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RESEARCH PAPER

Effect of *Cucurbita Pepo* Nanoshells on Some Antibiotic-Resistant Pathogenic Microorganisms

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ABSTRACT

The bio-based approach to synthesizing nanoparticles has been one of most interesting trends in nano science over the past decade. This is due to its environmentally friendly and green chemistry method, which is safe to use and free of chemical pollutants for biological applications. Study's results demonstrated the effectiveness of using zucchini peel nanoparticles in combination with silver nanoparticles as an alternative to inhibitory materials for drug-resistant microorganisms. The results of the FTIR analysis showed that $AgNO_3$ had four strong bands at (3211, 1632, 1520, 1390, and 1239) cm-1 while AgNPS had seven strong bands at (3211, 2940, 1632, 1523, 1395, 1241 and 1050) cm^{-1}. XRD analysis revealed four different diffraction peaks for $AgNO_3$ (32.77°, 38.11°, 49.66°and 67.54°), while AgNPS showed five strong diffraction peaks (32.77°, 56.13°, 58.22°, 67.54° and 75.88°). In terms of inhibiting *Pseudomonas aeruginosa*, AgNPS had the highest inhibition zone of 14 mm at a concentration of 10 mg/ml, the inhibition zone increased to 25 mm when the concentration was increased to 20 mg/ml. on the other hand $AgNO_3$ had 9 mm inhibition zone at a concentration of 10 mg/ml, which increased to 16 mm at concentration of 20 mg/ml. Nanotechnology is providing alternatives to treatments that have harmful effects on the body due to their imprecise delivery methods to targeted diseased cells, such as cancer chemotherapy. Researchers have utilized nanotechnology to attach special RNA with a small diameter of about 10 nanometers to nanoparticles filled with chemotherapy drugs to mitigate the pain and harmful effects associated with chemotherapy.

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INTRODUCTION

Plant Squash (*Cucurbita pepo)* is one of the most important vegetable crops in the *Cucurbitaceae* family, which includes many well-known plants used in food and medicine. This family also includes *Cucurbita maxima*, known locally as Anaki squash, *Cucumis melo*, *Cucumis sativus*, as well as wild species like *Citrullus*, *Bryonia multiflora*, wild gourd *Ecballium elaterium*, and other plants [1]. Squash is one of the most important and widely cultivated vegetable crops in Iraq, due to the high nutritional value of its fresh fruits, as well as the use of its seeds in nutrition. The seeds are characterized by their high content of oils 46%, proteins 34%, carbohydrates 10%, and fiber 2.8%. Additionally, squash used for medical purposes.

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Zucchini has many varieties both pure and hybrid, and each with its own advantage in terms of production quantity and quality. Most studies have focused on evaluating the fruits intended for fresh consumption. They are rich in niacin and have a medium content of riboflavin and ascorbic acid. The dry seeds of squash are considered one of the richest sources of protein and oils. They also contain a medium amount of mineral salts and small amounts of vitamins A, B, and C [2]. Multi-drug resistance (MDR) has become widespread among many bacterial species causing diseases worldwide. The excessive and repeated use of traditional antibiotics long with their development, has diminished their effectiveness in combating common infectious diseases. Consequently, new generations, strains and types of pathogenic bacteria that are resistant to existing antibiotics have emerged. This trend has raised concerns and posed a significant challenge in the healthcare sector [3, 4]. Multidrug-resistant organisms such as *Enterococcus* spp., *Staphylococcus aureus, Klebsiella pneumoniae*, *Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp. have recently been identified as ESKAPE pathogens. This term signifies their ability to evade the effects of antibacterial agents or the lack of new effective antibiotics. Antibiotic resistance is a complex issue that involves human behavior and various societal levels, impacting individuals worldwide [5]. Therefore, studies have been directed towards solving this problem by searching for nanomaterial's for certain metals and their oxides as a promising alternative for developing new, unconventional antimicrobial agents. These agents, called nanoantibiotics, have gained attention in recent years [6]. Nanoparticles of some materials posses unique physical and chemical properties compared to their larger counterparts, making them suitable for various applications across industries, ranging from electronics to medicine [7]. These antibiotics have clear advantages over conventional antibiotics. They have low toxicity, can overcome bacterial resistance, and are cost effective. Additionally, they work by mechanically disrupting the bacterial membrane through the bacterial defense system [8]. Many elements and metals have been deemed safe for microorganisms in their natural state. However, they have shown high toxicity at the nano level due to the increased specific surface area, the high reactivity of these particles, and their ability to overcome resistance development that interferes with multiple biological pathways [9]. Parts of plants and their alcoholic and aqueous extracts have been used as antidotes and treatments for bacterial infections in general. This is because they contain active compounds with therapeutic properties [10]. The study aimed to test the antagonistic effectiveness of microbial and nano zucchini peel powder on certain microorganisms that are important in the food industry. This microorganism play a crucial role in manufacturing processes and can also be present on food, causing damage. Finding alternatives to materials that inhibit microorganisms, especially those that have developed resistance due to the widespread use of antibiotics, is important.

MATERIALS AND METHODS

Sample collection

Cucurbita pepo was selected for the experiment and obtained from local markets in Baghdad city between February 2023 to April 2023. The squash was washed with tap water to remove suspended dust. The plant peel was then dried in sunlight, ground into a fine powder using an electric grinder, stored in an opaque box, wrapped in aluminum foil at the refrigerator temperature to prevent oxidation.

Preparation of Cucurbita pepo cortex

The cortex of *Cucurbita pepo L.* was collected from the region of Baghdad city The plants were washed multiple times in distilled water then dried and ground to form a powder. A plant extract was prepared by suspending 15 grams of the powder in 250 ml of solvent (water) and using ultra sonication for 1hour at 37ºC. The suspension was then shaken for 4 hours and finally filtered using a Whitman filter. Additionally, 15 grams of the powder in 250 ml of water was boiled for 30 minutes before completing the same steps as mentioned [11].

Preparation of Silver nanoparticles (AgNPS)

Mixing 200 mL of 1mM AgNPs solution with 200 mL of *Cucurbita pepo L.* extract for 8 hours at 60 ºC in the dark,with continuous magnetic stirring, will cause a precipitate to form and the color will change from yellow to black.The mixture is then placed at 37 ºC to reach room temperature. The *Cucurbita pepo L.* precipitate, AgNPs (1 mM) and the *Cucurbita pepo L.* extract without $AgNO_3$

served as controls. Throughout the incubation period, mixtures were tested for the presence of black color demonstrating that AgNPs were finally interacting [12].

Characterization of Silver nanoparticles (AgNPS)

In order to ensure that the *Cucurbita pepo L.* powder reached the nano size after addition with AgNPs, the following methods were adopted for its diagnosis, [13,14]. These methods included:

Scanning Election Microscope

To determine the size of silver nanoparticles, using SEM, a scanning electron microscope should be used. This type of microscope was the first electron microscope to be invented, and its functional mechanism is very similar to an optical transmission microscope, The main difference is that in TEM, an electron beam is used instead of light, and a magnetic lens is used instead of an optical lens. Methods of analysis and identification of materials are crucial because the physical and chemical properties of the extract depend on the type of raw materials and their microstructure. Therefore, it is necessary to have methods and identification equipment in place to determine the microstructure of each material and consequently, the properties of those materials, this is essential for conducting research and controling the quality of industrial products [15].

Fourier-transform infrared spectroscopy

Fourier Transform Infrared (FTIR) spectra were recorded using a Shimadzu/84005 spectrophotometer. The powdered sample was mixed with KBr. The scans were recorded in the range between 3800- 600 cm^{-1} [16].

X-ray diffraction (XRD) spectroscopy

If the biosynthesized AgNPs are characterized using X-Ray diffraction spectrum, it will show alterations in the depth of the deposit during the integration. The substrate height before and after the intercalation process. [17].

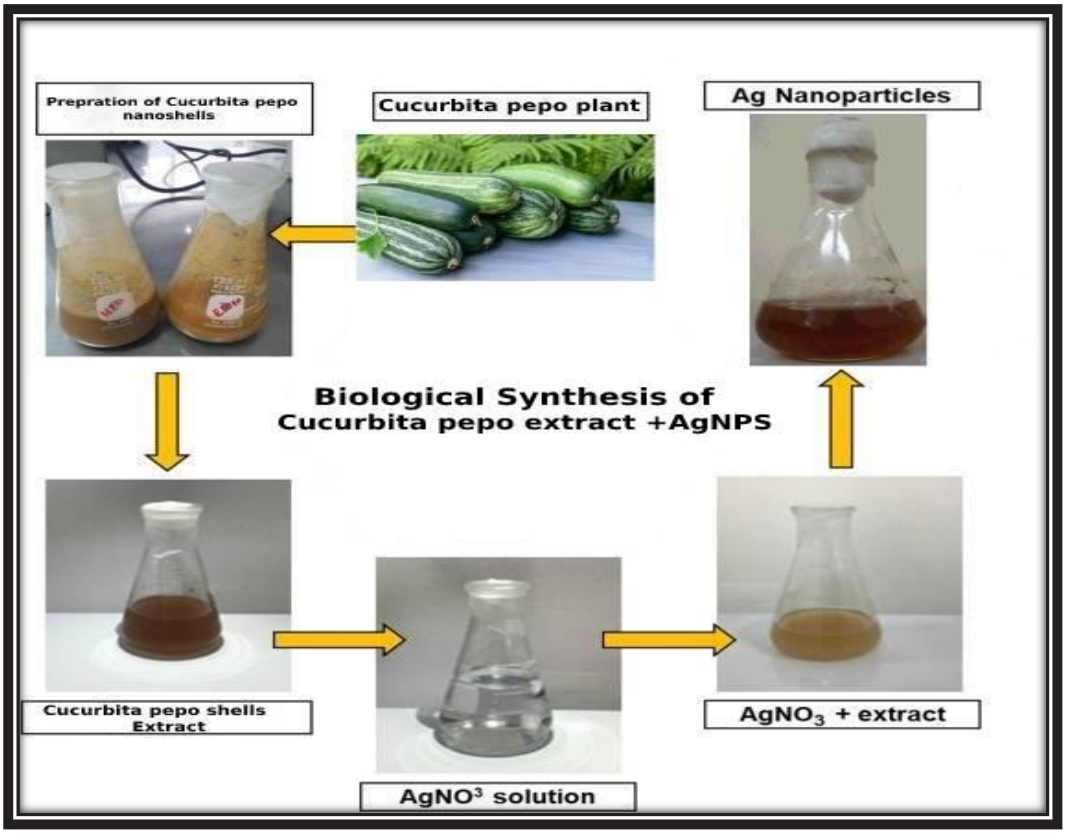

Fig. 1. Preparation of silver nanoparticles using *Cucurbita pepo* extract.

Isolation of study

The identified *Pseudomonas aeruginosa* isolate, which was isolated from cheese was selected by storing it on Brain Heart Infusion Broth (BHIB) slant agar at 4°C until use. [18].

Antibacterial activity of Cucurbita pepo L. sheel

This method was described by [19]. The inhibitory ability of microbial and nanos shell *Cucurbita pepo L* powder against *Pseudomonas aeruginosa* was tested in culture plates using agar

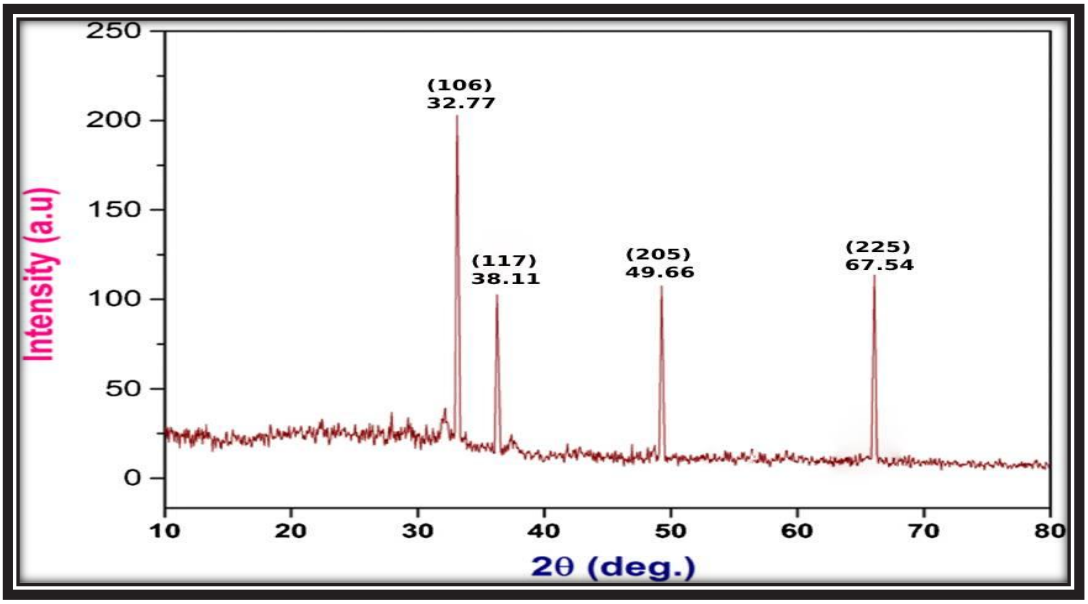

Fig. 2. XRD analysis of $AgNO_3$.

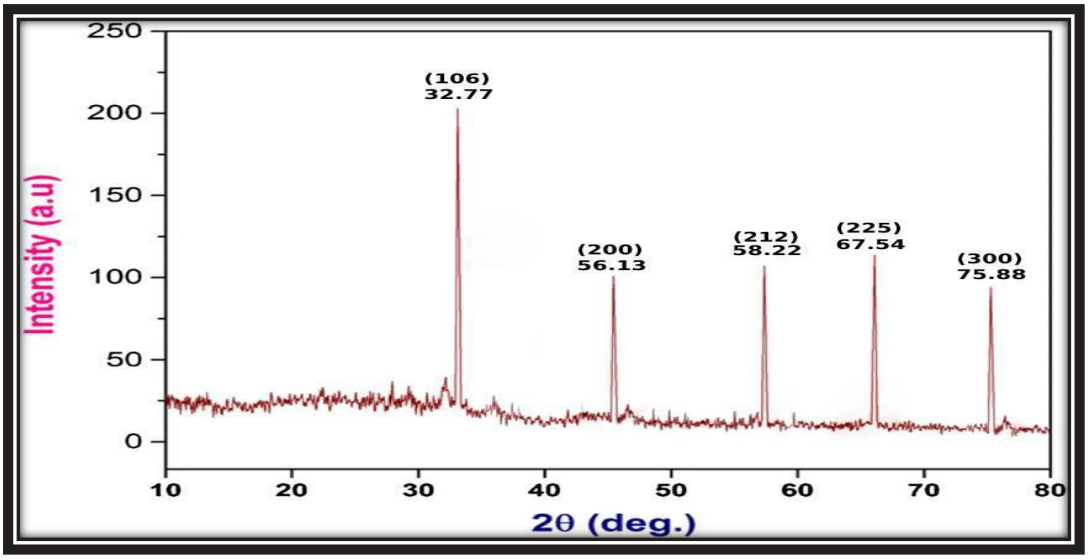

Fig. 3. XRD diffraction of AgNPs with *Cucurbita pepo* Extract.

well diffusion methods, twenty milliners of Mueller Hinton agar medium was prepared by seeding 0.1 ml of bacterial suspension of the study strain. The growth was comparing in liquid nutrient medium BHIB with a standard McFarland solution with an inoculum size of 3×10^8 cells/ml.Holes were made in the agar medium inoculated with bacteria using a sterile cork borer. Using a fine pipette, 50 microliters of the prepared concentrations (10, 20 mg/ml) were transferred and placed in the holes. The plates were then incubated at 37°C for 24 hours. After incubation, the diameter of growth inhibition around each hole was measured in millimeters.

Statistical Analyses

Laboratory experiments were conducted following a completely randomized design. The results were analyzed using an analysis of variance table and the means were compared using the Duncan test. To conduct a statistical analysis of the changes, the test was performed at a significance

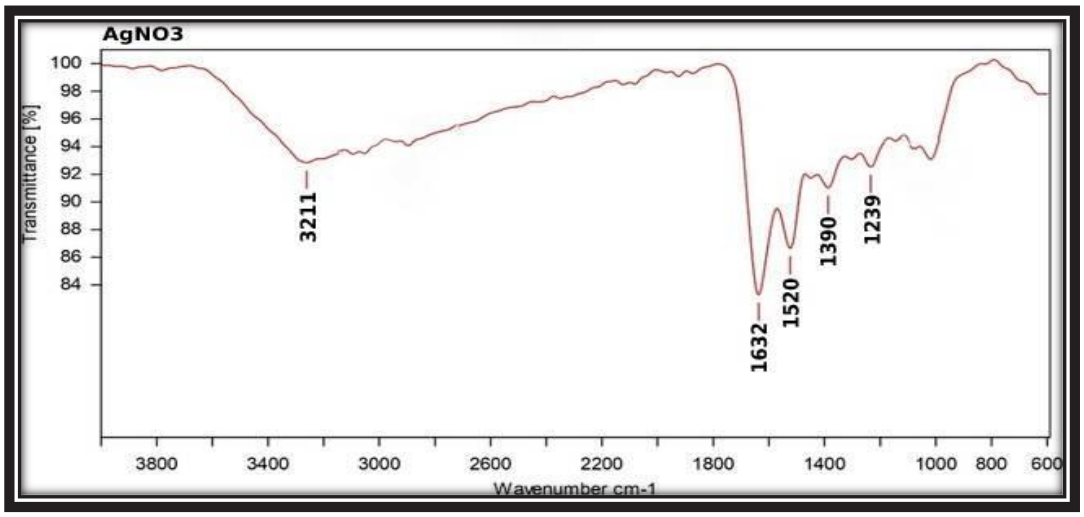

Fig. 4. Fourier Transform Infrared Spectroscopy analysis of $AgNO_3$.

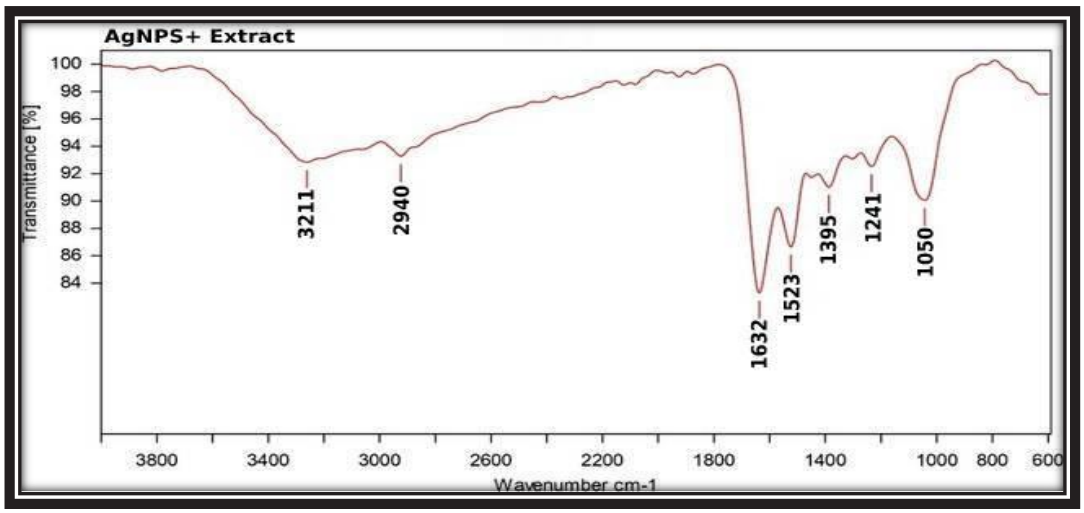

Fig. 5. Fourier transform infrared spectroscopy analysis of AgNPS+ extract.

level of P≤ 0.05 [20].

RESULTS AND DISCUSSION

In this study, silver nanoparticles (AgNPS) solutions were synthesized using *Cucurbita pepo* extract through biological methods. The color of the $AgNO_3$ solution before the addition of *Cucurbita pepo* extract was yellow. However, after treatment and mixing with the extract it showed a gradient color change from yellow to dark brown when heated at 80 ºC for 15 minutes. This indicated the formation of AgNPS as showed in (Fig. 1).

This study was in agreement with [21], as the charges on the surface created an electric field that attracted counter ions. The layer of surface charges and counter ions formed the electric double layer.

The results of $AgNO_3$ revealed the existence of four different diffraction peaks corresponding to the crystal planes of crystalline $AgNO_3$ with observed values of 32.77°, 38.11°, 49.66°and 67.54°. These peaks correspond to the reflections of the silver face-centered cubic structure, represented by the numbers (106), (117), (205), and (225) in (Fig. 2). While, results of AgNPS showed five strong diffraction peaks corresponding to the crystal planes of AgNPs with observed values of 32.77°, 56.13°, 58.22°, 67.54º and 75.88º. which are represented by the numbers (106), (200), (212), (225) and (300) in (Fig. 3).

The result agreement with [21] in how found the sharp peaks indicated that the AgNPs had a well-defined crystalline and pure structure. The observed peak broadening was also consistent with a small particle size.

These results were consistent with those of previous studies [22, 23]. Which concluded that the nanoparticles were super paramagnetic in nature. Nanomaterials with increased surface area have a significantly larger surface area compared to their volume. The smaller the particle size, greater the surface area, leading to increased chemical reactions and absorption. This unique property gives nanomaterial's distinctive characteristic in areas such as catalysis, gas absorption, and water purification. In terms of electrical properties: some materials exhibit unique behavior nanoscale. For example, certain materials that are electrical conductors at larger dimensions become electrical insulators at nanoscale dimensions. This property can be utilized in the production of microelectronic components.

Fig. 4 shows the FTIR spectrum of $AgNO_3$ with absorption bands located in the region of 3800 cm^{-1} to 600 cm^{-1}, characterized by strong bands at (3211, 1632, 1520, 1390, and 1239) cm^{-1}. Fig. 5, on the other hand, displays the FTIR spectrum of AgPNS + extract also with absorption bands in the region of 3800 cm^{-1} to 600 cm^{-1}, featuring strong bands at (3211, 2940, 1632, 1523, 1395, 1241 and 1050) cm^{-1}.

The band at 3211 cm^{-1} was assigned to the hydroxyl group –OH- (OH stretching). The band at 2940 cm^{-1} represented the (CH) groups, while the band at 1632 cm−1 was attributed to the stretching vibration of the conjugated carbonyl (–C=O) group indicating its interaction with the nanoparticles. The band at 1523 cm^{-1} associated with (OH-bend), is similar to phenolic compounds and may affect

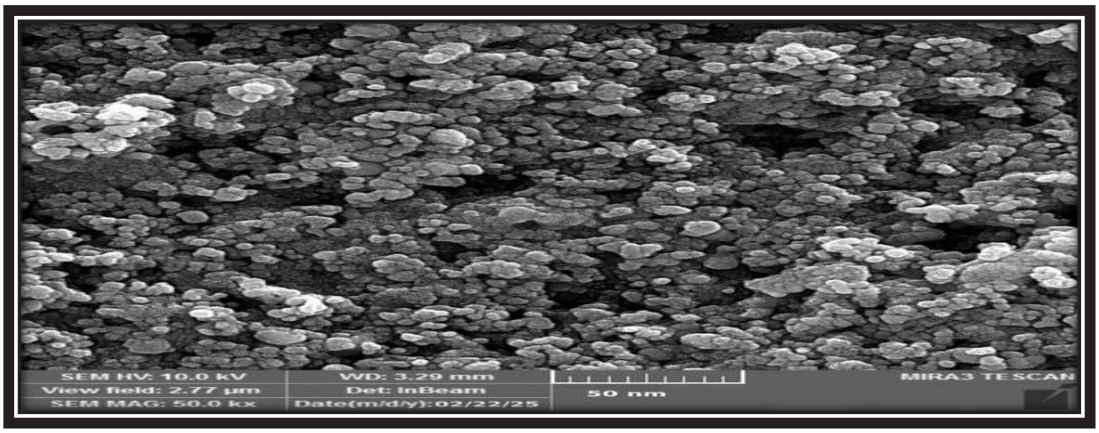

Fig. 6. Scanning Electron Microscopy images of AgNO3.

the synthesis and stability of AgNPs. The band at 1395 cm^{-1} indicated the stretching of (COO-) groups. The band at 1241 cm^{-1} demonstrated the (C-O) stretch, and the band at 1050 cm^{-1} corresponded to the (C-H) stretching of the phenyl ring in the substitution band.

The current study agreed with [24] who observed bands, similar to those reported for silver nanoparticles. The overall FTIR pattern confirmed the presence of the synthesized AgNPs. The locations and amounts of absorption peaks are dependent on the chemical composition, crystalline structure and morphology [25].

It is evident that the sample consists of a number of nanoparticles that exhibit a spherical shape, specifically AgNPS.The average size of the obtained sample was approximately 20 nm (Fig. 7). This indicates that homogeneous nanoparticles can be synthesized with a rather good size distribution. The crystallites of particles are less agglomerated. (Fig.6).

It is in agreement with previous research related to [4] that the basis of the scanning electron microscope is the creation of an electron beam by establishing a potential difference in the electron source. This beam initially passes through a condensing lens which serves to narrow the primary beam to a specific amount. The beam then moves through a series of coils (wires) known as Scan Coils, which shift the beam left and right by generating an electromagnetic force. Subsequently, the beam travels through the objective lens, which focuses the beam on the sample. once the beam contacts the sample, a series of electron beams are emitted from the surface, and detected by sensors positioned above the sample. Finally, the sample is identified by converting these electrons into signals.

Antibacterial activity of AgNPs against Resistance bacteria Pseudomonas aeruginosa

The results of the study showed varying inhibitory effectiveness in inhibiting *Pseudomonas aeruginosa* bacteria for both silver nitrate and silver nanoparticles with zucchini peel extract at concentrations of 10 and 20 mg/ml in Mueller Hinton agar medium. Silver nanoparticles with the extract produced the highest inhibition zone on *Pseudomonas aeruginosa* at 14 mm with a concentration of 10 mg/ml. The inhibition zone against bacteria increased to 25 mm with a concentration of 20 mg/ml of plant extract.in comparison, while silver nitrate resulted in 9 mm inhibition at a concentration of 10 mg/ml, which increased to 16 mm with a concentration of 20 mg/ml, respectively, compared to the control treatment.

This result was agreement with [8,21,26] which found the main components responsible for the antimicrobial effect may interact specifically with the bacterial cell wall. Research has shown that fruits, seeds and leaves of vegetable crops belonging to the Cucurbitaceae family have gained increasing research interest for their biological effects in treating allergies, antioxidants, inflammation, microbes, viruses and cancer.

The zucchini peel can be processed into powders and extracts that become a more

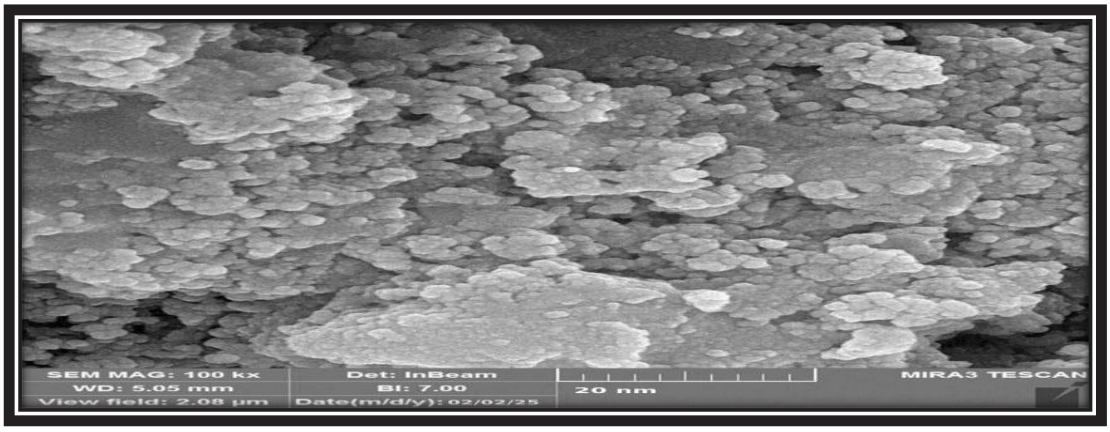

Fig.7. SEM images of AgNPS+ extract.

concentrated source of nutrients and can be used in new pharmaceuticals and food products. The phytochemicals and proteins present in the components of zucchini can be extracted to develop safe and novel antioxidant and antimicrobial drugs. After approval from the scientific community, pumpkin-based nutrients and cosmetics can be commercialized for human well-being.

CONCLUSION

Reducing the excessive use of antibiotics in infants and children to prevent the development of resistance and using alternative methods to strengthen the immune system through various methods, including the optimal use of existing vaccines, hygiene, and the use of therapeutic organisms and medicinal plants.

CONFLICT OF INTEREST

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

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