 و3بالنسبة لبكتريا** *Staphylococcus aureus* **،بينما بلغت قيم الـ** MIC **للمستخلص الميثانولي لقشور نبات القرع 8 ملغم/مل على جميع عزلات بكتريا** *Escherichia coli* **باستثناء العزلة رقم 2 اذ كانت بتركيز مثبط ادنى (**MIC**) بلغ 16 ملغم/مل و 64 ملغم/مل على جميع عزلات بكتريا** *Pseudomonas aeruginosa***، كما وبلغت قيم الـ** MIC **للمستخلص الميثانولي لقشور نبات القرع 8 و4 ملغم/مل على جميع عزلات كل من بكتريا** *Salmonella Typhi* **و** *Staphylococcus aureus* **على التوالي. كما اظهر المركب النانوي فعالية تثبيطية عالية مقارنة بالمستخلص الميثانولي ازاء الفطريات المختبرة**

الكلمات المفتاحية: قشور القرع، الكيتوسان،النانوي، مستخلص ميثانولي.

INTRODUCTION

Nanotechnology has become at the forefront of the most important fields in physics, chemistry, biology, engineering and many other fields. This technology has given great hope for scientific revolutions in the near future, and this technology will change many applications, as nanotechnology provides the means by which risks can be seen before To become fatal, it also enables the treatment of identified environmental risks in a way that reduces and addresses the effects of pollution (**Ghayada, 2016; Sefat, 2009**) has defined the importance of nanotechnology as a modern technology that is not expensive, in comparison with the technologies currently used, and its economic returns are very high. It also works and specializes in science and technology to move towards scientific applications, as its work begins from the basic components of matter (atoms and molecules), which makes its impact great, and includes all fields of science and technology. Bacteria resist antibiotics has also

become a serious global problem (**De Gregorio, *et al*., 2020**). The ability of microorganisms to generate resistance competes with the generation of new and effective antibiotics. In addition, current antimicrobial treatments have major drawbacks, such as their limited diversity and effects Incomplete antibiotic treatment leads to the acquisition of resistance to microorganisms, and the development of multi-resistant bacteria has outpaced the rate of development of new antibiotics; therefore, the generation of new and effective antimicrobial therapies is crucial (**Kung, *et al*. , 2020**), and for this reason, it has become increasingly important to develop new and powerful therapeutic approaches to eliminate and control resistant pathogens. An alternative to this global problem is the use of nanomaterials with antimicrobial properties. In this case, nanotechnology has emerged and taken the lead in designing nanomaterials using plant extracts to address the problem of control Antibiotics. Food wastes are end products of food processing industries that have not been recycled or used for other purposes, so they are disposed of as waste. These wastes can be considered valuable by-products, where there are appropriate technological means (**Ezzat, *et al*., 2021**). Fruit and vegetable peels are among the By-products, which accumulate in large quantities in the processing of various fruits and vegetables, and peel manufacturing operations represent a serious problem that have a harmful impact on the environment. They are an important part of a good diet and contain large quantities of life-active compounds (**Khamis, 2022**). There are huge amounts of food waste all over the world, from the manufacture of fruits, vegetables, oils, and fermentation, as waste is generated in the various stages of food processing, before and after harvest, and from food manufacturing, packaging and retail, and the availability of food waste reprocessing and the possibility of recycling this. By-products and benefiting from them instead of disposing of them, which causes harmful environmental pollution (**Ezzat, *et al*., 2021**) Fruit and vegetable wastes and by-products obtained from the food industry consist of seeds, peels, and pomace, and the effective use of these components can lead to the extraction of many bioactive compounds including essential components such as sugars, proteins, dietary fibers, etc. This, and other secondary components such as antioxidants, antimicrobials, and dyes, including natural dyes because they are natural (plant-based) and safe, can often find potential applications as well as can enhance the overall value of manufactured food commodities (**Sharma *et al*., 2021**). It is certain that consumption of Pumpkin produces large quantities of by-products such as peels and seeds. The by-products resulting from the separation of the edible portion of the Pumpkin constitute approximately 25%, which largely consists of peels (2.6-16%) and seeds (3.1 - 4.4%) (**Lovatto, *et al*,2020**), that Pumpkin contains antimicrobial compounds, as it was found that a concentration (2%) of Pumpkin extract inhibits the growth of (*Aeromonas veronii,* Candida, *Enterocicus feacalis, Escherichia coli, Salmonella entenica, and Staphylicoccus aureus*), and in addition to that, it has antifungal properties. It can inhibit pathogenic fungi, and also has antifungal properties, without toxicity to human red blood cells (**Syed, *et al*., 2019**). (**Mazzal, *et al*., 2022**) indicated the possibility of benefiting from pumpkin by-products in the food industry, as they made biscuits. Healthy from sprouted Pumpkin seeds. (**Ahmed, 2022**) also prepared nanoparticles of aqueous extract of pumpkin and iron salts to treat burns and wounds, and the prepared nanoparticles showed high effectiveness in inhibiting

all types of microorganisms. Therefore, the current study aimed to estimate the inhibitory effectiveness of methanolic and nano-extracts of Pumpkin peels prepared in our previous study on some microorganisms.

MATERIAIS AND METHODS

- **Bacterial isolates:** Four strains of each isolate of *Pseudomonas aeruginosa, Salmonella Typhi, Staphylococcus aureus, and Escherichia coli* from different sourees soil, contaminated food pieces and chicken) were obtained from the College of Agricultural Engineering Sciences - University of Baghdad, and the diagnosis was confirmed using the VITEK-2 system. The isolates were activated by re-culture on the nutrient medium. The plates were incubated at 37°C for 24 hours.

- **Collect Pumpkin fruits:** Pumpkin fruits were obtained from local markets Iraq and the plant was classified as Cucurbita pepo L.)) in the herbarium of the Department of Life Sciences Biologe/College of Science, University of Baghdad. The fruits are washed with water, the peels, dried at room temperature and ground using a home grinder, then stored in containers at 4°C until use. In a previous study, the methanolic extract of raw Pumpkin peels and the nano-extract of Pumpkin peels loaded with nano chitosan were prepared using the ionic gel method **Othman *et al.*, (2018)**. The prepared nanocomposites were described and characterized using techniques represented by ultraviolet (UV) spectroscopy and scanning electron microscopy (SEM), in addition to X-ray scattering (XRD) and infrared spectroscopy (FTIR) techniques.

The antibacterial activity of methanolic extract of Pumpkin peels and Cu/Cs-NPs

Disc Diffusion Method (DDM)

The method described by **Razmavar *et al.*, (2014**) was adopted to evaluate the antibacterial activity of the methanolic extract of Pumpkin peels, as well as of the Cu/Cs-NPs, individually, as the bacterial culture that was modified according to the McCafferland standard No. 0.5 was used to inoculate dishes containing Mueller's medium. The plates were dried for 15 minutes in a laboratory atmosphere. Sterile discs with a diameter of 6 mm were placed in 20 microliters of a buffer solution of the methanolic extract of Pumpkin peels, at a concentration of 100 mg/ml and another concentration of 200 mg/ml. Another set of these discs was also placed in 20 microliters of the Cu/Cs-NPs at concentrations of 16 and 32 mg/ml. the discs were also placed in 20 microliters of distilled water and DMSO to be used as negative control elements or as comparison elements. All discs were completely dried before placing them on the surface of Mueller-Hinton agar. The plates were then incubated at 37°C for 18 to 24 hours. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone around The discs, three discs for each concentration.

Determination of the Minimum Inhibitory Concentration (MIC) of the methanolic extract of Pumpkin peels and the Cu/Cs-NPs.

The liquid nutrient medium microdilution method was used to determine the minimum inhibitory concentration (MIC) of the methanolic extract of Pumpkin peels as well as the Cu/Cs-NPs using a 96-hole µl plate. The extract is prepared at a concentration of 256 mg/ml

and added to the broth. Two-fold serial dilutions of the extract are made directly on the plate to make concentrations (1, 2, 4, 8, 16, 32, 64, 128) mg/ml, and the concentration was also prepared. 32 mg/ml for the Cu/Cs-NPs and added to the broth. Two-fold serial dilutions of the Cu/Cs-NPs were prepared directly on the plate to make concentrations (0.125, 0.25, 0.5, 1, 2, 4, 8, 16) mg/ml. As well as a column containing 200 µL of liquid nutrient medium (negative control) that served as the comparison. 100 microliters of 1 × 108 CFU/ml bacterial inoculum was transferred to all pits except the negative control or control pits (positive control). The µl plates were incubated at 37°C for 18-20 hours, then 20 microliters of resazurin dye were added to all the pits and incubated for 30 minutes to observe the changes in color. Minimum inhibitory concentrations were then determined visually in a µl plate in which the color did not change from blue to pink (**Ohikhena et al.,2017**).

Study of the antifungal activity of methanolic extract of Pumpkin peels and Cu/Cs-NPs

The inhibitory activity of the methanolic extract of Pumpkin peels and the Cu/Cs-NPs was tested on two fungal isolates, including fungi isolated from soybean plants and other fungi identified by the Prevention Department/ Agricultural Research Department, by dissolving 0.25 mg/ml. of the methanolic extract of Pumpkin peels, and 0.25 mg/ml of Cu/Cs-NPs individually in 250 ml of sterilized PDA before solidifying in glass bottles, and after shaking well they were poured into sterile Petri dishes with a diameter of 5 cm, and after Hardening the medium: A disc was taken from the edge of the week-old fungal colonies using a cork borer with a diameter of 0.5 cm and placed in the center of the dish in sterile conditions. Then the dishes were incubated upside down at a temperature of 28°C for a week. The results were taken to calculate the average measurement of two perpendicular diameters for each fungal colony and were repeated for each of the methanolic extract of Pumpkin peels, the Cu/Cs-NPs and for each fungal isolate, as well as for a dish containing only PDA medium as a control sample. The results were also taken by daily photography. For Petri dishes, for a week, then the percentage of inhibition was taken (**Mostahar, *et al*.,2007**).

Statistical Analysis

The statistical program (**SAS, 2018**) was used to analyze the data to study the effect of different parameters on the studied traits according to a completely randomized design (CRD), and the significant differences between the means were compared using the least significant difference (LSD) test under the 0.05 level of significance.

RESULTS AND DISCUSSION

Bacterial isolates: Sixteen stains of four isolates from different sources were obtained from the College of Agricultural Engineering Sciences - University of Baghdad. The diagnosis was confirmed using the VITEK-2 system, where the results showed that all the samples obtained were *Pseudomonas aeruginosa p. aeruginosa, Salmonella enterica S. Typhi, Staphylococcus aureus S. aureus, E. coli*.

- **Antibacterial activity of methanolic extract of Pumpkin peels and Cu/Cs-NPs**

Disk propagation method: The antibacterial activity of both the methanolic extract of Pumpkin peels and the Cu/Cs-NPs was evaluated using the disk diffusion method on bacterial isolates (*Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Escherichia coli*). The bacterial isolates used in the study are sensitive to both the methanolic extract of Pumpkin peels and the Cu/Cs-NPs, as the zone of inhibition was measured to be more than 8 mm, according to (**CLSI, 2021**). The results showed that the Cu/Cs-NPs was more effective than the methanolic extract of Pumpkin peels on all types of bacteria used in the study, despite the use of the plant extract at higher concentrations, which were 100 and 200 mg/ml. As for the nanocomposite, it was used at lower concentrations. 16 and 32 mg/ml. The Cu/Cs-NPs gave the highest inhibition areas of (20.33, 17.33, 12.67, 16.33) mm at a concentration of 32 mg/ml for each of the bacteria *Staphylococcus aureus, Salmonella typhi , Pseudomonas aeruginosa, and E.coli,* respectively, with a significant difference between the concentrations and types of bacteria used in this study are at the probability level (P≤0.05), while there are no statistically significant differences between isolates at the same concentration, as shown in Table (1). While the methanolic extract of Pumpkin peels gave the highest inhibition zones of (19.67, 16.67, 10.13, 15.67) mm at a concentration of 200 mg/ml for each of the bacteria Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa and Escherichia coli, respectively, with a significant difference between the concentrations and types. The bacteria used in this study are at the probability level (P≤0.05), while there are no statistically significant differences between the isolates at the same concentration, as shown in Table (2).

Table (1): Effect of Cu/Cs-NPs on the growth of bacterial isolates.

LSD value	E. Coli		P. aeruginosa		S. Typhi		Staph. Aureus		Isolation number
	32	16	32	16	32	16	32	16	
*0.865	16.33±0.58	12.00±1.00	11.67±0.58	8.00±0.00	16.67±0.58	12.67±0.58	19.33±0.58	14.33±0.58	1
0.790*	15.67±0.58	11.67±0.58	11.00±0.00	8.67±0.58	17.00±1.00	13.33±0.58	20.33±0.58	15.00±0.00	2
*2.620	15.00±0.00	11.33±0.58	12.67±0.58	9.33±0.58	17.33±0.58	13.67±0.58	20.00±1.00	15.33±0.58	3
*0.790	16.00±0.00	12.67±0.58	11.00±0.00	8.67±0.58	16.67±0.58	12.00±0.00	19.33±0.58	14.33±0.58	4
*** (P≤0.05)** ***The diameter of the inhibition zone is measured in millimeters, *The concentration of the extract is measured in (milligrams/ml)**									

Table (2): Effect of methanolic extract of squash peels on the growth of bacterial isolates.

LSD value	E Coli		P. aeruginosa		S. Typhi		Staph. aureus		Isolation number
	200	100	200	100	200	100	200	100	
*0.999	15.67±0.58	12.33±0.58	9.33±0.58	8.68±0.58	16.67±0.58	12.33±0.58	18.33±0.58	12.67±0.58	1
*0.790	13.67±0.58	11.00±1.00	9.00±0.00	8.68±0.58	14.67±0.58	11.00±0.00	19.33±0.58	11.67±0.58	2
0.999*	14.33±0.58	11.67±0.58	10.33±0.58	9.00±1.00	14.67±0.57	11.33±0.58	19.67±0.58	12.33±0.58	3
0.935*	15.33±0.58	12.33±0.58	9.67±0.58	8.00±0.00	16.67±0.58	12.00±0.00	18.67±0.58	12.33±0.58	4
*** (P≤0.05)** ***The diameter of the inhibition zone is measured in millimeters, *The concentration of the extract is measured in (milligrams/ml)**									

The Cu/Cs-NPs had high activity against all bacterial isolates used in this study. Because of its size, it can easily reach the nuclear content of bacteria and exhibit a large surface area and thus be in contact with bacteria to a greater degree (**Khan, *et al*.,2019**). The presence of electrostatic force between the Cu/Cs-NPs and the bacterial cell promotes tighter contact with the charged molecules, allowing the Cu/Cs-NPs to pass through the cell wall. Moreover, Cu/Cs-NPs can alter the electron transport chain of bacteria (**Ivask, *et al*, 2014**). The mechanisms of action of Cu/Cs-NPs against bacterial isolates depend on their size, shape, surface charge, solubility, exposure time, and concentration (**Espinosa, *et al*., 2020**). (**Kong, *et al*., 2010**) found that nano chitosan has the strongest antibacterial activity due to their unique properties, such as their large surface area and affinity for bacterial cells, which results in a positive effect by killing bacterial cells. As particle size decreases to the nanoscale range, the specific surface area of nanoparticles increases, allowing greater interaction of the material with the surrounding environment such as the cell membrane of target pathogenic bacteria. A study by (**Perera, *et al*., 2020**) indicates that the degree of effectiveness of nanomaterials depends on size. As demonstrated (**Zhang, *et al*., 2020**), essential oils were successfully loaded into nano chitosan, thereby increasing their antibacterial activity to a greater degree. In addition, (**Hipalaswins, *et al*., 2016**) revealed that nano chitosan, which were manufactured by ionic gelation method, possess antibiotic activity against gram-negative bacteria. In addition, (**Zaki &Hafez, 2012**) reported that the use of nano chitosan leads to enhanced absorption of antibiotics by cells infected with bacteria or to increase the activity of antibiotics against multidrug-resistant bacteria. Through this, it was concluded that the nano chitosan used in the current study, which were manufactured by loading the methanolic extract of Pumpkin peels, increased the antibacterial activity of the methanolic extract compounds loaded on them. Cell attachment is the initial stage in biofilm formation after the formation of the membrane consisting of nutrients and organic and inorganic molecules absorbed on the surface (surface conditioning). Surface conditioning is vital for cell growth and often creates a favorable environment for bacterial growth, and enhances cell adhesion to surfaces, leading to infection (**Gorniak, *et al*., 2019**). Therefore, it can be assumed that the presence of plant extract with nano chitosan and the formation of Cu/Cs-NPs in the growth medium led to an unfavorable condition that could prevent cell attachment or reduce surface adhesion.These results also showed that different concentrations of the Cu/Cs-NPs have antibacterial activity on the studied bacterial isolates. These results were agreed upon with (**Kralova& Jampilek, 2022**) who showed that the Cs-NPs chitosan nanocomposite has a broad spectrum of antimicrobial activity against diseases. Various bacteria. (**Kadhum& Zaidan, 2020**) also reported this, as they found that the chitosan nanocomposite is effective in treating intestinal infections. These results also agreed with (**Hassan & Awad, 2023**) when they found it Schiff's chitosan/ capsaicin Inhibitory effectiveness against bacteria Gram positive and Gram negative. It also agreed with (**Almalah, *et al*, 2019**), who reported that nanocomposites are effective in inhibiting both Gram-positive and Gram-negative bacteria, giving an inhibition zone of 25 mm against S. aureus, 24 mm against K. pneumonia, and 22 mm against P. aeruginosa and A. baumanni, respectively, as observed (**Ansari& Alzhairy, 2018**), that the antibacterial activities

increased with increasing concentration of the nanocomposite, and it was found that the zones of inhibition were larger at higher concentrations (500 mg/ml) of the nanocomposite, while the zone of inhibition was smaller at lower concentrations. (**al-Tameemi , *et al*., 2023**) also reported that zinc nanoparticles showed a strong inhibitory effect against methicillin-resistant Staphylococcus aureus, as the minimum inhibitory concentration of the nanocomposite reached 0.625 mg/ml, while the minimum inhibitory concentration of the biocomposite Zno-NPs reached 1.25 mg/ml. (**Al-hafud ,2017**) reported that adding the alcoholic extract of male gum led to a reduction in the total number of aerobic bacteria, coliform bacteria, and Staphylococcus aureus bacteria. . As for (**AL-Gbouri & Hamzah, 2018**), they indicated the effectiveness of amla extract as an antibacterial, as the extract showed inhibitory activity at concentrations ranging from 20 mg/ml to 5 mg/ml for both Staphylococcus bacteria and coli bacilli, and at concentrations of 20 and 10 mg/ml for Pseudomonas aeruginosa bacteria. and he mentioned in (**Samah &AL-Janabi, 2023**) that the effectiveness of the aqueous extract of rice bran against the isolates Staphylococcus aureus, Salmonella Typhi, Pseudomonas aeruginosa, and Escherichia coli diameter rate 10.5, 7, 8.5 and 8 mm, respectively, after incubation at 37°C for 24 hours.

- Determination of the minimum inhibitory concentration (MIC) for both the methanolic extract of Pumpkin peels and the Cu/Cs-NPs

The lowest concentration of an antimicrobial drug that will inhibit the growth of bacterial isolates. The visible growth of microorganisms after an incubation period is known to determine the minimum inhibitory concentration (MIC). Clinical laboratories have used MICs primarily to confirm resistance, however they are also used as a research tool to determine the activity of new agents. antimicrobial properties as well as their MIC breakpoints (**Kowalska-Krochmal& Dudek-Wicher, 2021**). the color of the resazurin dye, which is an indicator of bacterial growth. Oxidoreductase enzymes in the cells reduce the non-fluorescent, non-toxic dye resazurin to resorfurin, which is pink in color. Resorufin upon further reduction becomes hydroresorufin and is colorless (**Elshikh, *et al*., 2016**). The MIC result showed that the Cu/Cs-NPs was more effective than the methanolic extract of Pumpkin peels. The results showed that the MIC values for the Cu/Cs-NPs on all strains of *Escherichia coli* and *Pseudomonas* aeruginosa reached 0.5 and 1 mg/ml, respectively, Figure (1), and 2 mg/ml on all isolates of *Salmonella* intestinalis bacteria, and 0.5 mg/ml. ml for strains 1 and 4, and 0.25 mg/ml for strain 2 and 3 for *Staphylococcus* aureus bacteria, Figure (2), and as shown in Table (3). While the MIC values for the methanolic extract of Pumpkin peels reached 8 mg/ml for all *Escherichia coli,* except for strains No. 2, which had a minimum inhibitory concentration (MIC) of 16 mg/ml, and 64 mg/ml for all Pseudomonas aeruginosa strains. (3), and the MIC values for the methanolic extract of Pumpkin peels also reached 8 and 4 mg/ml for all strains of Salmonella intestinalis and *Staphylococcus* aureus, respectively, in Figure (4), and as shown in Table (4).

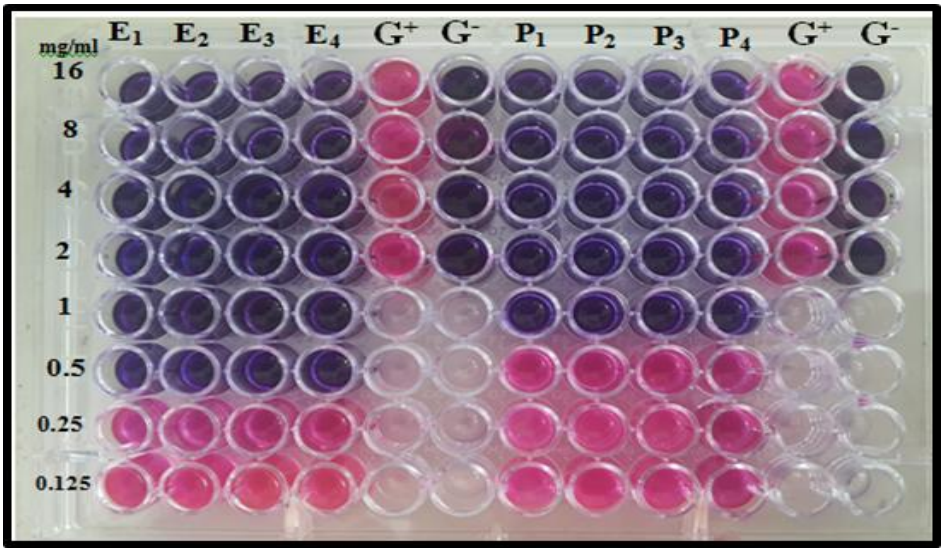

Figure (1): Minimum inhibitory concentration (MIC) of Cu/Cs-NPs nanocomposite on the growth of *E. coli and P. aeruginosa.*

(E): *E. coli isolate*, (P): *P. aeruginosa* isolate, (G^+): Control positive (Bacteria + Media) (G^-): Control negative (Media only)

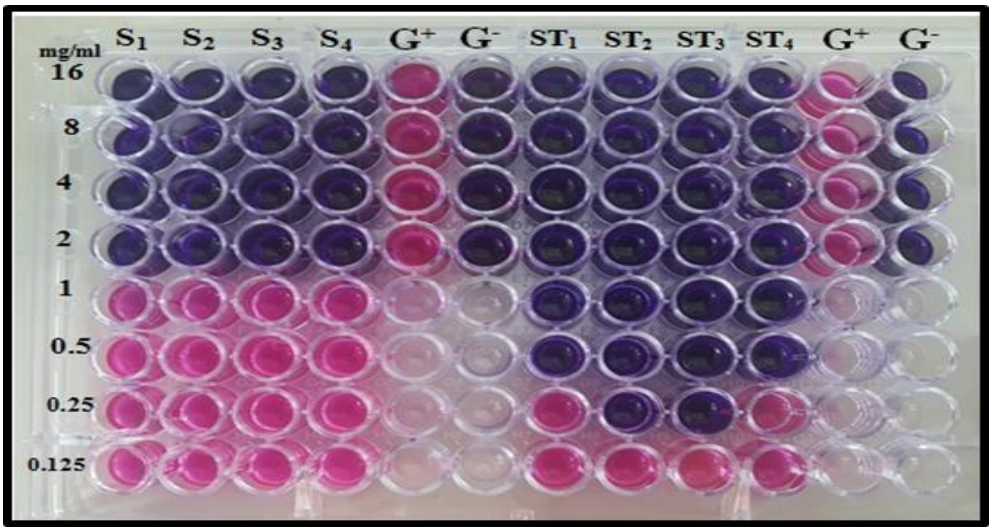

Figure (2): Minimum inhibitory concentration (MIC) of Cu/Cs-NPs nanocomposite on the growth of Salmonella Typhi and Staph bacteria. Aureus.

(S): S. aureus isolate, (ST): *S. Typhi* isolate, (G^+): Control positive (Bacteria + Media) (G^-): Control negative (Media only)

Table (3): Minimum inhibitory concentration (MIC) of Cu/Cs-NPs.

Cu/Cs-NPs (mg/Ml)				Isolation number
E Coli	**P. aeruginosa**	**Salmonella Typhi**	**Staph. Aureus**	
0.5	1	2	0.5	1
0.5	1	2	0.25	2
0.5	1	2	0.25	3
0.5	1	2	0.5	4

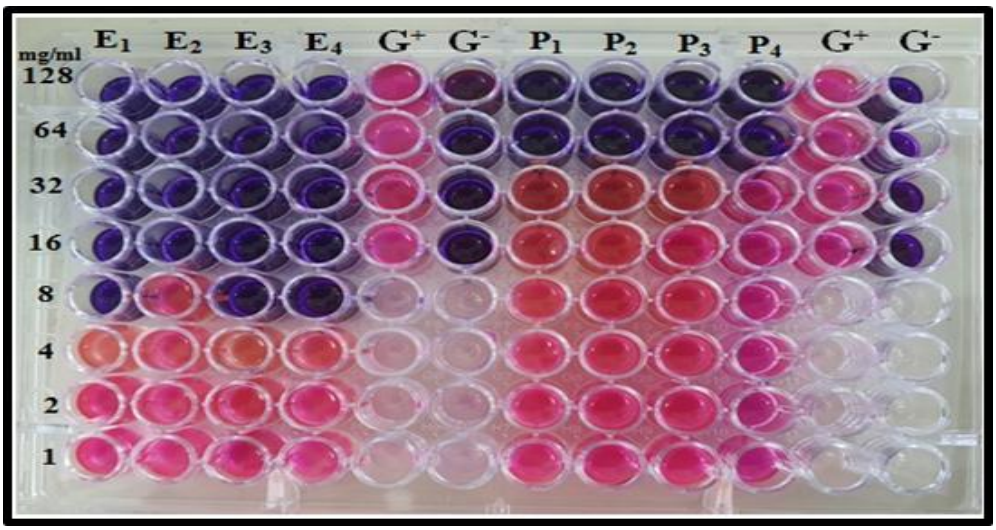

Figure (3): Minimum inhibitory concentration (MIC) of methanolic extract of Pumpkin peels on growth of E. coli and P. aeruginosa.

(S): S. aureus isolate, (ST): *S. Typhi* isolate, (G^+): Control positive (Bacteria + --+3Media) (G^-): Control negative (Media only)

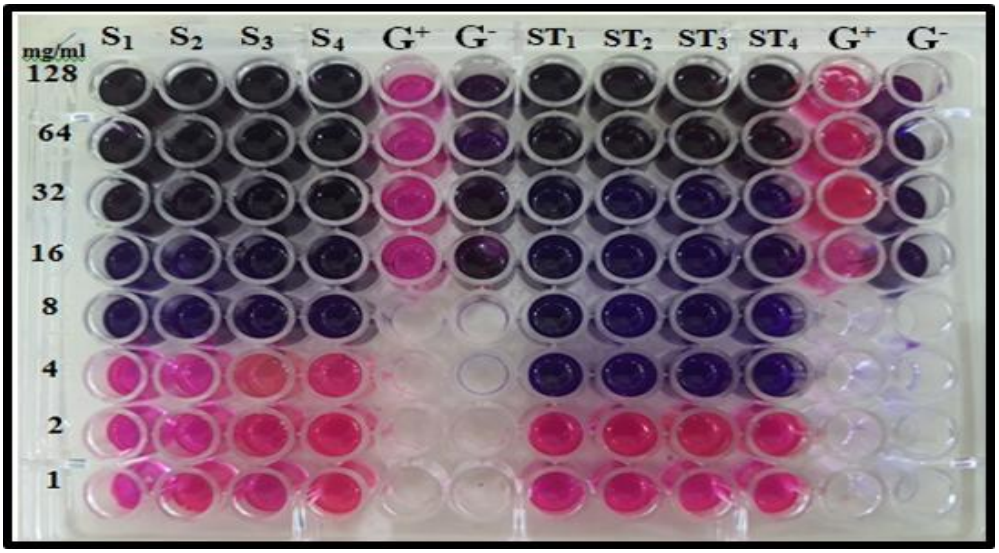

Figure (4): Minimum inhibitory concentration (MIC) of methanolic extract of Pumpkin peels on the growth of Salmonella Typhi and Staph bacteria. Aureus.

(E): *E. coli* isolate, (P): P. aeruginosa isolate, (G^+): Control positive (Bacteria + Media) (G^-): Control negative (Media only)

Table (4): Minimum inhibitory concentration (MIC) of methanolic extract of Pumpkin peels.

Methanolic extract (mg/mL)				Isolation number
E Coli	**P. aeruginosa**	**Salmonella Typhi**	**Staph. Aureus**	
8	64	8	4	1
16	64	8	4	2
8	64	8	4	3
8	64	8	4	4

The results of this study indicated that the nanoparticles had a higher biological activity against the bacterial isolates used in this study than the methanolic extract of Pumpkin peels. This may be due to the fact that the Cu/Cs-NPs has a large surface area and therefore contains quantitative and qualitative compounds. More than the methanolic extract of Pumpkin peels, these compounds can contribute to various antimicrobial activities. The importance of nanotechnology in the field of drug delivery lies in increasing the therapeutic effectiveness of drugs by increasing a small area with a small molecular size that enables the drugs to reach the targeted areas more effectively and in a small quantity, which reduces the side effects that can be caused by chemical drugs in addition to being Economic importance (**Chenthamara, *et al*.,2019**). The role of plant extracts loaded with nanoparticles is that the plant compounds act as reducing and stabilizing agents, for example, flavonoid chemicals act as reducing agents, while amino acids act as stabilizing agents (**Javed, *et al*.,2020**). These properties lead to Increase activity of nanoparticles, so the Cu/Cs-NPs was much better than the methanolic

extract of Pumpkin peels, leading to greater inhibition of bacterial isolates. (**Gorniak, *et al*.,2019**) also found that flavonoids should exist as effective antimicrobial agents against a wide range of pathogenic microorganisms in vitro. Therefore, the compounds present in the plant extract and loaded onto nano chitosan can exert antibacterial activities through multiple mechanisms, including disruption of the cytoplasmic membrane, inhibition of DNA synthesis, inhibition of energy metabolism, inhibition of folic acid synthesis, and inhibition of cell membrane function in addition to Anti-virulence mechanisms (**Al-Kamel & Al-Snafi, 2019**) It was reported (**Mustafa & Ogaidi, 2023**) that the minimum inhibitory concentration of ZnS-chitosan NPs against pathogenic bacteria is 100 µg/ml for Staphylococcus aureus and Acinetobacter baumannii, while 50 mg/ml for *Psedomonas aeruginosa*. (**ALbandary, 2023**) reported that the minimum inhibitory concentration of the methanolic extract of red onion peels against the bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *coli bacilli* is 200 micrograms/ml.

Antifungal activity of methanolic extract of Pumpkin peels and Cu/Cs-NPs

The results showed that the inhibitory effectiveness of the treatments against the tested fungi depended on the type of treatment (methanolic extract, Cu/Cs-NPs) and its concentration, as well as the type of fungal isolate. The Cu/Cs-NPs showed high inhibitory effectiveness, followed by the methanolic extract of plant peels. Pumpkin, as the results of this study indicate the effect of the methanolic extract of the peels of the Pumpkin plant and the nanocomposite Cu/Cs-NPs at a concentration of (0.25) mg/ml in potato dextrose agar (PDA) medium on which the *Penicillium* and *Aspergillus* fungi grow, as it gave a concentration of 0.25 mg/ml of The methanolic extract of the Pumpkin plant peels for the first fungi had a percentage of the rate of the two perpendicular diameters of 10%, and the percentage increased to 63% for the Cu/Cs-NPs , and when balanced with the percentage of inhibition for the second fungi, the same concentration recorded a percentage of 24% for the methanolic extract of the Pumpkin plant peels, and it increased The percentage also reached 64% for the Cu/Cs-NPs , balanced with the percentage of the two perpendicular diameters for the control treatment, which reached 80.6% and 90%, respectively, as shown in Tables (5 and 6) and Figures (5 and 6).

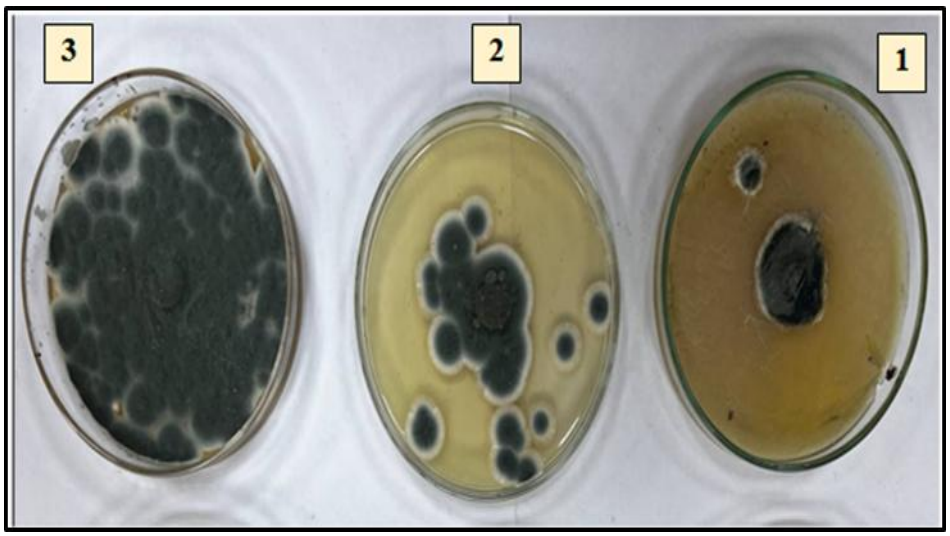

Figure (5): Effect of Pumpkin peel extracts on *Penicillium spp*.

(1): Nano extract, (2): Alcoholic extract, (3): Contro

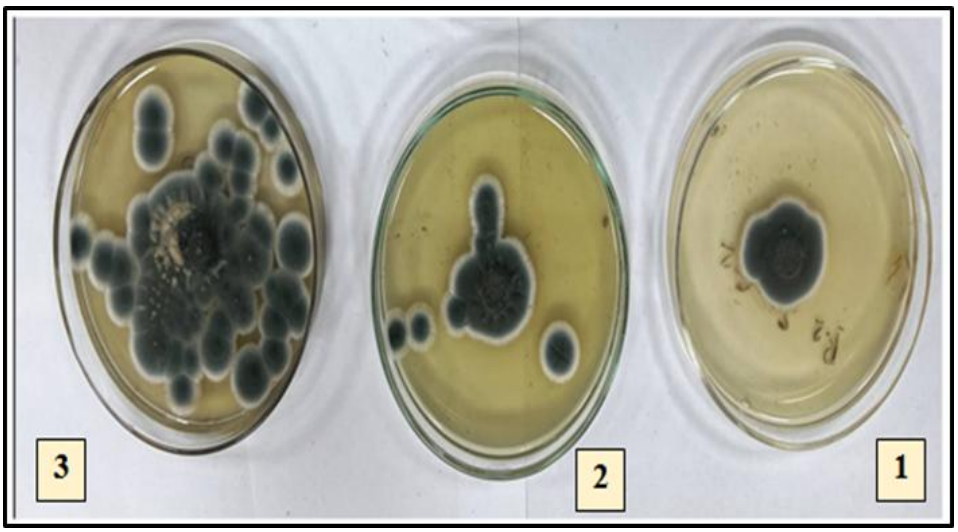

Figure (6): Effect of Pumpkin peel extracts on *Aspergillus spp*.

(1): Nano extract, (2): Alcoholic extract, (3): Control

Table (5): Effect of methanolic extract of Pumpkin peels on *Aspergillus spp* and *Penicillium spp* fungi.

Growth percentage	control	Percentage of inhibition	Methanolic extract	Fungi type
90 %	10	% 24	7.2	**Aspergillus spp**
	8		8.3	
	9		7.8	
80.6 %	8	10.4 %	3.2	**Penicillium spp**
	9		3.1	
	9		3.4	

Table (6): Effect of Cu/Cs-NPs on *Aspergillus spp* and *Penicillium spp* fungi.

Growth percentage	control	Percentage of inhibition	Cu/Cs-NPs	Fungi type
90 %	10	64 %	5.5	**Aspergillus spp**
	8		4.8	
	9		5.2	
80.6 %	8	63 %	3	**Penicillium spp**
	9		3.2	
	9		3.2	

Some studies have indicated that the active substances extracted from plants give better results than the same substance manufactured by chemical methods that are accompanied by toxic side effects, which indicates the possibility of the active substances in the secondary compounds contributing to enhancing the effective role of the plant (**Negeba, *et al.*, 2018**). Nano chitosan also have a high potential. It inhibits fungal growth due to its small size and its spread in the cell membrane of microorganisms (**Rozman, *et al.*, 2019**) A study conducted by (**Phongpaichit, *et al.*, 2004**), in Thailand showed that the alcoholic extract of acacia leaves showed inhibitory activity against the growth and spore formation of both Penicillium fungi and Candida albicans yeast. Also, the mechanism by which nanoparticles interact with microorganisms is that these organisms carry negative charges, while nanocomposites carry positive charges, which creates an electromagnetic attraction between the cell and the medium of the particles. The particles release ions that interact with the thiol group of proteins that transport nutrients that appear from the membrane. cell, which reduces the permeability of the membrane and thus cell death. Various factors have been proposed responsible for the antifungal activity of the nanocomposite, including the formation of hydrogen peroxide H2O2 on the surface of the nanocomposite due to the possibility of forming a hydrogen bond between the hydroxyl group of the cellulose molecules from the fungi with the oxygen atom from The nanocomposite leads to inhibit fungal growth, or the nanocomposite may cause damage to the cell membrane during its interaction with the contents of the fungal cell, which causes inhibition of DNA replication and then leads to the destruction of proteins and enzymes as well as inhibition of cell division (**Matai, *et al.*, 2014**) All of these factors lead to rupture of the fungal cell membrane, causing leakage of fungal cell contents, leading to a shrinkage in the size of the plasma membrane and cell death. These results are consistent with (**Hassan, *et al.*,**

2013), who indicated the effect of nanocomposites on fungi treated at 8 mg/ml, which It caused damage to the cell membrane of the conidia of the Aspergillus spp fungus, forming holes in the cell wall and creating some voids between the cells, which led to the leakage of cell components and ultimately its death.

CONCLUSION

According to the results of the current study, it can be concluded that the prepared Cu/Cs-NPs has an antibacterial effect against Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli in addition to Salmonella intestinalis. It also has an antifungal effect against Penicillium and Aspergillus fungi, as these were found. The study showed that the inhibitory effectiveness of the methanolic extract increased by loading it with nano chitosan.

REFRENCES

1. Ahmed, S. H., (2022). Synthesis of novel Iron Nanoparticles using the aqueous extract of the Pumpkin plant and using it in the treatment of burns. *Egyptian Journal of Chemistry*, 65 (3), 353 – 362.
2. AL- Hafud, A. S. (2017). Effect of the activity of Boswellia Carterii extracts on preservation of ground meat. *Journal of College of Education for Women*, 28(4).
3. Albandary, N. A. (2023). *Phenolic* compounds content, antioxidant, antibacterial and antifungal activities of red onions skin. *Iraqi Journal of Agricultural Sciences*, 54(4), 1050-1057
4. Al-Gbouri, N. M., & Hamzah, A. M. (2018). Evaluation of phyllanthus emblica extract as antibacterial and antibiofilm against biofilm formation bacteria. *Iraqi Journal of Agricultural Sciences*, 49(1).
5. Al-Kamel, M. L. & Al-Snafi, A. E. (2019). Antibacterial effect of the phenolic extract of Alhagi maurorum. *IOSR Journal of Pharmacy*, 9 (9): 47-55
6. Almalah, H. I.; Alzahrani, H. A. & Abdelkader, H. S. (2019). Green Synthesis of Silver Nanoparticles using Cinnamomum Zylinicum and their Synergistic Effect against Multi-Drug Resistance Bacteria. *Journal of Nanotechnology Research*. 1(3): 95-107.
7. Al-Tameemi, A. I., Masarudin, M. J., Rahim, R. A., Timms, V., Neilan, B., & Isa, N. M. (2023). Antibacterial properties of zinc oxide nanoparticles synthesized by the supernatant of weissella confusa upm22mt04. *Iraqi Journal of Agricultural Sciences*, 54(5), 1209-1222
8. Ansari, M. A. & Alzohairy, M. A. (2018). One-pot facile green synthesis of silver nanoparticles using seed extract of Phoenix dactylifera and their bactericidal potential against MRSA. *Evidence Based Complementary and Alternative Medicine*,
9. Chenthamara, D, S, Subramaniam, S. G, Ramakrishnan, S; Krishnaswamy, M. M, Essa, F. H; Lin, & Qoronfleh, M. W. (2019). Therapeutic efficacy of nanoparticles and routes of administration. *Biomaterials Research*, 23(1), 1–29.

10. Clinical Laboratory Standards Institute (CLSI). (2021). *Performance Standards for Antimicrobial Susceptibility Testing*. (31st ed.). CLSI supplement M100.Wayne, PA: Clinical and Laboratory Standards Institute.
11. De Gregorio, E., Esposito, A., Vollaro, A., De Fenza, M., D'Alonzo, D., Migliaccio, A. & Guaragna, A. (2020). N-Nonyloxypentyl-l-Deoxynojirimycin inhibits growth, biofilm *formation and virulence factors expression of Staphylococcus aureus. Antibiotics*. 9(6), 362.
12. Elshikh, M., Ahmed, S., Funston, S., Dunlop, P., McGaw, M. & Marchant, et al R. (2016). Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotechnology Letters., 38:1015-1019
13. Espinosa, J. C. M., Cerritos, R. C., Morales, M. A. R., Guerrero, K. P. S., Contreras, R. A. S. & Crystals, J. M. (2020). *Characterization of silver nanoparticles obtained by a green route and their evaluation in the bacterium of Pseudomonas aeruginosa*. Crystals, 10(5): 13
14. Ezzat, S., M, A., R., & Abdel-Sattar, E., (2021). *Pumpkin Bio-Wastes as Source of Functional Ingredients*, Department of Pharmacognosy, Faculty of Pharmacy, October University for *Modern Sciences and Arts*, Cairo, Egypt, (667-696).
15. Ghayada, H. N. S., (2016). *Nanotechnology requirements included in secondary school chemistry textbooks and the extent to which eleventh grade students acquire them*. MSc.Theses in, College of Education, Islamic University, Gaza
16. Gorniak, I.; Bartoszewski, R. & J. Kroliczewski, (2019). *Comprehensive review of antimicrobial activities of plant flavonoids*. Phytochemistry, 18(1): 241-272
17. Hassan, H. S., & Awad, S. H. (2023). Synthesis And Characterization of New Schiff Bases For Chitosan And Study Their Antimicrobial Activity. *Iraqi Journal of Market Research and Consumer Protection*, 15(2), 192-206.
18. Hassan, A. A.; Howayda, M. E. & H. H. Mahmoud, (2013). Effect of Zinc Oxide Nanoparticles on the Growth of *Mycotoxigenic Mould*. SCPT 1(4)66–74
19. Hipalaswins, W. M, Balakumaran, M. D, & Jagadeeswari, S. (2016). Synthesis, Characterization, and Antibacterial Activity of *Nano chitosan and their Impact on Seed Germination*. J. Acad. Ind. Res, 5(5), 65
20. Ivask, A, Elbadawy, A, Kaweeteerawat, C, Boren, D, Fischer, H; Chang, Z, Ji, C. Liu, H; Tolaymat, R, Telesca, T; Zink, D Cohen, J. I; Holden,Y; P. A; & Godwin, H. A. (2014). Toxicity mechanisms in *Escherichia coli vary for silver nanoparticles and differ from ionic silver*. ACS Nano, 8(1), 374–386.
21. Jampilek, J, & Kralova, K. (2022). Advances in Nanostructures for Antimicrobial Therapy. Materials, 15(7), 2388.
22. Javed, R, Zia, M, Naz, S, Aisida, S. O, & Ao, Q. (2020). Role of capping agents in the application of nanoparticles in biomedicine and environmental remediation: recent trends and future prospects. *Journal of Nanobiotechnology*, 18(1), 1–15

23. Kadhum, W. N, & Zaidan, I. A. (2020). The Synergistic Effects of Chitosan-Alginate Nanoparticles Loaded with Doxycycline Antibiotic Against Multidrug Resistant Proteus *Mirabilis*, Escherichia Coli and Enterococcus Faecalis. 61(12), 3187–3199.
24. Khamis, Sh. F. W., (2022). Using fruit waste (mango and banana) to reinforce laboratory cake and studying its qualitative and physical properties. MSc Thesis, University of Baghdad, College of Education for Girls, university of Baghdad. Iraq.
25. Khan, I, Saeed; K, & Khan, I. (2019). Nanoparticles: Properties, applications and *toxicities*. Arabian *JournalofChemistry*, 12(7), 908– 931.
26. Kong, M; X. Chen, G; Xing,K; & Park, H. J. (2010). Antimicrobial properties of chitosan and mode of action: a state of the art review. International *Journal of Food Microbiology*, 144(1), 51–63.
27. Kowalska-Krochmal, B; & Dudek-Wicher,R. (2021). The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, *Clinical Relevance*. *Pathogens*, 10(2).
28. Kung, J. C., Wang, W. H., Lee, C. L., Hsieh, H. C. & Shih,C. J. (2020). Antibacterial activity of silver nanoparticles (AgNP) confined to mesostructured, silica-based calcium phosphate against methicillin-resistant staphylococcus aureus. *Nanomaterials*, 10(7), 1264.
29. Lovatto, N. D. A., Loureiro, B. B., Bender, A.B.B., Loureiro, C. B., Goulart, F. R., Speroni, C.F.; Macagnan, F.T.; Piana, M. & Silva, L. P. D. (2020). Phosphorylated protein concentrate pumpkin seed (Cucurbita moschata): optimization by response surface methodology and nutritional characterization. Food Technology, 50(2): 1678-4596
30. Matai, I., Sachdev, A., Dubey,P., Kumar,S. U., Bhushan, B. & Gopinath, P. (2014). Antibacterial activity and mechanism of Ag–ZnO nanocomposite on S. aureus and GFP-expressing antibiotic resistant *E. coli. Colloids and Surfaces B: Biointerfaces*, 115, 359-367
31. Mazzaal, A. M., Abas, F. H. & Mousa, M. A. (2022). Preparation and Evaluation of Healthy Biscuits Using Germinated Wheat Flour and Germinated Pumpkin Seed Flour, NeuroQuantology, Volume 20, Issue 4, Page 244-252
32. Mostahar, S., Alam, S. & Islam, A. (2007). Cytotoxic and antimicrobial activities of two new synthetic 2'-oxygenated flavones reported from Andrographis viscosula. Journal of the *Serbian Chemical Society*, 72(4), 321-329
33. Mustafa, H. N., & Al-Ogaidi, I. (2023). Efficacy of zinc sulfide-nano chitosan against bacterial diabetic wound infection. *Iraqi journal of agricultural sciences*, 54(1), 1-17.
34. Ngegba, P. M., & Kanneh, S. M.m & Bayon, M. S.m & Ndoko, E. J. & Musa, P. D. (2018). Fungicidal effect of three plants extracts in control of four phytopathogenic fungi of tomato (Lycopersicum esculentum L.) fruit rot. International Journal of *Environment*, Agriculture and Biotechnology, 3(1), 112-117
35. Ohikhena, F. U.; Wintola, O. A. & Afolayan, A. J. (2017). Evaluation of the Antibacterial and Antifungal Properties of Phragmanthera capitata (Sprengel) Balle (Loranthaceae), a Mistletoe Growing on Rubber Tree, Using the Dilution Techniques. *The Scientific World Journal, Article* 8.

36. Othman, N., Masarudin, M. J., Kuen, C. Y., Dasuan, N. A., & Abdullah, L. C. (2018). Synthesis and optimization of chitosan nanoparticles loaded with L-ascorbic acid and thymoquinone. Nanomaterials, 8(11), 920.
37. Perera, W. P. T. D., Dissanayake, R. K., Ranatunga, U. I., Hettiarachchi, N. M., Perera,K. D. C.; & Unagolla,J. M.. (2020). *Curcumin loaded zinc oxide nanoparticles for activity-enhanced antibacterial and anticancer applications*. RSC Advances, 10(51): 30785-30795
38. Phongpaichit, S., Pujenjob, N., Rukachaisirikul, V. & Ongsakul, M. (2004). Antifungal activity from *leaf extracts of Cassia alata L.*, Cassia fistula L. and Cassia tora L. Songklanakarin Journal Sci Technol, 26(5), 741-748
39. Razmavar, S., Mahmood, A. A., Salmah, B. I. & Pouya, H. (2014). Antibacterial Activity of Leaf Extracts of Baeckea frutescens against Methicillin-Resistant Staphylococcus aureus. *BioMedical Research International*, Article ID 521287, 5.
40. Rozman, N. A. S., Tong, W. Y., Leong, C. R., Tan, W. N., Hasanolbasori, M. A. & Abdullah, S. Z. (2019). *Potential antimicrobial applications of nano chitosan* (ChNP).
41. SAS. (2018). *Statistical Analysis System, User's Guide. Statistical*. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA
42. Samah, R. A. B., & AL-Janabi, N. M. (2023). Testing The Inhibitory Effect Of Tricin Against Some Foodborne Bacteria And Estimate Its Phenol Coefficient. *Iraqi Journal of Market Research and Consumer Protection*, 15(2), 83-93.
43. Sefat, S, (2009). *Nanotechnology (Introduction to understanding nanotechnology)*. Arab House of Science – Beirut
44. Sharma, M., Usmani, Z., Gupta, V. K., & Bhat, R. (2021). Valorization of fruits and vegetable wastes and by-products to produce natural pigments. Crit. Rev. Biotechnol.
45. Syed, Q. A., Akram, M. & Shukat, R. (2019). Nutritional and Therapeutic Importance of the Pumpkin Seeds. Biomedical Journal of Scientific and Techincal Research Volume 21-Issue 2: 2574 -1241
46. Zaki, N. M; & Hafez,M. M. (2012). Enhanced Antibacterial Effect of Ceftriaxone Sodium-Loaded Nano chitosan Against Intracellular Salmonella typhimurium. AAPS PharmSciTech, 13(2), 411–421.
47. Zhang, F, Ramachandran, G, Mothana, R. A, Noman, O. M; Alobaid, W. A; Rajivgandhi, G; & Manoharan, N. (2020*). Anti-bacterial activity of chitosan loaded plant essential oil against multi drug resistant K. pneumoniae. Saudi Journal of Biological Sciences*, 27(12), 3449– 3455.