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Appraising Antioxidant and Antibacterial Activities of Zinc Oxide Nanoparticles Synthesized Biologically by Iraqi Propolis

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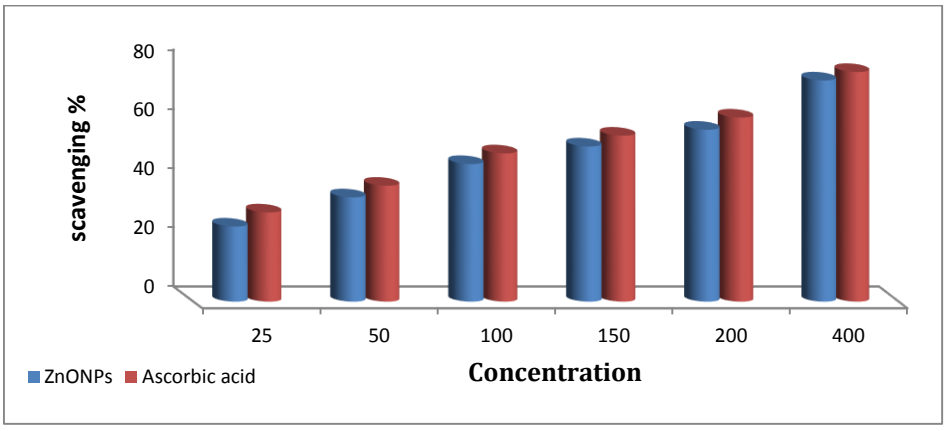
ABSTRACT

The biosynthesis of nanoparticles using natural products has recently attracted a lot of interest. The present study has investigated anti-biological activity of Zinc oxide nanoparticles (ZnONPs) synthesized biological by Iraqi propolis extract. ZnO NPs are well-known nanoparticles that are cost-effective, non-toxic and biocompatible. The antioxidant of ZnONPs was determined by using the free radical 2,2–diphenyl-1-picrylhydrazylhydrate (DPPH) assay, with different concentrations of the NPs. The results exhibited that with scavenging activity, the highest scavenging at 400µl/ml reached 74.70%, compared with ascorbic acid, which was used as standard at the same concentration reaching 77.56% ($P \leq 0.05$). The evolution of IC_{50} was 164.31 to ZnONPs compared with ascorbic acid IC_{50} =111.42. The antibacterial activity of the synthesized ZnONps was determined by using a disk diffusion assay. The results indicated effective antibacterial activity both in Gram-negative and Gram-positive bacteria in different concentration.

GRAPHICAL ABSTRACT



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Introduction

Zinc oxide (ZnO) is an inorganic metal oxide that can be used in a variety of nanostructures. It is found in the Earth's crust as the mineral zincite. It is a white-coloured powder that is almost insoluble in water. It is widely applied as an additive in many materials and products, such as glass, rubber, ceramics, cement, food, and sunscreen, also cosmetic products contain zinc oxide nanoparticles (ZnONPs) [1]. ZnONPs are attracting attention in a variety of sectors, including medicine, due to unique optical, chemical and electrical properties [2]. Thanks to its tremendous potential to produce excess reactive oxygen species (ROS), free zinc ions increase cell death; ZnONPs have shown promise in biomedicine, particularly in the fields of anticancer and antibacterial medicine [3]. Zinc oxide nanostructures have been shown to possess catalytic capabilities because of their enormous surface area and strong catalytic activity. As a result, these features can help improve a variety of synthetic processes [4]. There are a variety of ways for producing nanoparticles (NPs), including chemical, physical and biological approaches. Because physical routes need a lot of extra energy and chemical approaches produce undesirable by-products, biosynthesis methods have been developed [5-6]. NPs are synthesized by reducing minerals to their oxide form using compounds found in bioactive natural and plant products, resulting in NPs with good stability [7]. Plants [8], natural products [9] and microorganisms [10] are used in biological ways using the technique of self-assembly from fresh nuclei of atoms that grow into a NP. Propolis is a natural product, which is one of the fascinating honey bee products (bee glue). It also plays an important role in honey bee social immunity [11-12]. The chemical makeup of propolis varies; it changes according to the cell and seasonand place [13-14]. Many compounds have been reported in propolis, including flavonoid, phenolic acids, and phenolic aldehydes [15]. Propolis is a rich source of natural antioxidants that really can help protect against oxidative stress. Antioxidants (AH) play a key role in lowering the risk of a variety of chronic diseases [16]. It is also an anti-bacterial; it works on two levels. The first applies a direct effect on the microorganism, whereas the second is linked to immune system activation, which causes the organism's innate defences to be activated [17]. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is an organic nitrogen radical with a long life time that is commercially available and does not require pre-generation like other scavenging tests [18]. It is a stable, methanol-soluble molecule with a deep violet color. AH or other radical species can provide an electron or a hydrogen atom to this stable radical reducing it to DPPH-H [19-20].

The aim of this study was therefore to study the AH and bacteria activity for the ZnONPs that were biosynthesized from Iraqi propolis**.**

Materials and Methods

The Zinc acetate dihydrate was obtained from BDH/ England**,** Iraqi propolis from local markets in Baghdad, Iraq. DPPH was procured from Merck, Muller Hinton Agar/Hi. Media. All isolates were collected from the Environment Department/Ministry of Science and Technology and were *Staphylococcus aureus, pseudomonas, and Escherichia coli* bacteria.

Methods

ZnONPs were biosynthesized from Iraqi propolis by an eco-friendly method. X-ray diffraction and scanning electron microscope were used to characterize the nanoparticles.

AH activity

The free radical scavenging activity was determined by DPPH assay [21] with some modifications. Varied concentrations (25, 50, 100, 150, 200, 400 µg/ml) of ZnONPs and ascorbic were prepared. The standard was ascorbic acid. 2.5ml of (0.1 M) DPPH solution was added to 500 µl of the prepared solutions for ZnONPs. The mixture was kept in a dark environment for 30 minutes at 25 °C. Shimadzu UV-1650 spectrophotometer was used to read the absorbance of the reaction of the mixture at 517 nm, and the percentage for scavenging was calculated using the following equation:

$$\%\text{Scavenging}=\frac{Absorbance\ control-Absorbance\ sample}{Absorbance\ control}\times 100\%$$

Antimicrobial studies

The synthesized ZnONPs were evaluated for an antimicrobial activity for three different concentrations 3, 4, 5 mg/ml dissolved in distilled water. Gram-positive bacteria like *Staphylococcus aureus* were isolated; two types of gram-negative bacteria were *Escherichia coli* and *Pseudomonas aeruginosa* [22]. 33.9 g Muller Hinton Agar was dissolved in 1000 ml distilled water to make Agar Medium. The disintegrating media was autoclaved for 15 minutes at 15 lbs pressure and 121°C. While still liquid, it was thoroughly mixed and placed into Petri plates (25-30 ml/plate). Antimicrobial activity testing are performed on this medium [23-24]. The bacterium isolates were grown at 37°C for 24 hours after being introduced into sterile nutritive broth. Each disc was pressed down to ensure complete contact with the medium and the target isolates were infected using the spread plate method with a 24-hour-old culture. Each plate was checked after 24 hours of incubation. The zones of inhibition that followed were always circular, with a lawn of growth that was confluent. The activities were represented by the average diameter of the inhibition zone as a whole. Each test was repeated many times.

Statistical analysis

We used the Statistical Analysis System- SAS (2012) program [25]. The results were calculated as a mean, standard deviation from three separate DPPH tests performed under the same experimental conditions.

Results and Discussion

Characterization of ZnO nanoparticles

The synthesized ZnONPs are spherical in shape with an average size less than 50 nm (Figure1 and Figure 2).

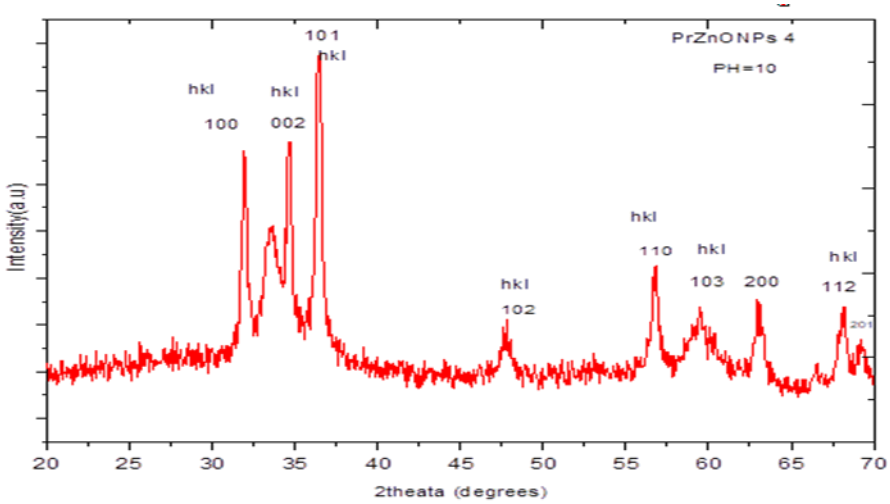

Figure 1: XRD patterns of ZnONPs

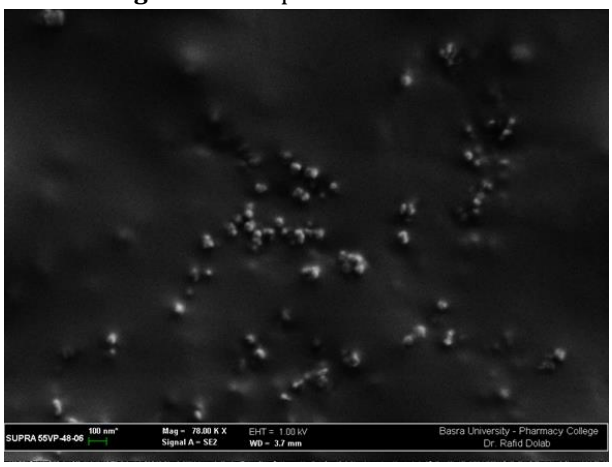

Figure 2: SEM image of ZnONPs

AH activity

The radical scavenging activity by ZnONPs on DPPH is shown in Table 1 and Figure 3. The free radical 1, 1- diphenyl-2- picrylhydrazyl is reduced by obtaining an electron or hydrogen from a donor atom [26]. When a DPPH radical solution is combined with an antioxidant/reducing agent, the colour of the resulting hydrazine changes from purple to yellow, observed an increase in scavenging as the concentration increases, reaching the highest scavenging at 400µl/ml, to 74.70%, while ascorbic acid at the same concentration reached 77.56%. (P≤0.05). Furthermore, there is no significant difference between the effect of Ascorbic Acid and ZnONPs on DPPH. The value of the half maximal inhibitory concentration (IC50), as shown in Figure 4, was 164.31 µg/ml for ZnONPs, while the value of IC_{50} of ascorbic acid was 111.42 µg /ml. ZnONPs performs scavenging hydroxyl radicals effectively because it can give an electron [26].

Table1: Scavenging of ZnOPNs and ascorbic acid at different concentration

Concentrations µg/ml	ZnONPs % scavenging	%scavenging Ascorbic acid	LSD value
25	25.42 ±0.45	30.20 ±0.73	5.039 NS
50	35.30 ±0.64	39.24 ±0.97	5.822 NS
100	46.55 ±0.51	50.15 ±1.31	5.64 NS
150	52.46 ±0.39	56.11 ±1.42	4.93 NS
200	58.12 ±0.87	62.21 ±1.69	5.79 NS
400	74.70 ±1.56	77.56 ±1.85	4.97 NS
(P≤0.05)			

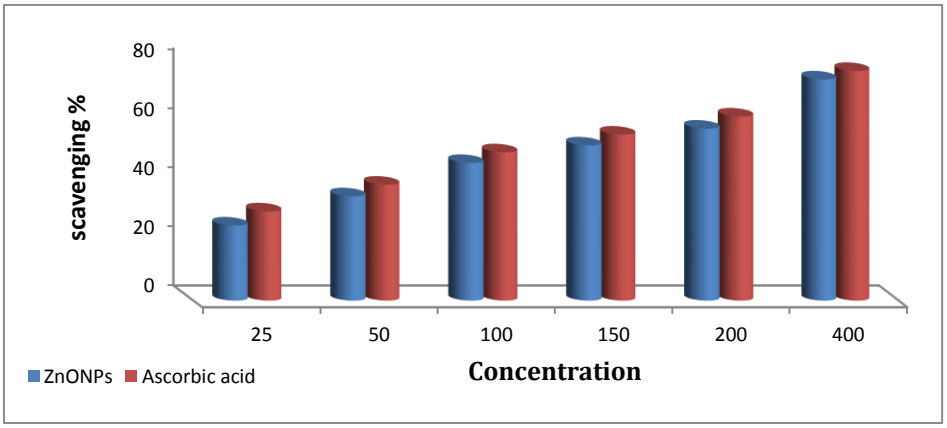

Figure 3: Scavenging activity of ZnONPs and ascorbic acid on DPPH

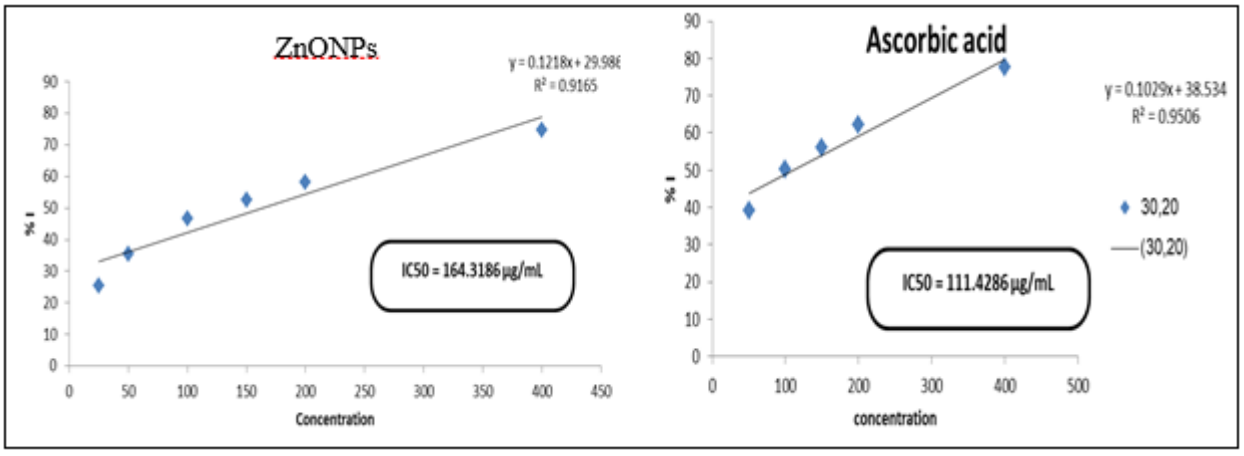

Figure 4: IC_{50} value of ZnONPs by Iraqi propolis and ascorbic acid through a regression equation

Antibacterial activity

Antibacterial activity of synthesized ZnONPs against Gram-positive (*Staphylococcus aureus*) and gram-negative *(Pseudomons* and *Escherichia coli*) bacteria were revealed and the zone of inhibition was measured (Table 2 and Figure 5). The results indicated that ZnONPs showed effective antibacterial activity in Gram-negative and Gram-positive bacteria in different concentrations. Furthermore, this activity increases with the increase of concentrations. NPs disrupt the cell wall and membrane permeability, which harms biomolecules like DNA and protein because they hinder key activities like DNA replication and protein synthesis and cut off energy access to bacteria [27].

Table 2: Antibacterial activity of ZnONPs of different concentrations

Average of inhibition zone (millimetre)				
C control	**concentration 3 mg/ml**	**concentration 4 mg/ml**	**Concentration 5 mg/ml**	**Pathogenic bacterial isolates**
-	12	16	23	S.aureus
-	16	18	23	E.coli
-	12	14	22	P.eroginosa

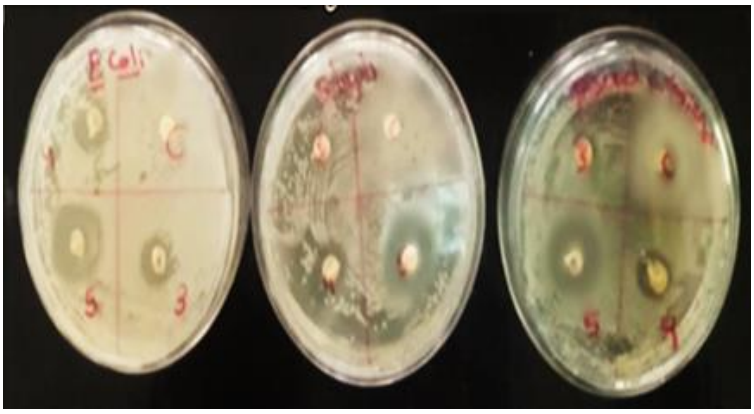

Figure 5: Zone of inhibition for synthesized ZnONPs using Iraqi propolis extract

Conclusion

It can be concluded that ZnONPs synthesized from Iraqi propolis extract is safe, non-toxic, and ecofriendly with high antioxidant and antibacterial properties, making it a good source of antioxidants, when compared with ascorbic acid. Based on the in vitro studies described above, it also possesses antibacterial activityand both the antioxidant and antibacterial activities increase with increasing concentrations.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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