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Characterization of Zn Nanoparticles of Platonus Orientalis Plant, Investigation of DPPH Radical Extinquishing and Antimicrobial Activity

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ABSTRACT

Zinc(Zn) is one of the favorite elements because of its nanostructures and properties. It is known to be involved in many tasks, such as DNA protection, membrane stabilization, protein, ribosome, and carbohydrate production. The aim of our present study is to examine some biochemical parameters directly related to human health as a result of the characterization of the *Platonus orientalis* plant with Zn. In our study, ZnO particles were obtained with the green synthesis method using Platonus orientalis plant extract and $ZnC_4H_6O_4$. Scanning electron microscopy was used for SEM and SEM / EDX images of synthesized ZnO NPs. The characterization process was performed using a UV visible spectrophotometer and fourier transformed infrared spectroscopy (FT-IR). As a result of DPPH(2,2-diphenyl-1-picrylhydrazyl) analysis, zinc nanostructures have an important antioxidant potential. The antimicrobial activity of synthesized metal nanoparticles against some pathogens causing disease in humans by the disk diffusion method was investigated. ZnO NPs/Po has been shown to have antibacterial properties against Bacillus subtilis ATCC 6633, Escherichia coli ATCC 25952, and Pseudomonas aeruginosa ATCC 27853. According to the results of the study, the effects of ZnO NPs / Po, we believe that this material will shed light on the scientific world, especially in the field of pharmacology.

Keywords: *Platonus orientalis*, antimicrobial, antioxidant, nanoparticle, DPPH

Introduction

The rapid advancement of nanotechnology creates a new field for scientific research. This turns (-) attention to the nanoparticles that constitute the core of nanatechnology (1). Since nanoparticles have important properties such as chemical catalytic and biological activity, they have an important place in scientific and economic terms in many fields. Using these features; in medical applications, it is used in areas such as antimicrobial and anti-cancer agents, the food and cosmetics industry, electronics, magnetics, optics, and the space industry. Metallic nanoparticles can be used in a variety of applications. The importance of metallic nanoparticles synthesized by biological applications for their antimicrobial and cytotoxic effects is increasing day by day. In particular, the antimicrobial effects of nanoparticles such as silver, copper, zinc, and gold have been reported (2).

While antibiotics are used in the treatment of bacterial diseases; it also affect the immune system negatively by eliminating beneficial bacteria that synthesize K vitamins in the intestines and absorb vitamins and digest food. Metal nanoparticles make an important contribution in the search for antimicrobial agents to eliminate these negative effects. Many studies have shown that metal nanoparticles have an antimicrobial effect (3). Antimicrobial effect activities; It depends on the type of plant, variety, concentration, variety of food, and processing conditions. Antibiotics used today affect the cell wall, protein structures, lipid structures, and DNA structures of bacteria. On the other hand, nano-particles come into direct contact with the bacterial cell wall without penetrating the cell. Thus, although bacteria develop resistance to antibiotics, they cannot develop resistance to NPs (4).

Medical or traditional medicine forms obtained from herbs and their preparations have been used by people for centuries(5). There are a lot of plants used for medicinal purposes by people(6). In recent years, studies on plants and interest in plants have increased for reasons such as the lack

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of sufficient financial resources and chemical industry in developing countries, dangerous side effects seen in synthetic compounds, and the cheaper and easier production of herbal drugs than synthetic ones (7). In a previous study, it was revealed that evening primrose oil reduced oxidative stress in rats with metabolic syndrome (8). Problems in the search for antimicrobial agents in metal nanoparticles can be solved with the green synthesis method. The plant itself, leaves, fruit, or roots are used to create nanoparticles with the green synthesis method. Plant extracts and metal ions are subjected to bioreduction. Plant metabolites play an important role in reducing and stabilizing metal ions into nanoparticles. Many plant species contain bioactive alkaloids, phenolic acids, polyphenols, proteins, sugars, and terpenoids (9). Plants provide important advantages due to their low cost and lack of harm to the environment. For this reason, plants are seen as the best choice for biological nanoparticle synthesis.

The plane tree (Platonus orientalis) is a tall, thick-diameter tree that forms the platonus genus in the platanaceae family (10). Its homeland is north America, east of Europe and Asia. The plane tree, which is found almost everywhere in Turkey, especially in the Marmara, Aegean, and Mediterranean regions, is considered (-) the national tree of our country. In our country, as in the forest areas along the streams and river beds, it is found as an ornamental plant shade tree on waterheads, roadsides, parks and gardens. Leaves fall off in autumn. These fragmented leaves remain clean as they are not affected by the dust from the environment. Today, sycamore leaf is used in the treatment of many diseases. Platonus orientalis is used as an antiseptic and antimicrobial agent in urinary system disorders. In addition, the cytotoxic activity of chempferol glycosides in human leukemia cells was determined (11).

Platonus orientalis collected in rural areas of Van was collected in accordance with the method. The washing and drying processes were carried out in the laboratory environment. Then, some biological and biochemical parameters were examined to investigate the effect on human health. Antioxidant capacity and antibacterial activity against gram positive and gram negative bacteria were investigated by the DPPH method. The ZnO NPs/Po structures obtained by the interaction of Platonus orientalis plant with Zn metal were characterized by SEM-EDX, FTIR andn UV.

Materials and Methods

Preparation of plant extract: The collected Platonus orientalis plant was washed with distilled water and then kept at room temperature to dry. The dried plant was thoroughly powdered with an electric blender. 50 grams of powdered plant were mixed into 250 mL of distilled water and heated for 15 minutes in a mixer at 80 ^{o}C. The extracted was filtered through Whatman No.1 filter paper and stored at 4 °C for future research.

Green Synthesis of Zinc Oxide Nanoparticles: For the synthesis of ZnO NPs, a 1 mM, 500 mL $ZnC_4H_6O_4$ aqueous solution was prepared. At room temperature, 100 mL of Platonus orientalis plant leaf extract was allowed to react in a 1000 mL flask at room temperature under constant conditions. The formation of zinc nanoparticles was determined by the change of color of the colorless solution to dark brown within 30–40 minutes. The nanoparticles obtained by green synthesis were separated by centrifugation at 10,000 rpm for 5 minutes. This process was repeated three times, free zinc ions were removed from the environment, and the resulting nanoparticles were determined as ZnO NP / Po. It was left to dry in the oven and stored at +4 °C for further processing.

Characterization of ZnO NPs / Po: The formation of zinc nanoparticles was confirmed using the Shimadzu UV-2450 instrument, measuring the ultraviolet-visible (UV-Vis) absorption spectrum in the 200-800 nm range. With the Fourier transform-infrared (FTIR) analysis performed on a spectrometer named Perkin Elmer-Spectrum in the range of 500-4000 cm^{-1}, ZnO revealed the presence of phytochemicals in NPs / Po. By scanning the sample surface with a scanning electron microscope or SEM (scanning electron microscope), the topography and composition of the surface were taken. At the same time, elemental analysis was performed with Zeiss Smart EDX-branded EDX.

Antioxidant activity (DPPH): The DPPH quenching activity of ZnO NPs/Po, the extract of the study subject, was calculated using the method found before. BHA and BHT were used as positive controls in this procedure. The experiment was carried out using 0.1 mg/mL DPPH methanol solutions. DPPH and extracts in the same ratio were prepared in 7 different concentrations of 5, 10, 15, 20, 25, 50, and 100 µg/mL. 3 mL of ZnO NPs/Po extract and a positive control were taken and DPPH solution was added to them. The mixtures formed in the tubes were incubated for 30 minutes at room

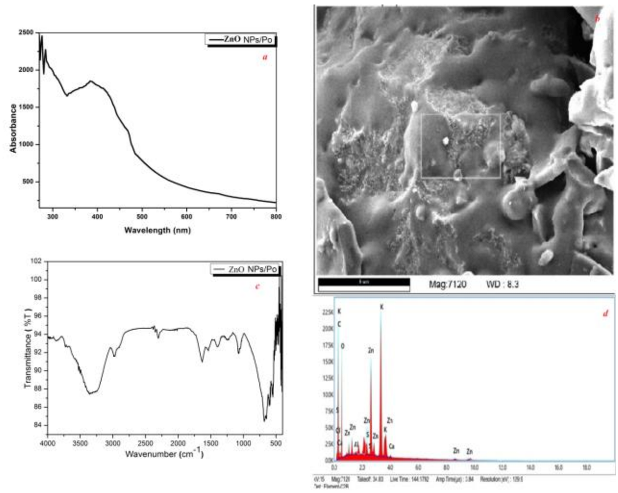

Fig. 1. a- UV-vis spectra, b- SEM ,c- FT-IR spectra and d- EDX of ZnO NPs/Po samples

temperature in the dark. At the end of this period, absorbance values were read at 517 nm.

% I = [(Acontrol-Asample) / Acontrol] × 100

As a result of these processes, a graph of ZnO NPs/Po concentration versus increasing DPPH ethanol concentration was obtained. This graph was obtained using the above equation.

Antimicrobial Activity: The ZnO NPs/Po clusters were obtained by using Zinc Acetate ($ZnC_4H_6O_4$) with the extract obtained from the leaves of the Platanus orientalis plant. The antimicrobial activity of plant extract and zinc nanoparticles were investigated. Six pathogenic microorganisms were used in the study. Pathogen isolates were obtained from Van Yüzüncü Yıl University, Department of Molecular Biology and Genetics. Antimicrobial activity tests were performed using the disk diffusion method (12). Neomycin (10 µg) was used as a positive control group. Inoculation was performed on Müller Hinton medium for the disk diffusion method and activation of microorganisms.

Results

Characterization of ZnO nanoparticles: For the structural and morphological characterization of ZnO nanoparticles prepared by green synthesis using the Platonus orientalis plant, SEM/SEM-EDX, FT-IR, and UV-vis techniques were used, respectively. Figure 1 shows the SEM images of the Zn NPs/Po sample taken at different scales and the EDX spectrum obtained from one of these images. From SEM images of different scales, it can be seen that there are Zn nanoparticles (Figure 1-b). In the EDX spectrum (Figure 1-d), Zn, C, and O elements, which form the structure of the Zn NPs/Po sample, as well as

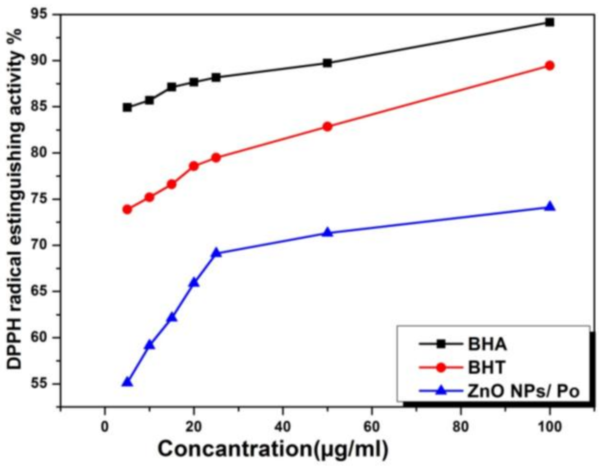

Fig. 2. DPPH Radical Scavenging Activity of ZnO NPs/Po. BHA (Buthly Hydroxy Anisol), BHT (Butyl Hydroxy Toluene)

different metals, are thought to be in the structure of the plant. It was determined that the UV image of ZnO NPs/Po (Figure 1-a) coincides with the reference studies (13). In the study, sharp peaks below 600 cm^{-1} correspond to the metal-oxygen (ZnO stretching vibrations) vibration mode. The peaks between 950-1600 cm^{-1} show the peaks formed by functional groups in the existing plant Platonus orientalis (Figure 1-c). It is thought that the peaks between 3400-2850 cm^{-1} belong to the hydroxyl organic component, and the sharp peak between 1800-2900 cm^{-1} belongs to many organic components.

Antioxidant activity (DPPH): Various methods are used to measure the antioxidant activity of pure compounds and plant extracts (14). To date, many methods have been developed to measure antioxidant capacity (15). DPPH radical quenching activity; it is the most widely used method due to its ease of use, low cost, ease of use accurate and quick results (16). This method is based on the scavenging of the DPPH radical by the antioxidant due to a redox reaction. It is a deep purple radical. By taking an antioxidant proton, it transforms into a colorless 2,2-diphenyl-1-picrylhydrazyl molecule (17). In our current study, the antioxidant activity of ZnO NPs/Po was compared with that of the BHA and BHT, which are positive controls (Figure 2). At the highest concentration of 100 µg/mL, DPPH radical quenching effect activities are 93.87 % for BHA, and 88.42 % for BHT, and 73.79 % for ZnO NPs/Po. The antioxidant activity of the Platanus orientalis plant has not been calculated in any previous study. When compared with studies with different plants, radical quenching activity appears to be significant (18).

Table 1. Zones Formed As A Result of Antimicrobial Activity

Test Mikroorganisms	Zone of Inhibition (mm)		
Bacteria	Extract	Po-ZnO-NPs	Neomycin
Bacillus cereus ATCC 10876	9±2.2		19±1.0
Bacillus subtilis ATCC 6633	11±1.0	8±1.5	22±1.0
Escherichia coli ATCC 25952	8±3.0	10±1.1	12±3.5
Pseudomonas aeruginosa ATCC 27853	10±2.0		
Staphylococcus aureus ATTC 29213			15±2.5
Fungus			
Candida albicans ATTC 90028			21±2.4

Antimicrobial Activity: In the study, it was observed that ZnO NPs/Po formed zones varying between 8 ± 3.0 and 11 ± 1.0 mm against Bacillus cereus ATCC 10876, Bacillus subtilis ATCC 6633, Escherichia coli ATCC 25952 and Pseudomonas aeruginosa ATCC 27853 bacteria. On the other hand, it was determined that Po-Zn-NPs clusters formed zones of 10 ± 1.1 mm against Escherichia coli ATCC 25952 bacteria and 8 ± 1.5 mm against Bacillus subtilis ATCC 6633. It was determined that zinc nanoparticles have antibacterial activity against some bacteria. In addition, it was observed that it had no antifungal effect on the Candida albicans fungus. Zones formed as a result of antimicrobial activity are given in Table-1. In many articles, the antimicrobial activities of plants have been examined, and it has been observed that these plants have antimicrobial effects (19,20,21). In our current study, the antimicrobial effect of ZnO NPs/Po was first demonstrated. According to the results obtained in our study, ZnO NPs/Po has an antimicrobial effect.

Our current study is the first study on the ZnO NPs/Po nanoparticles obtained as a result of the characterization of the Platonus orientalis plant with Zn. We believe that this work will contribute to the literature. According to the findings obtained as a result of the study, it is seen in the comparison study made with the positive controls BHA and BHT that ZnONPs/Po is a good antioxidant. It was determined that as the concentration was increased, DPPH increased radical quenching activity. In addition, the antimicrobial study of ZnONPs/Po was performed for the first time, and it was determined that ZnO NPs/Po has antibacterial activity against some bacteria. In addition, it was observed that it had no antifungal effect on the Candida albicans fungus. According to our study results, it is thought that ZnONPs/Po may have a protective effect against many diseases that may occur as a result of oxidative stress, especially cancer. In addition, we believe that conducting more comprehensive studies on ZnONPs/Po will contribute scientifically to pharmacology and other health fields.

References

1. Meydan I., Burhan H., Gür T. , Seçkin H. , Tanhaei B. , Şen F. Rheum kaburgalarının ZnO nanopartikül ile karakterizasyonu ve in vitro antidiyabetik, antibakteriyel, DNA hasarı önleme ve lipid peroksidasyon önleme aktivitesi . Çevre Araş. 2021 ;112363.
2. Umbreen Khattak., Rehman Ullah , Shafqat Ali Khan , Saiqa Afriq Jan , Abdur Rauf Mohamed Fawzy Ramadan . Synthesis, characteristics and biological activities of silver nanoparticles from Euphorbia dracunculoides Eurasian Journal of Biosciences Eurasia J Biosci 13, 2011: 2249-2260
3. Beykaya M., Çağlar A. Bitkisel Özütler Kullanılarak Gümüş-Nanopartikül (AgNP) Sentezlenmesi ve Antimikrobiyal Etkinlikleri Üzerine Bir Araştırma, Akü femübid 16 035403,2016: 631-641.
4. Cansız E.İ. Kırmusaoğlu S.. Nanoteknolojide Nano Gümüşün Antibakteriyel Özelliği. Haliç Üniversitesi Fen Bilimleri Dergisi, 2018;1: 119-130
5. Altındağ F., Özdek, U. Protective Eff ects of Chitosan and Chitosan Oligosaccharide on Sodium Fluoride-Induced Testicular Damage in Male Rats: A Stereological and Histopathological Study. Kafkas Univ Vet Fak Derg 2021; 27 (2): 183-189.
6. Pınar N., Topaloğlu M., Özer C., Alp H. Kardiyoloji Hastalarında Bitkisel Ürün Kullanımı. Turk Kardiyol Dern Ars 2017;45(7):614-622
7. Altindağ F. Rağbetli, M.Ç. The effect of maternal treatment with diclofenac sodium and thymoquinone on testicular parameters in rat

offspring. Revista Internacional de Andrología 2021; 9(1): 34-40

8. Mert H., İrak K., Çibuk S., Yıldırım S., Mert N. The effect of evening primrose oil (Oenothera biennis) on the level of adiponectin and some biochemical parameters in rats with fructose induced metabolic syndrome. Archives of Physiology and Biochemistry 2020
9. Shah M., Fawcett D., Sharma S., Tripathy S., & Poinern G. Green synthesis of metallic nanoparticles via biological entities. Materials, 2015: 8(11), 7278-7308
10. Baytop T. Türkçe Bitki Adları Sözlüğü. Ankara, Türk Tarih Kurumu Basımevi;1994
11. Dimas K., Demetzos C., Mitaku S., Marselos M., Tzavaras T. ve Kokkinopoulos D. Cytotoxic activity of kaempferol glycosides against human leukaemic cell lines in vitro. Pharmacol Res 2009; 41: 85-87
12. Seçkin H. *Diplotaenia turcica* Bitkisinden Yeşil Sentez ile Elde Edilen Nano-Bakır Parçacıkların Antimikrobiyal, Antioksidan ve DNA Hasarını Önleme Etkisi. Polonya Çevre Araştırmaları Dergisi. 2021;30(5):4187-4194. doi:10.15244/pjoes/132313.
13. Meydan İ., Seckin H. Green synthesis, characterization, antimicrobial and antioxidant activities of zinc oxide nanoparticles using Helichrysum arenarium extract. Int. J. of Agr. Env. and Food Sci.2021; 5(1):33-41. doi.org/10.31015
14. Scherer R., Godoy, H.T.. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chemistry 2009; 112(3): 654–658.
15. Albayrak S., Sağdıç O., Aksoy A.. Bitkisel ürün:erin ve gıdaların antioksidan kapasitelerinin belirlenmesinde kullanılan yöntemler. Erciyes Üniversitesi Fen Bilimleri. Enstitüsü Dergisi 2010; 26(4):401-409
16. Okan O.T., Varlıbaş H., Öz M., Deniz İ. Antioksidan Analiz Yöntemleri ve Doğu Karadeniz Bölgesinde Antioksidan Kaynağı Olarak Kullanılabilecek Odun Dışı Bazı Bitkisel Ürünler. Kastamonu Üni., Orman Fakültesi Dergisi 2013; 13 (1): 48-59
17. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agr Food Chem 2005;.53: 4290-302
18. Ceylan Ş.,Srarl Ö., Özcan M., Harşit B. Yaban mersininin (Vaccinium myrtillus L.) farklı çözücü ekstraktlarındaki antioksidan ve antimikrobiyal aktivitelerinin belirlenmesi. Artvin Çoruh Üniversitesi Orman Fakültesi Dergisi. 2017; Cilt: 18, Sayı:1, Sayfa:21-27
19. Meydan İ..Investıgatıon of Antıoxıdant, Antımıcrobıal and Dna Damage Preventıon Actıvıty of The Dıplotenıa Turcıca Plant Stabılızed Sılver Nanopartıcles. Fresenius Environmental Bulletin. 2021;30(2): 1231-1239.
20. Kung J.C., Chen Y.J., Chiang, Y.C., Lee C.L., Wang Y.T., Hung C.C., Shih C.J. Antibacterial activity of silver nanoparticle (AgNP) confined mesoporous structured bioactive powder against Enterococcus faecalis infecting root canal systems. Journal of Non-Crystalline Solids. 2018;502(15), 62-70
21. Uzun Y. Keleş A., İmalı A., Öğün E., Kaya A.. Antimicrobial Activity Of Urtica dioica L. And Rheum ribes L. Bio-Science Research Bulletin. 2002;V:18 (1) 43- 507