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Comparing the effect of zinc oxide nanoparticles biosynthesized by Aloe barbdensis and chemically manufactured on Klebsiella pneumoniae isolated from the inflamed middle ear

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***Abstract*---**The study included the collection of 100 samples of otitis media patients for the period between 15/9/2021 to 15/1/2022 from Samarra General Hospital and private clinics. Bacterial isolates were diagnosed with a set of microscopic and biochemical tests and the Vitek-2 system. The results of this diagnosis showed that 87(87%) samples gave a positive result for bacterial growth and showed that *Klebsiella pneumonia* was 16(18.39%). Zinc oxide was used in the synthesis of zinc nanoparticles by aloe Vera extract. The properties of the resulting nanomaterial were studied by adopting the diagnostic devices represented by the UV-compliant device, the atomic force microscope, the X-ray diffraction device, the infrared fluorescence spectroscopy, as well as the scanning electron microscope and the device for calculating the zeta potential difference was the results. The results of these devices indicated the formation of zinc oxide particles. In pure form and in different shapes and sizes ranging from 20-40 nm. The effectiveness of biosynthetic nanoparticles was tested against bacteria isolated from the inflamed middle ear, as it was based on four different concentrations 2.5, 5, 10, 20 mg/ml. All concentrations showed effectiveness in inhibiting the growth of bacterial isolates, while 2.5 mg/ml was not given. no activity in inhibiting the growth of the isolates was compared with the effectiveness of commercial zinc particles that were purchased from one of the offices that supplied

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laboratory materials with the same concentrations above and on all bacterial isolates. The results showed the superiority of zinc nanoparticles biosynthesized by aloe Vera extract in the level of bacterial growth inhibition on commercial zinc nanoparticles.

***Keywords*---**effect zinc, nanoparticles biosynthesized, Aloe barbdensis.

Introduction

Chronic suppurative otitis media (CSOM) is one of the most common ear infections in humans, affecting about 65-330 million people a year, especially children, school children are more susceptible than adults, it is a chronic inflammation of the middle ear and mastoid cavity. With perforation of the tympanic membrane accompanied by purulent secretions, inflammation usually occurs after acute otitis media or after acute upper respiratory infections (Parrish *et al.*, 2019).

Several types of microorganisms are involved in the increased incidence of disease and most of the bacterial strains that cause otitis media are *Pseudomonas* spp, *Staphylococcus*. spp, *Proteus* Spp and *Klebsiella* spp. several external factors contribute to infection and its spread in the population, including lack of personal hygiene, overcrowding, malnutrition and passive exposure to smoking (Khan *et al.*, 2019). Also, the factors produced by bacteria help to increase the pathogenesis of disease, and the occurrence of chronic infection is virulence factors to help the bacteria invade the host and cause disease and avoid the host's defenses (Ghosh *et al.*, 2019). Excessive and frequent use of classic antibiotics has reduced their ability against widespread infectious diseases, and it has become necessary to carry out several medical interventions to avoid this condition (Coulson *et al.*, 2017).

The increasing resistance of bacteria and fungi to classic antibiotics has created enormous clinical problems, especially in immunodeficiency patients (AIDS) and cancer. Moreover, the identification and treatment of antibiotic-resistant microorganisms is difficult and costly (Ghosh *et al.*, 2019). Nanotechnology has been adopted as a way to develop new non-traditional antimicrobial agents called Nano-antibiotics, which are an effective treatment for infectious diseases that have many advantages over traditional antibiotics, including the absence of harmful effects and increased effectiveness against drug-resistant species (Huh and Kwon, 2011). The Nano antibiotics show antimicrobial activity on their own or increase the efficacy and safety of the administration of conventional antibiotics, leading to the formation of high and effective local concentrations (Hajipour *et al.*, 2012).

Aim of the study

Comparison of the antimicrobial effect of biosynthetic by aloe Vera and chemical (commercial) zinc nanoparticles on *K. pneumoniae* isolated from otitis media patients.

Material and Methods

Clinical specimens were collected from patients with chronic otitis media using a cotton swab underneath Doctor supervision. The samples were cultured and diagnosed under the microscope Screening and cultural characteristics, including growth of colonies on different media (nutrient agar, Blood agar, McConkey agar, eosin methylene blue (EMB) agar and mannitol salt agar. The growing bacteria were distinguished in terms of shape, size, color, edge, hemolysis pattern and fermentation of lactose; Biochemical tests were also carried out to verify the properties of the isolate bacteria. These tests included an indole test to check the production of indole, methyl red test to investigate sugar fermentation and acid production, Vogus-Proscauer test for acetone complex detection, citrate utilization test to check citrate consumption as a single Carbon source and sodium carbonate composition, urease test indicates the hydrolysis of Urea and ammonium composition, oxidase test to check cytochrome production, Catalase and fermentation tests for sugars, motility screening test, and coagulation test in two of methods based on a previously described method (Brown and Smith, 2017).

Preparation of Aloe Vera suspension

The aqueous extract of the aloe Vera plant was prepared according to the method of Mofid *et al.*, (2020), with a weight of 10 g of the dry powder of the plant, and 100 ml of non-ionic water was added to it instead of hot distilled water and left for 24 hours in the refrigerator, then on the shaker for 24 hours as well. The extract was filtered by several layers of gauze to get rid of the large parts of the plant, then using Whatman No.1 filter paper, and then it was taken from it and added to the zinc oxide solution with a concentration of 1 mM.

Biological formation of zinc oxide nanoparticles

Mixing 10 ml of aloe Vera extract with 90 ml of 1 mM zinc oxide and adjusting the pH of the mixture to 7, two laboratory vials were added, the first containing the extract only without adding zinc oxide, and the second containing zinc oxide only without adding the plant extract as control agents, then it was placed on a magnetic stirrer for 35 minutes, we then notice the formation of a white precipitate at the bottom of the laboratory flask, and this is an indication of the formation of zinc oxide nanoparticles, the white precipitate formed at the bottom of the laboratory flask was collected and washed three times using non-ionic water and centrifuged at a speed of 10000 revolutions per minute and for 10 minutes, the formed precipitate was dried by a convection oven at a temperature of 105° C for a period ranging between 5-6 hours until the precipitate dried to obtain secondary zinc oxide minutes (Baskar *et al.*, 2013).

Techniques for the diagnosis of prepared zinc nanoparticle

The zinc oxide nanoparticles were extensively investigated using UV-Vi's spectroscopy, Atomic Force Microscopy (AFM), Fourier transform infrared spectroscopy (FT-IR), Zeta Potential and Energy-dispersive X-rays of zinc nanoparticles (EDX), while electro-kinetic properties were assessed by using

Scanning Electron Microscopy (SEM)and Transmission electron microscope (TEM).

Statistical Analysis

The results were statistically analyzed by applying ANOVA randomized complete design (CRD) test, and the arithmetic means were compared with Duncan's polynomial test with a probability level of 0.05% (SAS, 2012).

Results and Discussion

Isolation and diagnosis

100 samples were collected from patients with otitis media who attended Samarra General Hospital, and some private clinics, from both sexes and from different age groups, which ranged between 1-70 years within the period, within a period of time from 15/9/2021 to 15/2/2022 The results showed that 87 (87%) samples gave a positive result for bacterial growth on the media used the reason for the absence of growth in these samples may be due to the use of antibiotics before taking the sample, which reduces the percentage of bacterial growth, or the pathogen may be fungal or viral (Brain, 2005), and these results were in agreement with the results of Kuczkowskizz *et al.*, (2004) who found that 13.2% did not give bacterial growth. Bacterial isolates were diagnosed based on their phenotypic and microscopic culture characteristics and a set of biochemical tests, where the initial diagnosis was based on the shape and strength of the developing bacterial colony. The final identification of bacterial isolates was carried out using Vitek-2 system, and the results showed that out 16 (18.39%) isolates were identified as *K. pneuumoniae*. As for the isolates of *K. pneumonia*, it was found in a percentage of 18.2%, and this result was similar with the results of Saranya *et al.*, (2015) and Basnet *et al.*, (2017), but Al-Mosawi (2018) found that 9% of the isolates were *K. pneumonia*.

Biosynthesis of ZnO Nanoparticles

The extract of the aloe Vera plant was used as a reducing agent and stabilizer in the biosynthesis of zinc oxide nanoparticles. The results of the study showed the appearance of a precipitate at the bottom of the reaction bulge, and this is evidence of the process of synthesis of zinc oxide nanoparticles, Figure 1. One of the most important reasons for using Nano-zinc oxide biosynthesis is the cheap price, safe for the environment, no risk, ease of operation, and low toxicity (Heer *et al.*, 2017).

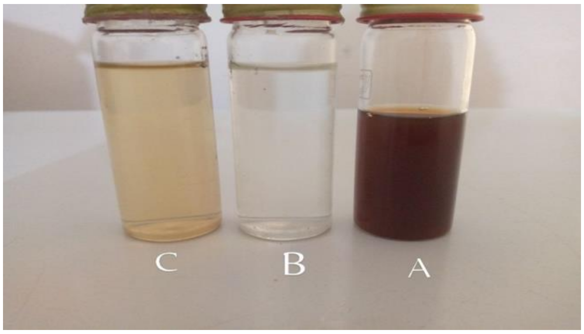

Figure 1: Biosynthesis of Nano Zinc Oxide by Aloe Vera Extract. A- Aloe Vera plant extract zinc oxide solution B- Zinc oxide solution C- Zinc oxide solution with aloe Vera plant

Characterization of ZnO Nanoparticles
UV-visible spectrum of zinc nanoparticles (UV-Vis)

The maximum wavelength λmax of the solution of zinc nanoparticles was determined by using UV-Vis spectroscopy. The results of this study agree with Jayaseelan *et al.*, (2012), in which zinc oxide particles were bio-synthesized using fungal filtrate and plant extracts, and the absorption peak was within the average 360-380 nanometers.

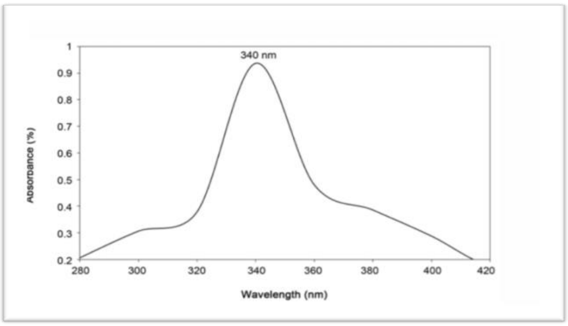

Figure 2: UV-visible spectrum of zinc nanoparticles

Fourier transforms infrared spectroscopy (FT-IR) of ZnO nanoparticles

The zinc oxide nanoparticles synthesized by aloe Vera extract were analyzed by Furet infrared spectroscopy to investigate the active groups present in aloe Vera extract that participated in the process of reducing and stabilizing zinc oxide nanoparticles. Figure 3 shows the fluorescent infrared spectrum recorded for the nanoparticles under investigation and in the range of 400-4000 cm.

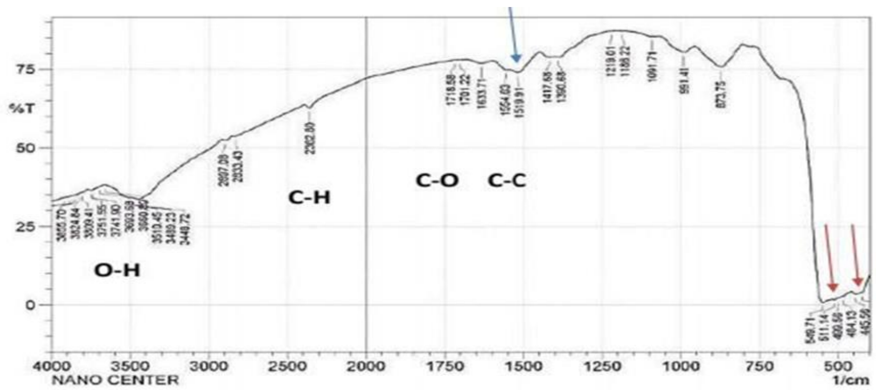

Figure 3: Furet infrared spectrum of biosynthetic zinc oxide nanoparticles.

The graph of the Fureh infrared spectrum analysis showed two sections, which are 445.56 cm-1 and 484.13 cm-1, and this indicates the transfer of the bonds between the oxygen molecule and the zinc molecule to two types of vibrations. We also note that there is a weak and severe peak of 1519.91 cm-1 with a range of 1500 to 1600 cm-1, which indicates the presence of a carbon-carrion group (Kennye group or aromatic ring), and these results are consistent with what was found by Rajan *et al.*, (2016).

Measurement of analysis and potential of the zeta potential difference

If the zeta potential analysis gave values that are either greater than +30 millivolts or less than -30 millivolts, in which the suspension dimension is stable, the high value confirms the repulsion between the molecules, which leads to the stability of the formula (Clogston and Patri, 2011). The zeta potential is found to be −50 mV as shown in Figure 4, A high value confirms the repulsion between the minutes and thus increases the stability of the formula.

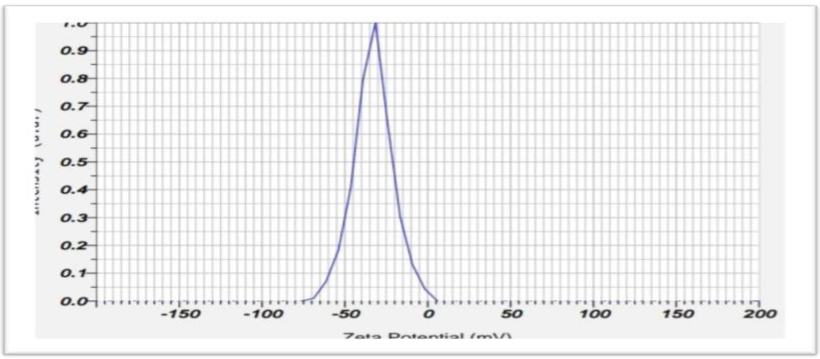

Figure 4: Zeta potential difference analysis curve of the as-prepared zinc nanoparticles.

Scanning electron microscope for zinc nanoparticles (SEM)

The scanning electron microscope measurements showed the shape and size of the prepared zinc nanoparticles with a magnification force of 200 nm, in the form of spherical and semi-symmetric nanoparticles, as the sizes of the prepared zinc nanoparticles appeared approximately between 31.16 - 86.13 nm, as shown in Figure 5. The current study succeeded in achieving good results in reaching a narrow range of sizes of zinc oxide nanoparticles, which were less than what was found by Kulkarni and Shirsat (2015), and also with the results of Pavani *et al* (2011), as the average size of the nanoparticles referred to in its research ranges between 50-120 nm.

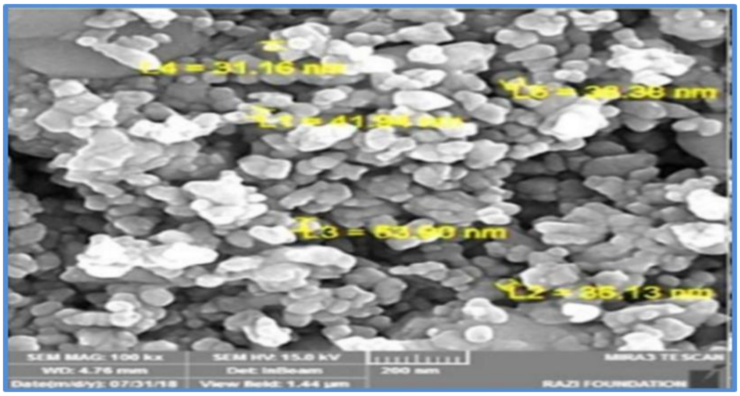

Figure 5: Scanning electron microscope (SEM) image with magnification (200nm) of the as-prepared zinc nanoparticles.

X-ray diffraction of ZnO Nanoparticles

These results confirm that the examined material is zinc oxide nanoparticles and that it is of high strength, and the average size of zinc oxide nanoparticles was extracted using the Debye-Scherrer equation, and the average size of the minutes was 20-40 nm, the obtained peaks were compared with the X-ray diffraction database as This base was strongly supported by the presence of zinc oxide nanoparticles.

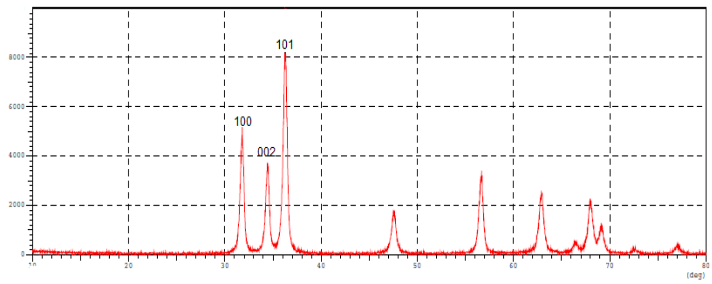

Figure 6: X-ray diffraction of zinc oxide

Atomic Force Microscopy AFM of ZnO Nanoparticles

Atomic force microscopy was used to validate the surface shape of zinc oxide nanoparticles biosynthesized by cactus aloe Vera extract, images were taken by 2D and 3D atomic force microscopy with an offline drop-coated, the results showed the variation in the phenotypic features of zinc oxide nanoparticles by adding through The three-dimensional image of the prepared zinc nanoparticles shows sharp protrusions with a maximum height of 6.98 nm, as shown in Figure 7.

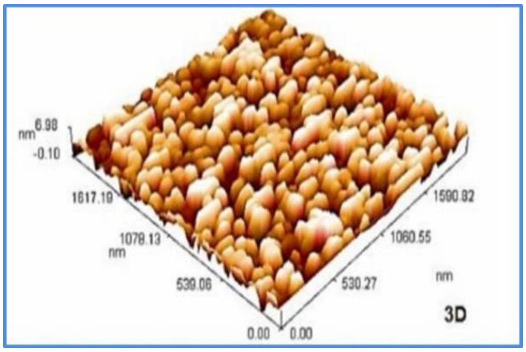

Figure 7: 3D AFM profile image of as-prepared zinc nanoparticles

Energy-dispersive X-rays of zinc nanoparticles (EDX) -7

The measurement of energy dispersion in X-rays shows the presence of zinc nanoparticles in very large quantities, as we note the band within region 1 and 8 evidence of the presence of prepared zinc grains as shown in Figure 8, and Table 1 gives the percentages of the sample elements to verify the chemical composition and purity prepared sample. As the results show that the sample contains zinc in the range of 74%, as we notice from Figure 7 the presence of zinc particles in the area 9.3,8,1.

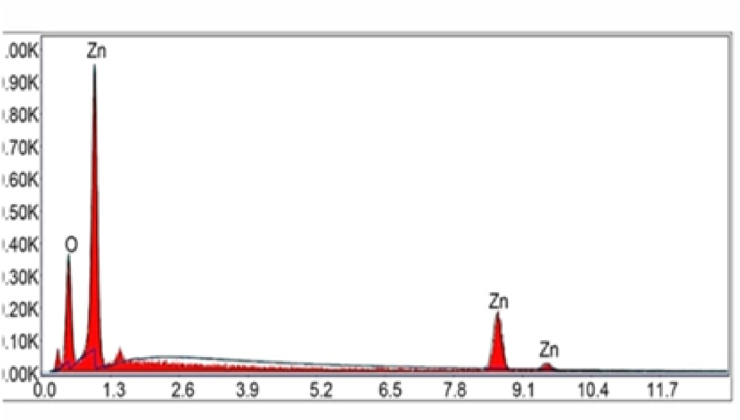

Figure 8: Energy X-ray scattering (EDX) of zinc nanoparticles

Table 1: Percentages of the components of the prepared zinc nanoparticle sample

Atomic %	Weight %	Elements
51.00	74.04	Zn
39.57	26.06	O

Transmission electron microscope for zinc nanoparticles (TEM) 8

The results shown in Figure 9 at the magnification strength of 150 nm show that the prepared zinc nanoparticles are within the Nano scale and have a spherical shape.

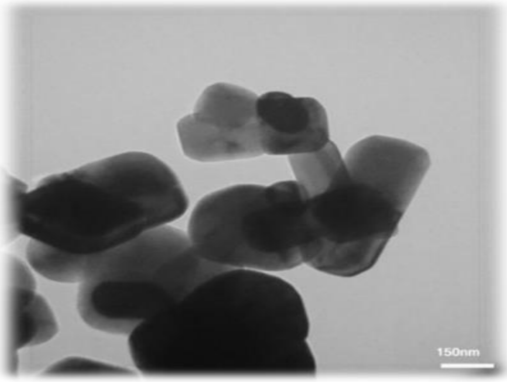

Figure 9: Transmission electron microscopy (TEM) image of the prepared zinc nanoparticles at 150nm magnification

Antimicrobial activity of zinc oxide nanoparticles biosynthesized by aloe Vera extracts against isolated bacteria and compared with that of commercial zinc particles.

Table 2 showed that there were significant differences between the inhibitory effect of all concentrations of commercial and biosynthetic zinc nanoparticles on *K. pneumoniae* isolated from otitis media patients. The two types of zinc Nano solutions, where the concentrations of commercial and synthetic zinc nanoparticles, which are 2.5, 5, 10, 20 mg/ml, gave inhibition rates of 10, 13, 15, 17 mm, respectively for the solution of biosynthetic zinc particles, while the commercial zinc particles solution gave the rates of retarding diameters are 0, 12, 12, 13 mm, respectively, it was observed that the highest inhibitory activity of the biosynthetic zinc nanoparticles solution was at a concentration of 20 mg/ml with an average inhibition diameter of 17 mm, while the lowest inhibitory activity was at a concentration of 2.5 mg/ml with an average inhibition diameter of 10 mm towards the above bacteria, while the commercial zinc nanoparticles solution gave the highest inhibitory activity was at a concentration of 20 mg/ml with an average inhibition diameter of 13 mm, and the lowest inhibitory activity was at a concentration of 5 mg/ml with an average inhibition diameter of 12 mm. The result we obtained is in agreement with the findings of Rasha *et al.* (2021), where zinc nanoparticles biosynthesized by acacia plant extract showed an inhibitory effect on *K. pneumoniae* bacteria. It also agrees with the findings of Farzana and Iqra, (2017) who observed the zinc nanoparticle solution has inhibitory activity against *K. pneumonia*.

Table 2: Inhibitory activity of commercial and biosynthetic zinc nanoparticles on *K. pneumonia*

Con. / Zinc nanoparticles	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	Average zinc nanoparticles
Commercial	13 c	12 c	12 c	0 e	9.3 B
Biosynthetic	17 a	15 b	13 c	10 d	13.8 A
Average concentration	15.0 a	13.5 b	12.5 b	5.0 c	

Similar letters mean that there are no significant differences between them with a probability level of 0.05

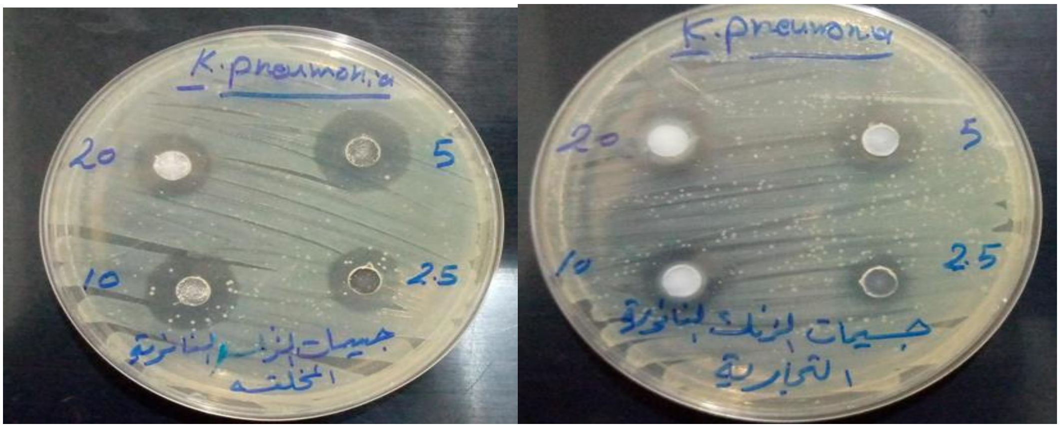

Figure 10: Effect of biosynthetic and commercial zinc nanoparticles on *K. pneumonia*

We conclude from the above that the most inhibiting activity of zinc nanoparticles against *K. pneumonia* is the solution of biosynthesized zinc particles by aloe Vera extract and its superiority over commercial zinc particles solution. Synthesis of nanoparticles and this is consistent with the results of Rasli *et al.*, (2020), which proved that aloe Vera extract was effective in the synthesis of zinc nanoparticles and tested its effectiveness against *E. coli*.

Studies indicate that nanoparticles have a disruptive effect on the integrity of the bacterial cell membrane, reduce the resistance of the cell surface to water, and down regulate the transcription of antioxidant resistance genes in bacteria (Pati *et al.*, 2014). Zinc oxide nanoparticles first damage the bacterial cell membrane and then penetrate into it (Stoimenov *et al.*, 2002).

However, zinc oxide nanoparticles are highly effective in inhibiting many pathogenic bacteria (Sultan *et al.*, 2015). It was shown by Rauf *et al.*, (2017), that the synthesized zinc oxide nanoparticles have an effect on *Ps. aeruginosa*, *K.*

pneumoniae and *Staph. aureus*. Some studies showed that conformation and oxidative stress are responsible for the inhibition activity of zinc oxide nanoparticles towards bacteria (Sourabh *et al.*, 2014). However, some studies indicated that the antibacterial activity may be due to a disturbance in the cell membrane activity (Brayner *et al.*, 2006). Another important mechanism is the activity of reactive oxygen species between cells that release free radicals, including H_2O_2, which is harmful to bacterial cells (Jones *et al.*, 2008).

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