Original Article

Green synthesis and characterization of zinc oxide nanoparticles using Capsella bursa-pastoris (L.) leaf extract and its effect on pathogenic bacteria

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Abstract

Zinc oxide nanoparticles (ZnONPs) have received great attention as a special antibacterial agent and have been widely entered into the field of medicine. An extracellular procedure was performed in a short period. The formation of nanoparticles for visible and ultraviolet radiation was confirmed. This study was tested using plant zinc nanoparticles for diffusion along with disc diffusion. Test media was used E. coli, Corynobacterium , P. aeruginosa, St. salivarius, St. pneumonia , St. mutans, Staph. Epidermis and staph. aureus. Microbial property analysis of zinc nanoparticles by measuring the area of inhibition. zinc nanoparticles prepared from Capsella bursa-pastoris extracts, were toxic and gave the highest E. coli, staph. aureus and St. Edipermidis were 22,23,25mm respectively, while the growth of St. mutans, staph. aureus and 11, 13mm respectively, and the extract was maximized by SNPs synthesized from the extract comparison papers, the results indicate that nanoparticles could have an advantage in the conventional method as antibiotics.

Keywords: antimicrobial activity, green synthesis, zinc nanoparticles, plant synthesis.

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1. INTRODUCTION

Capsella bursa-pastoris(L.) Medic is one of the common medicinal plants among most Asia, African and Middle Eastern countries. The plant is popular in Iraq for its numerous qualities and application in the treatment of many metabolic and infectious disease conditions, management of menorrhagia, haematemesis, haematuria, diarrhoea and acute catarrhal cystitis [1]. It is abundant and flourishes in almost all parts of the world. The leaves are large and arranged in the form of a flower, spear-shaped serrated, and its location is close to the surface of the soil. The stems are distinguished by being slightly branched, rising in the center of the main leaves and bearing small numbers of small leaves .

The appearance is heart-shaped, the flowers are white and are self-pollinated, and the seeds are flat-shaped, yellow in color, characterized by an activity that attracts insects because they contain the sticky mucilage that is food for them [2]. Fatty acids, organic acids [3], The synthesis of nanoparticles by biological methods, especially plant extracts, is a proposal as possible environmentally friendly alternatives to chemical and physical methods [4]. Plants include natural chemical components such as aldehydes and alkaloids that contribute to the process of reducing and converting metallic elements into nanoparticles [5].

ZnO is superior to titanium dioxide in terms of biocompatibility, and it possesses the highest photocatalytic efficiency. [6], In addition, because of its increased selectivity, improved durability, and resistance to heat, it can be used to combat a wide variety of microorganisms, including S. aureus, E. coli, and Candida albicans, among others. [7], and when ZnO is reduced to the nanoscale, it shows good antimicrobial activity due to its large surface area to volume ratio and its distinctive chemical and physical properties [8]. It has a greater capacity to combat the growth of microorganisms. When it comes to the treatment of microorganisms, using inorganic compounds rather than organic ones has a number of benefits, including a lower risk of toxicity, increased durability, decreased resistance, and improved selectivity [9,10], However, bacteria that come into direct contact with zinc nanoparticles can be damaged. This is because the bacterial cell wall includes negative charges, such as roots Hydroxyl and superoxide, which prevents these molecules from penetrating the membrane. It is possible for hydrogen peroxide to penetrate the cell wall of bacteria, where it can then be taken up by the cell and eventually cause cell death[11,13,12]

One of the mechanisms responsible for bacteria wall damage is photoconductivity, which is photocatalytic process and ZnO has high photocatalytic efficiency [14]; This could contribute to its antimicrobial effect [15], Because it is not toxic to animal cells but is extremely toxic to bacteria, zinc nanoparticles make for an excellent bactericidal metal that is both safe and effective. [16,17], ZnO NPs showed high toxicity for all tested strains, which was attributed to the activity of NPs possibly being related to the toxicity of intrinsic mineral particles. The antibacterial activity of ZnO nanoparticles at a concentration of 125 mg/ml was examined, and the bacteria were shown to be affected by their contact with ions. All of the strains that were tested exhibited a significant amount of inhibition, ranging from 2% to 3%. [18]. Techniques Analyticales Zinc nanoparticles may also be toxic because of their interaction with the cell wall, which is another potential mechanism of toxicity. [19], and the increase in the permeability of bacteria cells, which leads to a loss of bacterial integrity [20] , Significantly increase the rate of bacterial inhibition and reduce inflammation after treatment with zinc nanoparticles [21], zinc nanoparticles were tested against Pseudomonas and Aspergillus niger, showing inhibition region [22].

This article's goals are to report a green synthesis of zinc nanoparticles using C. bursa-pastori leaf and to study the effectiveness of those nanoparticles on some pathogen-causing bacterial isolates.

2. Materials and methods

2.1. Plant extract and of zinc nanoparticles Preparation:

The plant extract was prepared from the leaves of the wild C. bursa-pastori from the central regions of Iraq in the period before flowering in the month of March. The dust and other particles are removed from the leaves of the plant by giving them a thorough washing in distilled water. After that, the section of the plant that had been washed is allowed to dry at room temperature, after which it is then cut into small pieces and crushed using a mortar, 100g of leaves were weighed and a sufficient amount of water was added to it and placed at a boiling point at 80°C for 60 min. with continuous stirring and after cooling, then filtered using Whatman No. 1 filter paper [23]. To purify the extract for later use in the preparation of zinc nanoparticles 5ml of 10mM Zn(NO3)2 6H2O was poured into the prepared homogeneous leaf extracts[24], At a temperature of 65 degrees Celsius, the mixture was stirred for twenty minutes, and the following was This paste, which was initially a thick yellow paste, was then completely dried at 400 degrees Celsius for two hours before being collected and packaged separately for additional characterization. During the sintering process, any impurities present in the sample are eliminated, resulting in a more pure form of NP. [25].

2.2.Analytical Methods

The UV-visible spectrophotometer, SEM, and XRD were evaluated and measured to finally confirm them as ZnO nanoparticles:

2.2.1. Ultraviolet Visible spectrophotometer: 1.7g of the synthesized zinc oxide nanoparticles were dissolved in 17ml of Sulfoxide Dimethyl, and it was measured in a UV spectrophotometer, as both visual examination and absorbance measurements were monitored. After that, the result of the spectroscopic reading of zinc oxide nanoparticles varies. This assay is used to verify the formation of zinc oxide nanoparticles [26].

2.2.2. The structure of Zn NPs was performed by XRD: The crystal size of the Zn NPs synthesis was calculated using Scherrer's constant ($D = 0.9\ \lambda/\beta\ \cos\theta$, where θ is the diffraction angle, $\lambda = 1.5406$ Å, and β is the peak width at half maximum. D-0.942, /B COS 0, where D is the average crystal field size perpendicular to the reflective planes, à is the X-ray wavelength. A laminated film of Zn NPs was formed on a glass plate and the XRD pattern was investigated in the range of 10 to 80 [27].

2.2.3. Scanning Electron Microscopy: This device depends on scanning the surface of the sample with a focused electron beam accurately for the purpose of obtaining microscopic images of the prepared zinc oxide particles.

2.3. The antibacterial activity of the biosynthesized ZnO NPs against pathogenic microorganisms , E. coli, Corynobacterium , P. aeruginosa, St. salivarius, St. pneumonia , St. mutans, Staph. Epidermis and staph. aureus were carried out on nutrient agar dishes using the disk diffusion method . Pure cultures of the microorganisms were provided by the Erbil hospital, Iraq. Cultured infectious bacteria and A sterile cork borer No. 4 was used to cut 3–4 10mm-diameter cups in each plate after the agar set. The cups were filled with 0.1 ml of ZnNPs samples and plant extract after the agar disks were removed. The plates were then placed in an incubator at 37 degrees Celsius for 24 hours, and the diameter of the results and growth inhibition zones were measured, averaged, and recorded. [28].

3. Results and discussion

3.1. Physicochemical Characterization

The present results indicated that adding 0.2g of the extract to 50 ml of water resulted from 5mM aqueous zinc oxides (ZnO) in the formation of the reddish solution after a whole day of incubation in the dark at room temperature, which indicates the generation of zinc nanoparticles (Fig. 1).

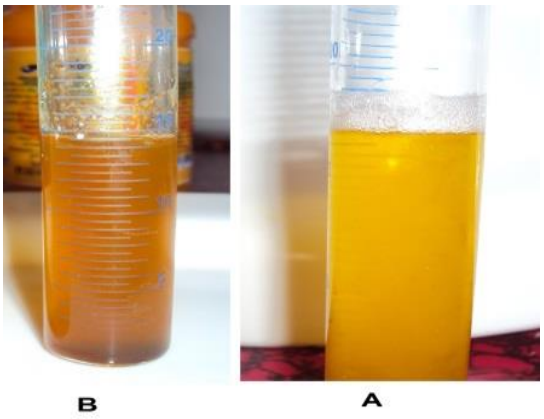

Fig-1: Color change of Zinc Oxide to Zinc nanoparticles by the addition of C. bursa-pastori leaf extract :(A) : Plant extracts; (B): Zinc nanoparticles

Zinc ions were reduced to ZnNPs after mixing with C. bursa-pastori leaf extract, followed by incubation for 24 h. in the dark, the color turned reddish-caffeine and this color change was previously observed by several investigators, the literature suggested that the color change appeared due to the surface resolution of the plasmon [29,30,31].

UV/VIS Spectrophotometry

This was established by conducting UV visible spectrophotometry in the range of 100-900 nm with a Shimadzu UV / VIS 2401PC instrument. The presence of secondary metabolites in plants, such as phenols and flavonoids, reduces the concentration of zinc ions in zinc oxide solution, which resulted in the maximum absorption being observed at 320 nm (Fig. 2)[32].

The plant extract has reducing and stabilizing effects, and its role in these processes can be thought of as ZnO's intrinsic absorption peak, which is caused by electron transitions from the valence band to the conduction band.[33].

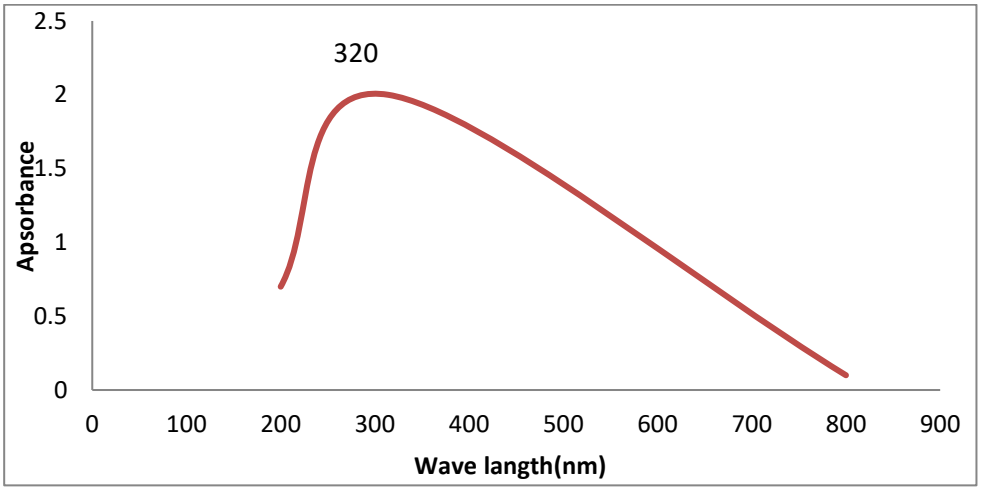

Fig-2: UV-Vis absorption spectroscopy of Zinc nanoparticles

Diagnosing nanoparticles with the XRD

The definite center line of the XRD peaks that can be seen in the X-ray diffraction pattern of ZnO nanoparticles indicates that the as-prepared material is composed of nanoscale particles. Following an examination of the XRD patterns, the diffraction peaks found at 32.66 degrees, 34.54 degrees, 34.31 degrees, 47.61 degrees, and 55.61 degrees were identified as belonging to the hexagonal phase of the particles. It was determined that the produced nanoparticles had an average crystalline size of 52.24 nanometers (nm). ICDD card number 01-079-0207 was a match for the peaks. The shape of the nanoparticle was a hexagon, and its lattice parameters were 3.3267 A 0 and 5.2114 A 0 respectively (previously reported). [34].

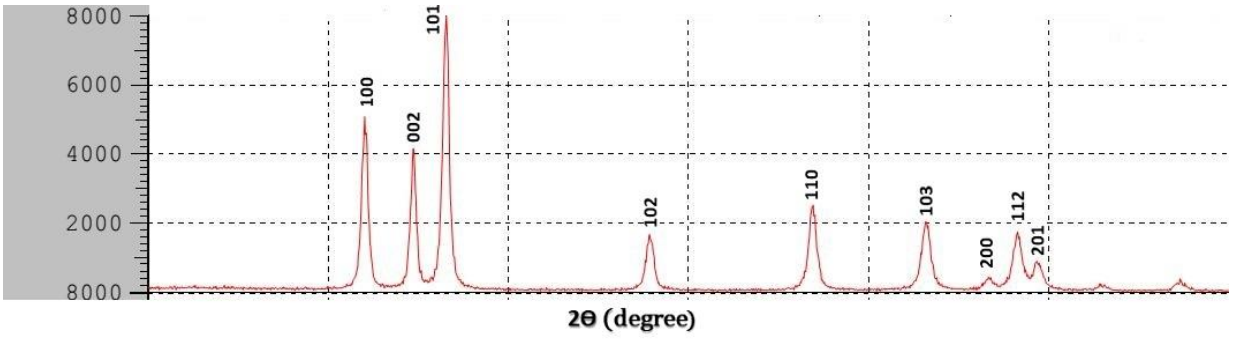

Fig. 3: ZnO nanoparticles X-ray diffraction spectrum

Diagnosis of nanoparticles by FESEM

A scanning electron microscope was used to determine the surface shapes of nanoparticles with an average diameter calculation. Fig. 4 shows clumps of individual zinc nanoparticles with many nanoaggregates, Zinc oxide nanoparticles showed agglomeration with the presence of some individual crystals, TEM analysis revealed that the size of ZN-NPs ranged between 30 and 53 nm Fig.4, and this is consistent with what was found by [35,36].

Fig. 4. FESEM image of ZnO nanoparticles

3.2. Antibacterial activity

The findings of the research project, which are summarized in Table 1, demonstrated the antimicrobial activity of zinc and C. bursa-pastoris leaf extract through the process of diffusion around the pits. This is a straightforward and speedy method that differentiates the antimicrobial activity of the samples that were tested and is explained by increasing the net area while keeping the concentration constant. Zn NPs from the plant showed the greatest antimicrobial activity against the tested microorganisms, as it led to the death of bacterial cells. Zinc nanoparticles have shown good antimicrobial activity due to their large surface area to volume ratio and their thinness. The results indicated that the leaf extract alone did not show a potent antimicrobial effect Tab.1 against G+ve bacteria. Zinc oxide showed a significant positive effect against the tested microorganisms (low visibility area). [37]. .

Table 1: Antibacterial activity of C. bursa-pastoris extract. (LSD 0.05= 0.023)

Tested bacteria	Inhibition Diameter Rate (mm)					
	Concentration of Zno NPs(mg\ml)			Concentration of Plant extract(mg\ml)		
Concentrations	10	20	30	10	20	30
Klebsiella	14	21	22	16	16	18
Escherichia Coli	13	18	25	15	16	18
Corynobacterium	12	14	21	11	13	14
Pseudomonas aeruginosa	7	13	20	10	11	12
Streptococcus pneumonia	14	16	22	13	16	18
Streptococcus salivarius	15	18	20	14	16	19
Streptococcus mutans	0	9	11	11	13	20
Staphylococcus epidermidis	11	15	23	15	17	19
Staphylococcus aureus	11	12	13	16	17	19

ZnO NPs enter cells by proliferation and endocytosis and interfere with mitochondrial activity, releasing ROS and Zn 2+.

These discharged ions might enter the membrane and damage the DNA, causing cell death. [38,39] , Bechambi found that aqueous ROS may cause cell death. Also, visible light [40], ZnO NPs increased cell death, and Gram-positive bacteria are more vulnerable to ZnO NPs than Gram-negative bacteria. [41] , and that inactivation of Gram-negative bacteria requires higher concentrations of ZnO NPs.

This is probable because Gram-positive bacteria's peptidoglycan layer can boost ZnO's attack inside the cell, whereas Gram-negative bacteria's lipopolysaccharides can resist it[42]. Also, Gram-positive bacteria were more sensitive to hydrogen peroxide than negative bacteria [43], and some microorganisms showed resistance to external factors because of their outer membrane and structure [44]. Dutta found zinc nanoparticles were used to inhibit the growth of E. Coli bacteria. by destroying the cell membrane and increasing the permeability of the cell membrane of bacteria[45].

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