

Camellia sinensis* (Green Tea) Mediated Synthesis of Zinc Oxide Nanoparticles and Detect its Antibacterial Activity against *Escherichia coli*, *Staphylococcus aureus* and *Acinetobacter baumannii

الشاي الاخضر كوسيط لتصنيع دقائق اوكسيد الزنك النانوية والتحري عن فعاليتها المضادة لبكتريا *Staphylococcus aureus*, *Escherichia coli*, and *Acinetobacter baumannii*

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Abstract

In the current study, synthesis and characterization of Zinc oxide nanoparticles (ZnONPs) and its application as anti-pathogenic bacteria were investigated. ZnO which has been prepared by using aqueous of green tea leaves extract (*Camellia sinensis*) as a reducing agent. The wavelength range was measured by Ultraviolet-visible spectroscopy (UV-Vis) for monitoring the formation of the nanoparticles, which showed sharp peak at 360 nm. The average size and shape of the nanoparticles were detected by using Atomic Force Microscopy (AFM) which was 88 nm with spherical shape. Fourier transform-infrared (FTIR). FT-IR spectra was documented for the ZnO nanoparticles synthesized by green tea extract to detect the biomolecules involved in the synthesis process. The antibacterial activity of crystal Zinc Oxide (ZnO) nanoparticles was explored against pathogenic bacteria that included *Escherichia coli*, *Staphylococcus aureus*, and *Acinetobacter baumannii*. The antibacterial test was conducted in solid media using different concentrations of ZnO and disk diffusion method, 100 µg/ml presented the best antibacterial activity, and further studies on the damage of bacterial genomic DNA of *Escherichia coli* and *Acinetobacter baumannii* were carried out using gel electrophoresis exposed the DNA fragment bands, this activity may be caused by the interactions between the surface charge of cell and nanoparticles. Reactive oxygen species (ROS) properties of the particles might disturb cell wall and great antimicrobial action

Key words: Nanoparticles, Zinc oxide, Pathogenic bacteria, DNA damage

المخلص

هدف البحث الى دراسة تأثير دقائق اوكسيد الزنك النانوية المحضرة بطريقة المستخلص المائي لاوراق الشاي الاخضر كمضاد بكتيري وصنفت هذه الدقائق باستخدام جهاز المطياف الضوئي والذي اظهر قمة امتصاص بحدود 360 نانوميتر لدقائق اوكسيد الزنك النانوية. وقد اظهرت نتائج القوة الذرية ان معدل حجم الدقائق النانوية بمقدار 88 نانوميتر وذات شكل كروي. وقد تم فحص التركيب الكيميائي الدقائق النانوية باستخدام مطياف الاشعة تحت الحمراء اذ اظهر وجود المركبات الحيوية على سطح دقائق الزنك النانوية اُختبرت فعالية دقائق اوكسيد الزنك المصنعة كمضادات لثلاثة انواع بكتيرية هي *Staphylococcus aureus* *Acinetobacter baumannii*, *Escherichia coli* في الوسط الصلب اظهرت النتائج ان تركيز 100 ميكروغرام /مل اعطى افضل نتيجة تثبيط، باعتماد قياس اقطار التثبيط لمراقبة تأثير دقائق اوكسيد الزنك النانوية. فضلا عن ذلك درس تاثير الدقائق النانوية على المادة الوراثية من خلال الترحيل الكهربائي وملاحظة تقطيع المادة الوراثية والتي من الممكن ان تعود الى التداخل مابين السطح المشحون للخلية والدقائق النانوية. او ان يعود سبب التأثير الى انواع الاوكسجين الاحادي.

الكلمات الدالة: الدقائق النانوية، اوكسيد الزنك، البكتريا الممرضة، تحطيم الدنا

Introduction

In the recent years different bacterial strains have developed resistance to conventional antimicrobial drugs, such strains include multi-drug-resistant isolates of *Staphylococcus aureus*, *Acinetobacter baumannii* and *Escherichia coli* [1]. The rapid spread of these isolates and the dangerous infections caused by them require the urge to find the replacement for the treatment of these MDR isolates derived the medical community to use them as novel antimicrobial agents. These new replacements must have the ability to interact and block microbial targets [2]. Silver and zinc oxide nanoparticles recently used as antibacterial agents, the mechanism of action of Ag NPs summarized by; Ag ions are released in aqueous solutions which cause the antimicrobial effect [3]. The exact mechanism of ZnO is yet unknown but it has been proven that the nanoscale ZnO has more antimicrobial activity than microscale ZnO and the smaller

concentration have better activity [4]. However; silver and zinc oxide NPs have a toxic effect on the prokaryotic and eukaryotic cells and have a damaging effect on the DNA [3,5]. ZnO nanoparticles can be synthesized using different chemical, physical and biological methods [6]. The biological techniques, using plant extract, microorganisms and enzymes, have been recommended as possible eco-friendly replacements [7]. The benefits of using plant or plant extract as reducing and coating agents during synthesizing nanoparticles is the preferred one over other biological methods, other biological methods, because it is a one-step biosynthesis process, shorten the long process of culturing and preserving of the cell, safe for human therapeutic use, and can also be scaled up for synthesis of large-scale nanoparticles. Many approaches are engaged for the synthesis of silver nanoparticles including reduction silver nitrate in solutions; microwave assisted, laser ablation, and biological or green synthesis methods [6, 8]. The latter is the most favorite ecofriendly fast method for synthesizing nanoparticles [9]. The current study differs from previous reports by using the green method instead of chemical method to synthesis ZnONPs. This study aims to use nanomaterials to achieve antibacterial activity against MDRs isolates through testing the activity of ZnO against MDRs isolates and test its ability to damage DNA of *Escherichia coli* and *Acinetobacter baumannii*.

Materials and Methods

Synthesis ZnO Nanoparticles

Zinc acetate dihydrate with 90% purity was perused from sigma and distilled water was utilized through all the experiments. 0.2 M of zinc acetate dehydrate was dissolved in 70 ml of distilled water and mixed for few minutes. Five gm of dried form of green tea leaf was added to 100 ml of distilled water and kept stir for 2h at 80°C by magnetic stirrer. Later on, the extract was kept to cool at room temperature and separated using filter paper (whatman No. 1); zinc acetate dihydrate then mixed with 30 ml of green tea extract. Finally the solution was dried at 60 °C in vacuum oven overnight to yield pale-white ZnO nanoparticles [10].

Characterizations of ZnO Nanoparticles

The maximum wavelength of UV–Vis (PD-303, Apel, Japan) absorption was ranged between 200–1200 nm to estimate the diameter and the shape of the ZnO. The shape and size of nanoparticles were analyzed by Atomic Force Microscopy (AFM) (Park Systems, Suwan, South Korea). Fourier transform–infrared (FTIR) spectra of the samples were recorded using FTIR (8400S Shimadzu / Japan).

Antibacterial Activity of the ZnO Nanoparticles

Three kinds of multi drug resistance bacteria were provided for this study from department of biotechnology –College of science/ Baghdad University, which were *Escherichia coli*, *Acinetobacter baumannii* and *Staphylococcus aureus*. To determine the antibacterial activity of ZnO nanoparticles the earlier mentioned bacteria cultivated separately in Luria–Bertani (LB) medium that prepared by adding 4.0 g, 2.0 g, and 5.0 g from peptone, yeast extract and NaCl respectively to 400 mL H₂O with pH value 7.2-7.5 and 1 mol L⁻¹ of NaOH before autoclaving. For producing LB agar 6.8 g of the agar is added to 1 L of LB medium. The activity of ZnONPs against *Staphylococcus aureus*, *E. coli* and *A.baumannii* were evaluated by disc diffusion method. Briefly, impregnated 6mm well with different concentrations of ZnONPs which were 25,50,75, and 100 µg/ml. Inhibition zones were checked after 24h at 37°C incubation [11,12].

Effect of ZnONPs on the DNA of Bacteria

E. coli and *A.baumannii* were incubated with the appropriate concentration of ZnO nanoparticles. After 24 hours, DNA was extracted by using Exiprep instrument with DNA extraction kit provided by Bioneer (Korea). Yield and purity of DNA samples were estimated by using nanodrop, and the quantity of extracted DNA was estimated by DNA electrophoresis performed in 1% agarose gel holding 10µg/mL ethidium bromide at 70 V, and the DNA fragments were observed by exposing the gel to ultraviolet light and documented by gel documentation [13].

Results and discussion

Producing nanoparticles by eco-friendly methods has recently become a hot topic of research, because of the growing applications in the biomedical areas. Various environmentally friendly and biological methods of ZnO nanoparticles synthesis have recently been reported [14, 15]. Meanwhile, decreasing the problem

of multidrug resistance bacteria becomes more important these years [16]. Herein, ZnO NPs were synthesized in the presence of green tea extract. The green tea extracts contains catechins and flavanols [17] which serve as reducing agents for the zinc acetate to ZnO nanoparticles. These bioorganic contents also serve as capping agents during the nanoparticle synthesis [18]. The mechanisms of action of the bioorganic molecules have been reported to provide a good activity giving the ability to stimulate the reduction precursor of nanomaterial in the formation of nanoparticles [19]. Phenolic composite shows great antioxidant agent and this property is a very good to reduce metal particles, consequently supporting the green synthesis of nanoparticles. Furthermore, higher substance of proteins, lipids and amino acids help the development of nanoparticles and prevent molecule agglomeration [20].

At the end of the ZnONPs synthesis, the presences of nanoparticles were detected through the UV-vis spectra. The absorbance peak was recorded at 360 nm in Figure (1). The green tea leaf extract reduction of the zinc acetic dehydrates to ZnO nanoparticles were produced as a powder with a pale white color. During synthesis, the addition of the tea extracts was accompanied by an immediate change in color which indicated the start of the formation of the ZnONPs. The color of the reaction went from colorless to white color; the color change indicates the formation of ZnO nanoparticles as confirmed by the UV-vis spectra. In Figure (1), the spectra showed a pronounced peak around 360 nm which had been previously reported for the synthesis of ZnONPs [21]. The highly blue-shifted absorption maximum occurring around 360 nm confirms the formation of ZnO product in nanoscale, because the absorption maximum for the bulk ZnO occurs at about 385 nm [11].

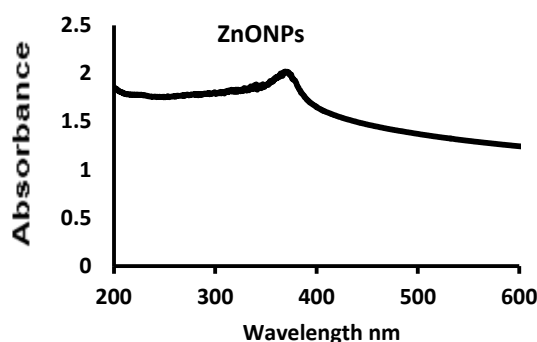


Fig. (1): UV-Vis spectrum of synthesized ZnO NPs by green tea.

Figure (2) presents the FTIR spectra of the ZnO nanoparticles synthesized by green tea, which illustrated the composition and quality of the product. FTIR spectroscopy estimate the retention of IR radiations by a sample plotted against the wavelength. The interpretation of the IR range includes the relationship of the absorption bands (vibrational groups) with the chemical composites in the sample [22]. Along these lines, the biomolecules present in plant extract that were served as of the reduction and capping agent in events of the green synthesis of nanoparticles can be recognized.

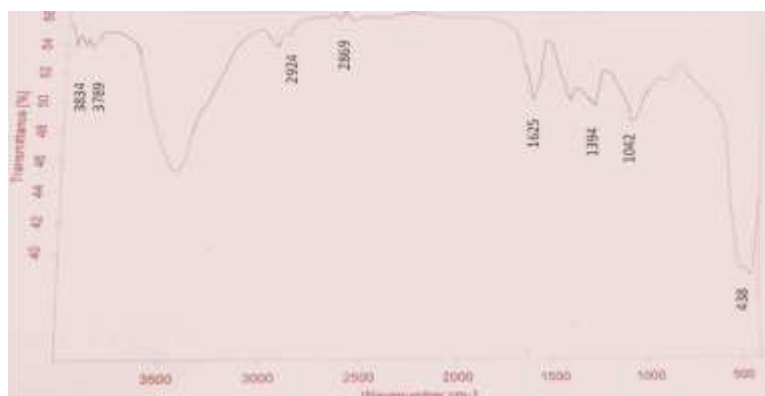


Fig. (2): FT-IR spectra of ZnO NPs synthesized by green tea.

In the IR range of green tea, the band around 3000 cm^{-1} because of extending vibrations of O–H group in water, alcohol and phenols and N–H extending in amines. The C–H stretch in alkanes and O–H stretch in carboxylic corrosive show up at 2924 and 2869 cm^{-1} individually. The band at 1625 cm^{-1} was ascribed to the C=C stretch in fragrant ring and C=O stretch in polyphenols. The C–N stretch of amide-I in protein gives the band at 1394 cm^{-1} . The C–O extending in amino corrosive causes a band at 1042 cm^{-1} . At long last the powerless band at 820 cm^{-1} is the after effect of C–H out of plane bend [22]. In this manner from the IR range it can be observed that green tea test was rich in polyphenols, carboxylic corrosive, polysaccharide, amino corrosive and proteins. The presence of the phenol may work as a good reducing agent and amide group in protein was responsible for the stabilization ZnO nanoparticles, the peak showing up at 438 cm^{-1} can attributed of ZnO molecules [22,23]. The surface morphology and size range of the ZnO nanoparticles were measured by AFM. The two-and three-dimensional geography of the ZnO nanoparticles was presented in Figure (3A) direct observation of the image exposed spherical shape of ZnO nanoparticles. The size distribution of the ZnO particles were showed in Figure (3B) which were in the range of 50–140 nm, and it clearly showed that most of the ZnO nanoparticles were at 88 nm sizes. This construction of ZnO NPs was produced during the reduction process by green tea extract that lead to nucleation of ZnO nanoparticles.

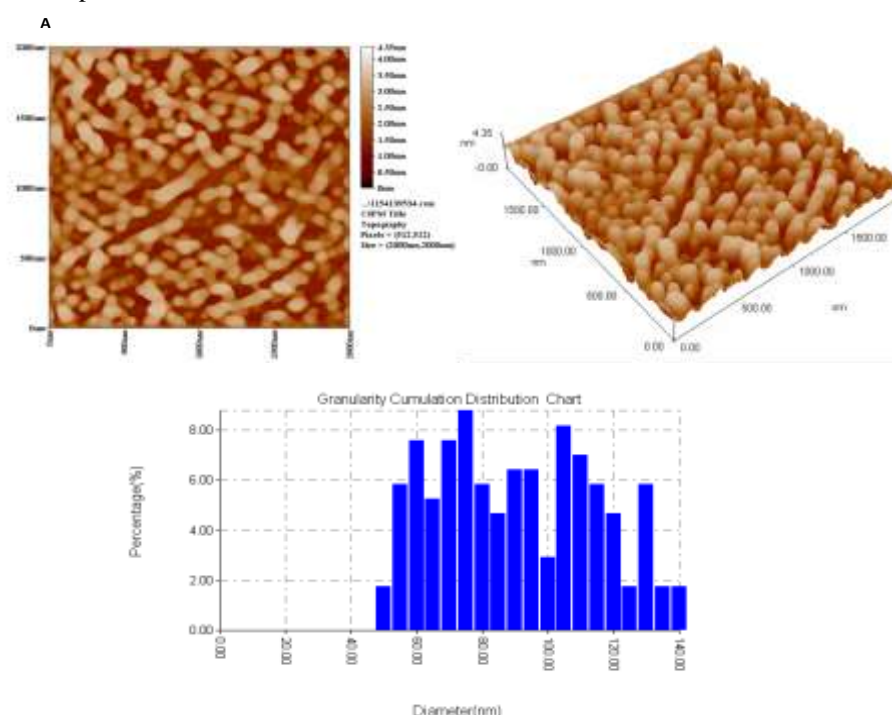


Fig. (3): AFM characterization of ZnO nanoparticles. (A) Surface morphology (B) chart for granularity distributed ZnO of nanoparticles

In this study ZnONPs showed remarkable antibacterial action against gram positive and negative bacteria even at low concentration as shown in Table (1). Based on the different in the bacterial structure the activity of ZnO on the gram positive bacteria was more than its activity on gram negative bacteria, because the interaction between nanoparticles and cell surfaces would be differ that lead to effect on the penetrability of membranes, since the entrance of nanoparticles inside bacterial cell induce oxidative stress consequently leading to inhibit cell growth and ultimately cell death [13,21,24]. There was a significant effect on *S.aureus* bacteria than on both the *E.coli* and *A.baumanii* bacteria. These results agree with the early study that reported the stronger antibacterial effect of ZnO on gram positive bacteria than gram negative bacteria [12]. The mechanism of action of ZnO nanoparticles was still unidentified; however, the predicted action that ZnO could adhere to the cell surface and caused damaged of the cell membrane or they can electrostatically interact with the surface of the cell [25-28]. Moreover, the activity of metal oxides could produce oxygen species like reactive oxygen species (ROS); at high levels ROS can diminish the physiological function of cell through diminishing cellular proteins, lipids, damage of DNA and further macromolecules [29-31].

Table (1): Zone of inhibition of antibacterial test of ZnO nanoparticles

Bioactive agent	Concentration $\mu\text{g/ml}$	zone of inhibition [Diameter, mm]		
		<i>E.coli</i>	<i>aureus</i>	<i>baumannii</i>
ZnO NPs	25	0	0	0
	50	11	14	6
	75	15	19	8
	100	20	23	9

To investigate the effect of ZnONPs on the genomic DNA of *E. coli* and *A.baumannii*, different concentrations of ZnO nanoparticles were tested. These isolates were evaluated by agarose gel electrophoresis as shown in Figure (4). The results were revealed destructive effects of ZnO on the genome of bacteria. It was clearly showed that there were single bands for chromosome and plasmids for normal *E. coli* and *A. baumannii* cell while DNA of *E. coli* and *A. baumannii* treated with ZnO was fragmented showing the evidence of action of nano-ZnO particle effect on the DNA damage thereby increasing antibacterial activity and these results are comparable with Prasad *et al.*, [13] investigation.

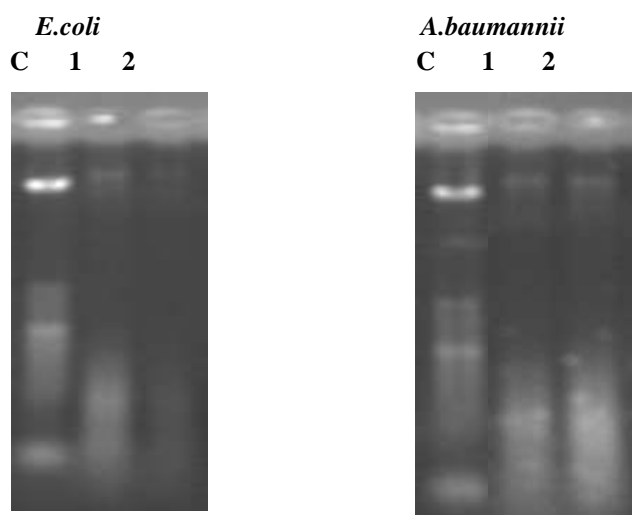


Fig. (4): The amount of DNA from normal *E. coli* and *A.baumannii* cells and bacterial cells treated by nano ZnO on agarose gel C: control, 1: 50 $\mu\text{g/ml}$ ZnO, 2: 100 $\mu\text{g/ml}$.

Conclusion

From the current investigation successful preparation of ZnO by economically method using leave extract of green tea. ZnO is achieved and characterized by UV-vis, FTIR and AFM. The FTIR studies obviously indicated the capping and reduction biomolecules being in the leave extract of green tea, the presence of high amount of biomolecules was responsible for the reduction process and the amino acids and amide bonds in protein were worked as stabilizer agents of the ZnO nanoparticles. The antibacterial tests demonstrated that 100 $\mu\text{g/ml}$ was the best antibacterial activity and the integrity of bacterial DNA was affected after treated with ZnONPs. The results have shown a destructive effect on DNA resulting in DNA degradation, therefore, inhibiting growth of bacteria. Hence conclude that using of ZnO nanoparticles can help in treating pathogenic bacteria and can be additionally used as potential production agent against bacteria

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