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Effectiveness evaluation of Escherichia coli as a reducing agent in synthesized zinc oxide nanoparticles

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This study is conducted to evaluate the effectiveness of using Escherichia coli as a reducing agent in the synthesis of ZnO nanoparticles. E. coli isolated from stool samples as a reducing agent for zinc sulfate to ZnO nanoparticles during the preparation process, a color change from a clear color to a white color is observed, which is preliminary evidence of the reduction process. The results of the biosynthesis of nanoparticles are then confirmed by UV/vis spectrophotometer, where an absorption peak appeared with a wavelength of 273 nm, and this length is within the specified range for nanoparticles (200-800 nm). The results of the examination of the crystal shape and crystal size by X-ray diffraction showed the appearance of 9 clear diffraction peaks at 2θ values, namely 31.65, 34.38, 36.19, 47.50, 56.53, 62.81, 69.01, 72.49, and 76.89, which corresponded to peaks 100, 002, 101, 102, 110, 103, 201, and 202, respectively, in the standard diagram under JCPDS card no. 36-1451. The XRD pattern also shows strong, narrow diffraction peaks, indicating that the biosynthesized ZnO nanoparticles are crystalline in nature, and the nanoparticle size of the crystal is 11.8 nm. Field-emission Scanning Electron Microscope results have revealed that the ZnO nanoparticles have an elongated oval to spherical and cluster-shaped shape with a nanosized ranging from 37.93 to 61.82 nm and an average size of 45.15 nm.

Keywords: Biological Reducing agent; Escherichia coli; Zinc Oxide Nanoparticle.

1. INTRODUCTION

Nanoparticles can be synthesized through physical, chemical, or biological methods. Physical and chemical methods offer uniformity and stability but are expensive and environmentally harmful. Chemical methods also raise safety concerns for medical, cosmetic, and food applications. To enhance biocompatibility, recent focus has shifted to eco-friendly biological methods using bacteria, fungi, yeasts, or plants [1-3]. Meanwhile, rising multidrug resistance has increased the demand for new treatments. However, drug development is costly and time-consuming. Nanoparticles, with their unique size, shape, and surface properties, have emerged as promising tools to address antibiotic resistance [4].

On the other hand, the main concern of scientists is the methods of preparing nanoparticles, as there are physical and chemical methods, but due to what these methods cause and produce residues that may be toxic and harmful to the environment, so researchers sought to find a safer, and less harmful method that is available in the environment, so there is the method of biosynthesis, a study found the possibility of preparing zinc oxide nanoparticles biologically and through the use of pomegranate leaf extract [5,6]. A study of nanoparticle synthesis using the bacterium Bacillus licheniformis as a reducing agent for nanoparticles and its use as an antibacterial agent is also found [7]. Several bacterial species have been used as reducing agents for oxides such as ZnO, including Escherichia coli and Enterobacter hormaechei. Where bacteria can absorb metal ions from the environment or surrounding solutions and convert these metal ions into nanoparticles form by enzymatic reduction inside or outside the cell [8,9]. In a recent study, the bacterium Lactobacillus spp. is used as an eco-friendly bio-agent in the synthesis of zinc oxide nanoparticles. These nanoparticles are characterized by antibacterial activity against pathogenic bacteria [10,11].

Due to the lack of studies on the use of E. coli in the synthesis of nanoparticles such as zinc oxide, this study is conducted to evaluate the efficacy of E. coli as a reducing agent in the synthesis of zinc oxide nanoparticles.

2. EXPERIMENTAL

2.1 Preparation of biomass from E. coli

The isolate of E. coli from stool samples is cultured on nutrient agar medium at 37°C. To prepare the biomass of the bacteria, Muller Hinton broth is prepared according to the manufacturer's instructions. This medium is sterilized and cooled, then is inoculated with E. coli and placed in a shaking incubator at 37°C for 48 hours. Centrifugation is performed to collect the bacterial biomass, then it is washed several times with deionized water and then stored in a refrigerator [12].

2.2 Biosynthesis of ZnO nanoparticles using E. coli

A preparation of $ZnSO_4$ zinc sulfate solution with a concentration of 0.1 mol/L is prepared by weighing 28 g of $ZnSO_4$ into a 1000 ml beaker, then 800 ml of deionized water is added. The solution is sterilized by filtration and placed at 4°C until use [13]. For the biosynthesis of ZnO nanoparticles, E. coli biomass is used as a reducing agent to reduce zinc sulfate to ZnO nanoparticles. The pre-prepared bacterial biomass is placed in 500 ml of pre-prepared zinc sulfate solution with a concentration of 0.1 M, and the flask is placed in a shaking incubator for 48 hours at 37°C while observing the color change of the solution from clear to milky white, indicating that the reduction process and the formation of ZnO nanoparticles occurred [12].

2.3 Nanoassays

After the color change and formation of ZnO nanoparticles, several tests are performed to confirm the reduction of zinc sulfate to ZnO nanoparticles mediated by E. coli. Several tests are conducted at the PHI Science Centre, where a UV-visible spectrophotometer (UV/vis spectroscopy) is used in the wavelength range of 200-800 nm, after which the nanoparticle solution is centrifuged at 5000 rpm for one minute. The ZnO nanoparticles are then centrifuged at 5000 rpm for 15 min and washed several times with deionized water, and then the samples are sent for X-ray diffraction examination (XRD scheme) in order to study the crystal shape and to determine the size of the ZnO nanoparticles. Field emission scanning electron microscopy (FE-SEM) is used to know the size and shape of the nanoparticles, and the elemental analysis and chemical properties are investigated by Energy Dispersive X-ray (EDX) [11,14].

3. RESULTS AND DISCUSSION

To biosynthesize ZnO nanoparticles, E. coli is used as a biological agent to reduce $ZnSO_4$ zinc sulfate to ZnO nanoparticles The results have showed that ZnO nanoparticles can be biosynthesized as the color changes of the zinc sulfate solution, starting from clear to milky or white after the addition of the biomass of E. coli with continuous stirring. The color change is an initial sign of the reduction of zinc sulfate to zinc oxide nanoparticles as shown in Figure 1.

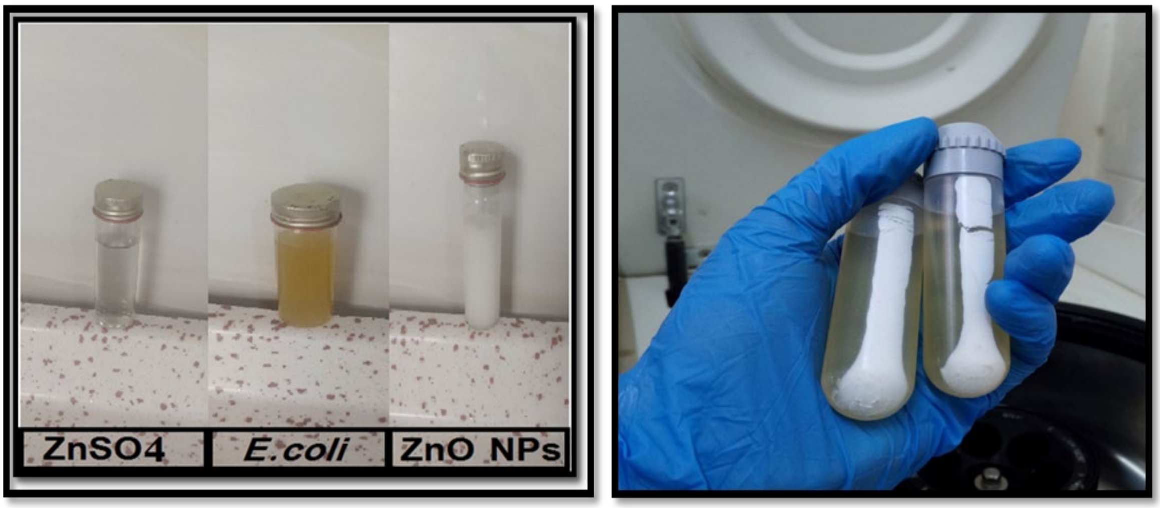

Figure 1 Color change of E. coli-mediated reduction of zinc sulfate to ZnO nanoparticles.

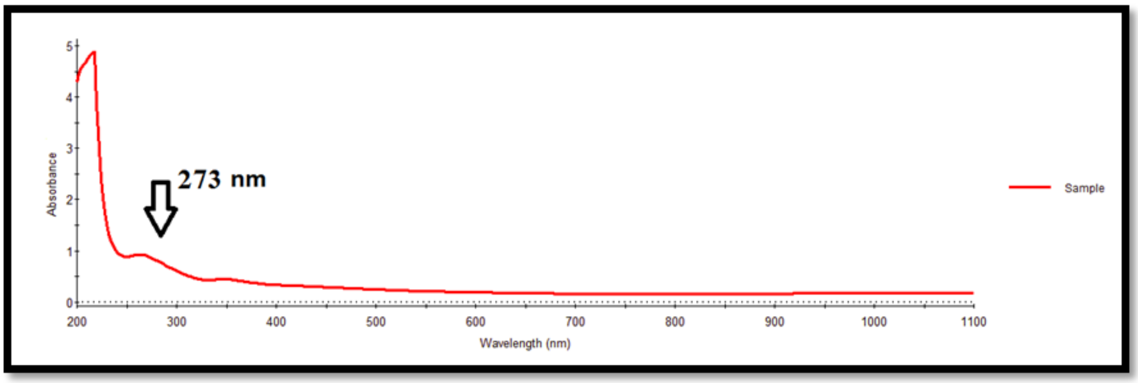

Figure 2 Screening of bioprepared ZnO nanoparticles by UV/vis spectroscopy.

The synthesis of ZnO nanoparticles prepared from E. coli is confirmed by XRD to determine the crystalline nature and size of the nanoparticles. Figure 3 has showed that 9 clear diffraction peaks appear at 2θ values of: 31.65°, 34.38°, 36.19°, 47.50°, 56.53°, 62.81°, 69.01°, 72.49° and 76.89° which correspond to 100, 002, 101, 102, 110, 110, 103, 201 and 202 respectively with the standard chart under JCPDS card no: 36-1451 and from the figure, the XRD pattern shows peaks with narrow and strong diffraction, indicating that the biosynthesized ZnO nanoparticles are crystalline.

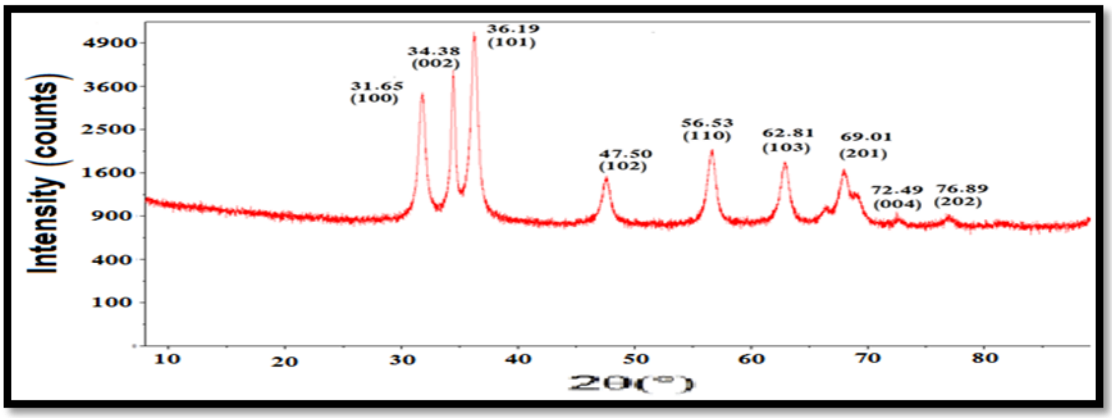

Figure 3 XRD scheme of E. coli-mediated biosynthesized ZnO nanoparticles.

The diffraction values of 2θ and 9 peaks are shown in Table 1, with the first and second peaks being the same, which lead to the neglect of one of them. Through the Scherrer Equation, the crystal size is extracted from the diffraction values, and the crystalline nature as the average crystal size of the ZnO nanoparticles during XRD examination is 11.8 nm.

Table 1 Structural properties of bioprepared ZnO nanoparticles.

	2θ	**D (Å)**	**Height (cts)**	**Rel. Int. (%)**	**FWHM Left [°2θ]**	**Area cal.**	**Area (cts*°2θ)**	**Backgr. (cts)**	**Crystallite Size (Å)**	**Micro Strain (%)**
1	31.652	2.824	919.55	30.89	0.4735	563.18	563.18	845.16	166	0.85188
2	31.806	2.811	898.81	30.2	0.6659	1054.98	1054.98	844.53	84	1.67563
3	34.385	2.606	2086.68	70.11	0.3402	1114.85	1114.85	834.79	202	0.64531
4	36.191	2.48	2976.37	100	0.5707	2603.03	2603.03	828.68	118	1.05335
5	47.500	1.912	471.53	15.84	0.7841	548.72	548.72	800.45	88	1.08518
6	56.536	1.626	847.34	28.47	0.7386	929.28	929.28	787.03	98	0.8322
7	62.819	1.478	846.84	28.45	0.4279	746.85	746.85	781.34	129	0.57232
8	69.019	1.359	251.26	8.44	0.9136	311.66	311.66	778.25	90	0.75348
9	72.492	1.302	68.5	2.3	0.342	48.28	48.28	777.55	176	0.36988
10	76.896	1.238	61.7	2.07	1.0053	63.97	63.97	777.65	109	0.56851

The size of ZnO nanoparticles prepared using E. coli as the reducing agent is also studied and confirmed by SEM examination. The results have shown that the ZnO nanoparticles have an elongated oval to spherical shape and clusters with a nano size ranging from 37.93-61.82 nm with an average size of 45.15 nm when examined at 500 nm, as shown in Figure 4.

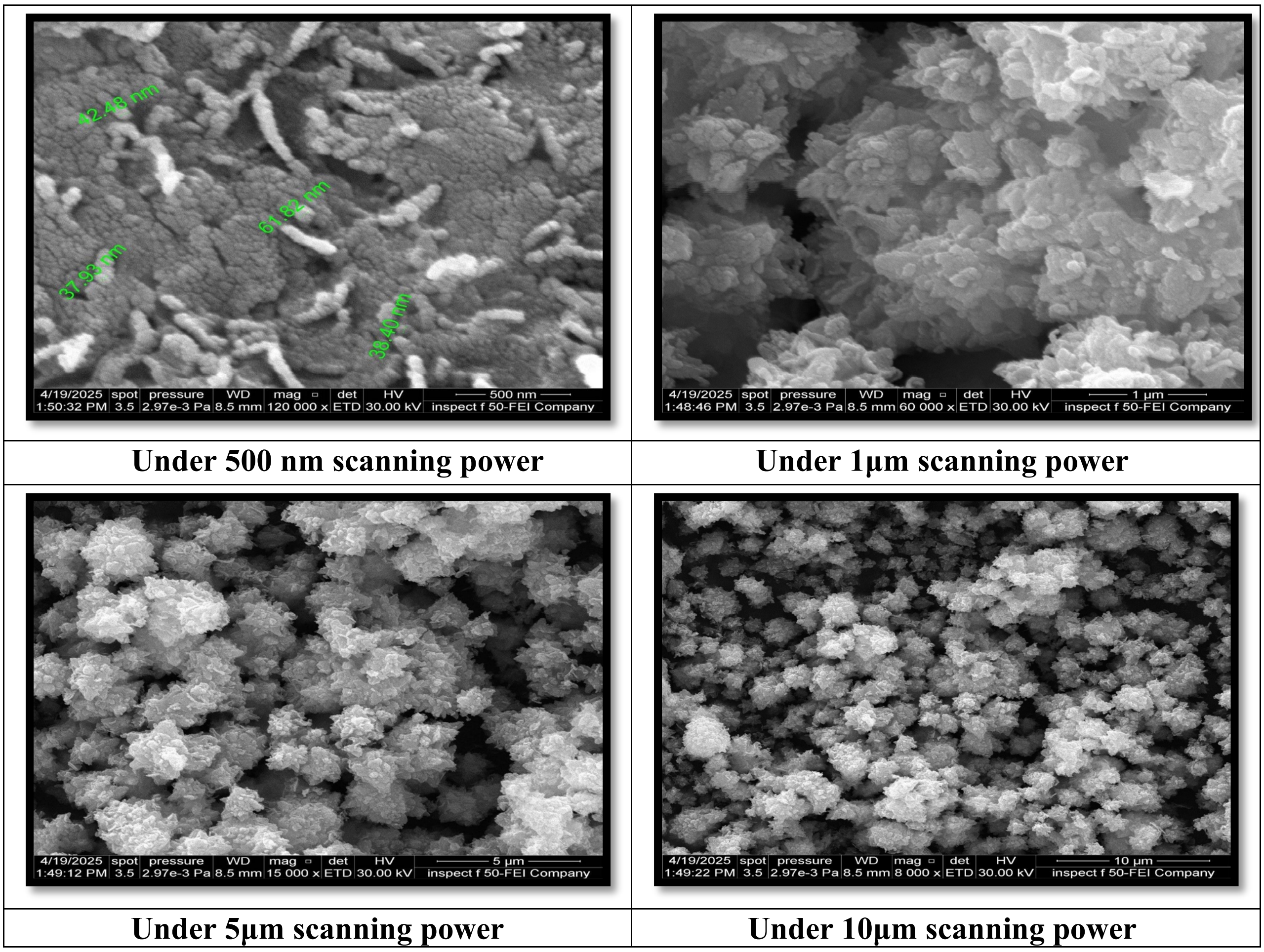

Figure 4 Bioprepared ZnO nanoparticles in FE-SEM under different units of measurement.

The analysis of the ZnO nanoparticles conducted by EDX is important in elemental analysis in order to know the chemical properties of the sample. The elemental composition of ZnO nanoparticles prepared biologically using E. coli is identified. Figure 5 shows the elemental composition of the sample, in which the presence of zinc and oxygen elements is clear, where the zinc element Zn appeared in three clear and high peaks at 1 keV, 8.8 keV and 9.7 keV, while oxygen is appeared with a single peak at 0.5 keV, with separate peaks for carbon at 0.2 keV and two for iron at 1.7 keV and 6.5 keV.

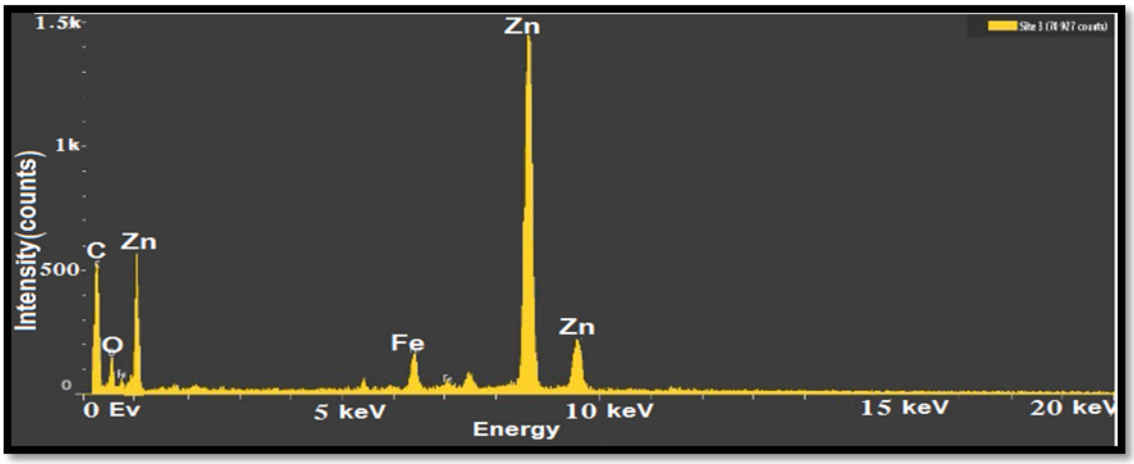

Figure 5 Analysis of ZnO nanoparticles in the EDX scheme.

The study indicates that ZnO nanoparticles can be biosynthesized using E. coli as a biological reducing agent, which is consistent with a local study in Iraq that indicates that E. coli bacteria that is isolated from stool samples can be used as a biological agent in the reduction and synthesis of ZnO nanoparticles. The researchers have showed that the bio- preparation method using bacteria is a safe and environmentally friendly method [11,15,16], are able to synthesize ZnO nanoparticles using bacteria and pointed out the importance of using microorganisms such as bacteria in biosynthesis. Because of their rapid growth and reproduction speed and thus the synthesis process is fast and does not require effort and time, in addition to being a harmless method to the environment and its by-products are non-toxic unlike chemical and physical methods, A recent study has shown that the microbial-mediated biosynthetic method in which microorganisms such as bacteria are used as a reducing agent in the preparation of ZnO nanoparticles Biosynthesis provides a more acceptable alternative to chemical and physical methods that may cause contamination in the environment, minimizing hazardous environmental impacts, as the metabolic compounds present in the microbial solutions during the biosynthesis process act as reductants and biostabilizers, while the metabolic compounds present in the microbial solutions during the biosynthesis process act as reductants. This microbial method is environmentally friendly, non-toxic, and biodegradable, and can occur either intracellular within microbial cells or extracellular using proteins, enzymes, and other biomolecules secreted by microbes [17].

The reason for the color change when manufacturing nanoparticles is due to the presence of secondary metabolic compounds, enzymes, proteins and organic molecules that can reduce and stabilize metals and elements to nano-size by encapsulating or covering these metals or elements with active complexes of secondary metabolic compounds, and the color change can also be due to the Surface Plasmon phenomenon, and that the color change is due to the appearance of the surface plasmon resonance absorption band, which is an advantage of nanoparticles [18,19].

Regarding UV/vis spectroscopy. Studies have shown that nano-sized materials exhibit absorbance between 200 and 800 nm when examined by UV/vis spectrophotometer, which is our study has shown. The absorbance peak is within the specified range for nanoparticles [11, 20, 21]. On the other hand, found that ZnO nanoparticles, when prepared by biological methods using Enterobacter hormaechei as a reducing agent, show optical and absorption properties at a wavelength of 320 nm, which did not agree with the result of our study [22], and this may be due to the different type of bacteria as well as the different base material containing the zinc element, different reaction conditions, the method of diluting the sample at the time of examination, and the time Studies have indicated that the concentration and stability of the nanoparticle solution affect the reading process when examining the

UV/vis absorption spectrum of the UV/vis spectrophotometer, and the longer the incubation time, the greater the stability and reduction process and the stability of the nanoparticle, and the absorption wavelength increases over time due to the increased reduction and stability process. [16,23]. The stabilization process may be due to the presence of effective aggregates in the bacterial solution that are found in the substances produced by bacteria in cases of extracellular stress, including exopolysaccharides. Among the aggregates that play a role in the reduction and stabilization of nanoparticles are carboxyl groups resulting from the secretion of acids by bacteria, methyl groups, aldehydes and acetates, and proteins that act as a reducing agent, coating, and stabilizer of zinc oxide [17]. Bacteria can also absorb metal ions from the environment or surrounding solutions and convert these metal ions into nanoparticle elemental form by enzymatic reduction [24].

Studies have shown that X-ray diffraction spectroscopy is important to gain a deeper understanding of structural changes during electrochemical reactions such as phase formation or growth orientation [25], The X-ray diffraction XRD of ZnO nanoparticles biosynthesised from Escherichia coli bacteria E. coli indicated the presence of 7 peaks that appeared in our study and these peaks are 32.45°, 34.73°, 36.56°, 47.70°, 55.86°, 62.12° and 63.10° which are corresponding to diffraction peaks 100, 002, 101, 102, 110, 103 and 112, respectively [11], The study has revealed that the manufacture of zinc oxide nanoparticles by the biological method and through the use of microorganisms has a small size compared to other methods, as their size ranged between 13-15 nm [26]. The reason for the small crystalline size is due to the role of the biological method, especially microorganisms, due to the role of these organisms in the crystallization process and control. Unlike the chemical and physical method, in which the size is affected by the nature and quantity of the reactants, and the reaction conditions of high pressure and high temperature. In our study, the absence of additional peaks indicate the purity of zinc oxide nanoparticle prepared by E. coli bacteria and the sharp peaks show the good crystallinity of the nanoparticle as some studies have indicated that when the sample is not completely pure, other peaks appear, which may be caused by bacteria or organic matter originating from bacteria [27]. FE-SEM results have showed that the use of FE-SEM is important in analyzing the morphology and surface morphology of the fabricated nanoparticles layer [28,11], demonstrated that ZnO nanoparticles prepared using E. coli bacteria have a nanoparticle size range between 32-45 nm, Zinc oxide nanoparticles prepared by Bacillus subtilis have a spherical or hexagonal cluster shape [29]. And ZnO nanoparticles bio-prepared from the extracts have a nanoparticle size of 45- 65 nm and their shapes are spherical [30]. It is observed from the images in the scanning electron microscopy results that there are some agglomerations that are due to the increase in the surface area of the nanoparticles and the strong attraction of these materials due to the Vander Waals force or due to the reduction of the kinetic energy exhibited by the nanoparticles or due to the agglomeration of organic matter present in the extracts or due to the H-bonding force present in the bioactive molecules are the cause of these aggregations [31].

According to the other studies, each element has a distinctive atomic structure, so each element has a set of distinctive peaks in EDX scheme. Subsequently, it EDX that have an energy corresponding to the energy difference between the atomic orbitals, this energy difference is a feature of each chemical element and the presence of this peak indicates the presence of the element. EDX energy dispersive spectroscopy has shown in identifying the components of the sample of zinc oxide nanocomposite prepared by the biological method, where the presence of zinc and oxygen elements is detected. EDX is also used to gain additional insight into the topographies of ZnO nanoparticles as the nanoparticles show three distinct Zn peaks at 1 keV, 8.7 keV and 9.8 keV, respectively, as well as a single oxygen peak at 0.5 keV [32]. The reason for the appearance of a peak for the carbon element is due to the traces and residues of the coating of the nanoparticle zinc oxide sample with carbon, which is added as a coating that increases electrical conductivity in scanning electron microscopy as well as X-ray scattering spectroscopy. Since the EDX examination is very sensitive and detects all elements present in the sample, the carbon element appeared among the peaks in the peak plot of the studied sample [33-36].

4. CONCLUSIONS

Despite increasing research in recent years towards biological methods for the preparation of nanoparticles, these methods have several drawbacks including, the difficulty in obtaining a completely pure material, the difficulty in controlling the required size, as well as the process of homogenization and shape variation. Also, the use of biological agents such as bacteria such as E. coli may lead to several different results when repeating the experiment, so chemical and physical methods remain more accurate in obtaining the required characteristics of size, shape and purity, especially in areas that require accurate results such as therapeutic and medical aspects.

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