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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Assessment of the Bioactivity of Zinc Nanoparticles Synthesized Using *Erica arborea* Plant

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Abstract: Nanoparticles (NPs) have been the subject of much research in the fields of synthesis, medicine and industry with environmentally friendly, cost-effective and simple methods. In particular, it offers a promising approach for the development of next-generation nano-based drugs. In this study, *Erica arborea* (*E. arborea*) leaf extract was used as a stabilizing and reducing agent and zinc oxide nanoparticles (ZnO NPs) were synthesized. The prepared ZnO NPs were characterized by ultraviolet-visible spectroscopy (UV-vis), fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) analysis. SEM and EDX analysis showed that the NPs were spherical in shape and showed strong signals of zinc metal. UV-vis analysis confirmed the formation of NPs with the color of the solution changing to light yellow, exhibiting an absorption peak at 350 nm. FT-IR confirmed that the formation of nanoparticles was accompanied by metabolites of the leaf extract. The biological activity of ZnO NPs synthesized by *E. arborea* was carried out by antimicrobial, antioxidant and lipid peroxidation assays. The biogenic ZnO NPs were found to be sensitive against *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus* pathogenic bacteria and *Candida albicans* fungus. Especially *Bacillus subtilis* and *Candida albicans* were more effective than rifampin antibiotic. ZnO NPs exhibited better antioxidant activity compared to plant extract. The IC_{50} values of DPPH radical scavenging activity of ZnO NPs and leaf extract were 9.21± 0.10 and 18.71±0.35, respectively. In addition, the IC_{50} value for the lipid peroxidation inhibitory activity of ZnO NPs were found to be 5.33±0.09. The findings suggest that synthesizing naturally synthesized ZnO NPs could be an alternative agent to physical and chemical methods. In addition, the biological activity of NPs will contribute to next-generation drug development studies.

Keywords: Antibacterial, Antioxidant, Lipid peroxidation, *Erica arborea*, Zinc oxide nanoparticles.

Erica arborea Bitkisi Kullanılarak Sentezlenen Çinko Nanopartiküllerin Biyoaktivitesinin Değerlendirilmesi

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Öz Nanopartiküller (NP'ler), çevre dostu, uygun maliyetli ve basit yöntemlerle sentezi, tıp ve endüstri alanlarında pek çok araştırmaya konu olmuştur. NP'ler özellikle yeni nesil nano tabanlı ilaçların geliştirilmesi için umut verici bir yaklaşım sunmaktadır. Bu çalışmada, *Erica arborea* (*E. arborea*) yaprak özütü stabilize edici ve indirgeyici ajan olarak kullanıldı ve çinko oksit NP'ler (ZnO NP'ler) sentezlendi. Hazırlanan ZnO NP'ler ultraviyole-görünür spektroskopisi (UV-vis), fourier dönüşümlü kızılötesi spektroskopisi (FTIR), taramalı elektron mikroskopisi (SEM) and enerji dağılımlı X-ışını spektroskopisi (EDX) analizleri ile karakterize edildi. SEM ve EDX analizlerinde, NP'lerin küresel şekilde olduğu ve çinko metalinin güçlü sinyalleri görüldü. UV-vis analizi, çözeltinin renginin açık sarıya dönüşüyle NP'lerin oluşumunu doğruladı ve 350 nm'de bir absorpsiyon piki sergiledi. FT-IR, NP'lerin oluşumuna yaprak ekstresinin metabolitlerinin eşlik ettiğini doğruladı. *E. arborea* tarafından sentezlenen ZnO NP'lerin biyolojik aktivitesi antimikrobiyal, antioksidan ve lipid peroksidasyon analizleri ile gerçekleştirildi. Biyojenik ZnO NP'ler, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis* ve *Staphylococcus aureus* patojen bakterileri ve *Candida albicans* mantarına karşı duyarlı olduğu bulundu. Özellikle *Bacillus subtilis* ve *Candida albicans* rifampin antibiyotiğine göre daha etkiliydi. ZnO NP'ler, bitki ekstraktına kıyasla daha iyi antioksidan aktivite sergiledi. ZnO NP'ler ve yaprak ekstraktının DPPH radikal süpürme aktivitesinin IC_{50} değerleri sırasıyla 9.21±0.10 ve 18.71±0.35 idi. Ayrıca ZnO NP'lerin lipid peroksidasyon inhibitör aktivitesi için IC_{50} değeri 5.33±0.09 olarak bulunmuştur. Bulgular, doğal olarak sentezlenen çinko NP'lerin sentezlenmesi, fiziksel ve kimyasal yöntemlere alternatif bir ajan olabileceğini göstermektedir. Ayrıca NP'lerin biyolojik aktivitesi yeni nesil ilaç geliştirme çalışmalarına katkı sağlayacaktır.

Anahtar kelimeler: Antibakteriyel, Antioksidan, Lipid peroksidasyon, *Erica arborea*, Çinko oksit nanopartiküller.

INTRODUCTION

Nanotechnology is a branch of pharmaceutical science that studies active ingredient design, synthesis and drug development. This technology investigates the synthesis and characterization of nanostructures in the range of 1-100 nm (Gur et al., 2022). Nanoparticles (NPs) have become the focus of medicine, dentistry, pharmacy and other scientific fields. In recent years, the harmful effects of NPs produced by physical and chemical methods on the environment and human health have led researchers to find different methods. Today, the focus is on green synthesis for the production of nanostructures. Green synthesis is environmentally friendly, economical, and safer than other methods because it does not involve toxic chemicals for living organisms. In this method, NPs are synthesized using plants, fungi or their metabolites (Gour & Jain, 2019).

Green synthesis is usually carried out using metallic salts such as gold, silver, palladium and copper. In the last years, there has been an increased interest in the use of zinc metal salts (Agarwal et al., 2017). Zinc oxide (ZnO) NPs have a wide range of applications in optics, electronics and pharmacology (Agarwal et al., 2017; Pulit-Prociak et al., 2016). ZnO NPs have been the subject of various types of research such as anti-cancer, wound healing, anti-diabetic, anti-inflammatory and antibacterial due to their acceptance as safe metal oxide and simpler, cost-effective (Agarwal et al., 2017; Rahimi Kalateh Shah Mohammad et al., 2019; Singh et al., 2020; Velsankar et al., 2022). It is also widely used in cosmetics due to its ultraviolet filtering properties (Agarwal et al., 2017).

Antioxidants are one of the body's defense mechanisms that prevent damage caused by oxidative stress. Disruption of this balance mechanism leads to increased lipid peroxidation (LPO) in the cell. The consequence of this contributes to the occurrence of various diseases such as cardiovascular, diabetes and cancer (Kocak et al., 2023). Therefore, the demand for research to prevent oxidative stress and strengthen the antioxidant defense system has increased. In this context, NPs produced by reacting bioactive compounds found in plants with metal salts make a promising contribution to studies in this field (Gur et al., 2022; Kocak et al., 2022; Ravichandran et al., 2020; Seckin et al., 2022).

Antibiotic resistance has become an important problem threatening the health of living organisms (Eldaw & Çiftci, 2023). However, advances in nanotechnology and the synthesis of NPs from natural sources have opened a new horizon in preventing bacterial resistance to multiple drugs. Previous studies have presented potent antibacterial effects of plant-mediated NPs (Happy Agarwal et al., 2018; Pillai et al., 2020).

Erica arborea (*E. arborea*) plant was used in the present study. The genus *Erica* has a large family in the world and is widespread in the coastal regions of Turkey (Yüksel et al., 2021). This species is rich in phenolics and flavonoids. It is used by the public for urinary tract infections, anti-edema and constipation. It has also been reported to have antimicrobial (Ertürk, 2006), antiseptic, antiviral, wound healing (Yüksel et al., 2021) and analgesic effects (Akkol et al., 2008). The aim of this study was to synthesize, characterize and evaluate the Bioactivity of *E. arborea*-mediated ZnO NPs.

MATERIAL AND METHOD

Preparation of E. arborea leaf extract; *E. arborea* leaves were obtained from the local market and the plant specimen was identified. Plant residues were removed using distilled water and dried in shade. The dried plant sample was pulverized in an electric mill. Plant powder (25 g) was stirred in 250 mL of distilled water with a rotary shaker for 24 hours. It was then kept in a magnetic stirrer at 50 °C for one hour. After cooling, the mixture was filtered through filter paper (Whatman No. 1). The resulting aqueous leaf extract was centrifuged and the supernatants were removed. The extraction method was inspired by the previous study with minor modifications (Meydan et al., 2022).

***Preparation of ZnO NPs*:** ZnO NPs were produced using *E. arborea* leaf extract. 25 mL of leaf extract was added dropwise to 100 mL of zinc acetate solution and stirred continuously. ZnO NPs were centrifuged at 10 000 rpm for 10 min to obtain pellets. Then, the pellets were allowed to dry in an oven (50 °C, 48 h) (Gur et al., 2022; Sampath et al., 2023).

Characterization of ZnO NPs: The optical properties of ZnO NPs were measured by ultraviolet-visible spectroscopy (UV-vis). The size, shape and elemental analysis of the NPs were performed by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) analysis. Fourier transform infrared spectroscopy (FTIR) was used to evaluate the binding capacity of bio components in leaf extract to NPs (Gur et al., 2022).

Antimicrobial assay: The effects of *E. arborea* aqueous extract and ZnO NPs on pathogenic bacteria were carried out by disk diffusion method (Gur et al., 2022). The bacteria *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853, *Staphylococcus aureus* (*S. aureus*) ATTC 29213, *Bacillus subtilis* (*B. subtilis*) ATCC 6633, *Bacillus cereus* (*B. cereus*) ATCC 10876 and the fungus *Candida albicans* (*C. albicans*) ATTC 90028 were obtained from Van Yüzüncü Yıl University, Faculty of Science, Department of Biology. Rifampin was used as a reference antibiotic. Müller Hinton medium was used for this experiment.

Antioxidant assay: Antioxidant activity assay was performed by DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging method. ZnO NPs and leaf extract were added to the DPPH solution and incubated in the dark for 60 min. The absorbance of the color change at the end of the reaction was measured spectrophotometrically at 515 nm. The results were expressed as IC_{50} values (Pyo et al., 2004). The IC_{50} value is the concentration of a substance that halves the activity of a particular biological or biochemical function. In this study, a decrease of 50 % of the initial DPPH concentration was considered (Yıldız et al., 2019). The Inhibition values were measured by preparing the samples at different concentrations of 2-10 mg/ml and IC_{50} value was found from the curve prepared with these values. The % inhibition values were calculated using the following formula 1 (Kocak et al., 2023).

$$\%\text{Inhibition (DPPH)} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (1)$$

Where $A_{control}$ is the absorbance of the control sample and A_{Sample} is the absorbance of the samples at different concentrations and positive control.

LPO inhibition activity assay: LPO inhibition activity assay was performed with minor modifications in the study by Meydan et al.,(2022). LPO results were found using thiobarbituric acid (TBA) method. α-Tocopherol was preferred as a positive control. All samples were prepared at different concentrations (2-10 mg/mL). These samples were mixed and incubated in the dark at 37°C for 1.5 h. Then 28% TCA was added and centrifuged at 3000 rpm for 15 min. 1.2 ml of TBA was added to the supernatant obtained. The samples were boiled at 100°C for 10 minutes and after rapid cooling, the absorbance values were read at 532 nm in the spectrometer. The results were expressed as IC_{50} values. The % inhibition of LPO was calculated according to formula 2 below.

$$\%\text{ inhibition (LPO)} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (2)$$

Where % LPO is the percent inhibition of lipid peroxidation, $A_{control}$ is the absorbance of the control and A_{sample} absorbance of samples.

Statistical analysis: The results of the analysis of this study were carried out using the SPSS package (Ver. 22). Values were expressed as mean ± standard deviation. The data obtained were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple-test comparison test ($p < 0.05$).

RESULTS AND DISCUSSION

***Characterization of ZNO NPs*:** Characterization of *E. arborea*-mediated zinc nanoparticles (EA-ZnO NPs) was carried out by SEM, EDX, FT-IR and UV-vis methods. SEM is a method in which surface images of NPs are obtained at the microscopic level using electron beams. SEM images of EA-ZnO NPs are shown in Figure 1. SEM images showed that the NPs were spherical and homogeneously distributed with clusters in some regions. Similarly, SEM images of ZnO NPs synthesized with different plant extracts reported that the NPs were close to spherical (Pillai et al., 2020). This study exhibited similar characteristics to the research in which Tetraselmis indicia-mediated ZnO NPs exhibited spherical surface smooth structures (Thirumoorthy et al., 2021). The SEM image showed that the average size of ZnO NPs ranged from 12 to 27 in size. These sizes were consistent with ZnO NPs formed using dried ginger (Janaki et al., 2015). The EDX analysis graph shows the energy in KEV. The presence of a strong signal in EDX at 1 KEV, the specific binding energy of the element Zn, confirms the formation of NPs. The carbon (C) and oxygen (O) elements seen in EDX indicate that other components in *E. arborea* contribute to NP formation (Gur et al., 2022; Janaki et al., 2015). The unidentified peak belongs to the gold element used in SEM image acquisition (Figure 1d). Indeed, the gold peak was shown in the EDX image in the previous study (Singh et al., 2013).

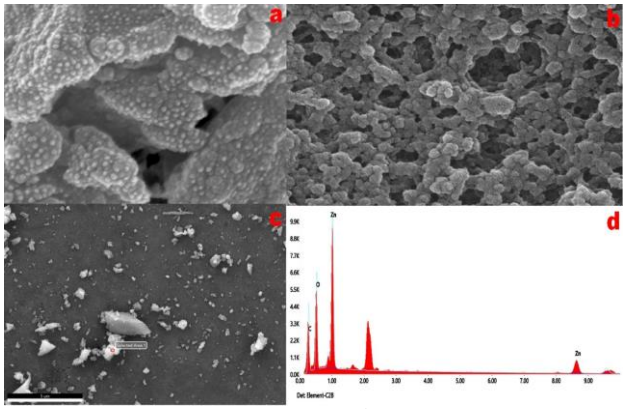

Figure 1. SEM (a-c) and EDX (d) image of zinc nanoparticles synthesized using *E. arborea* extract. EA-ZnO NPs*; E. arborea*-mediated biogenic zinc oxide nanoparticles. The unidentified peak in EDX belongs to the gold metal used in the SEM analysis shot.

UV-vis spectroscopy is one of the methods used to determine the structural characterization of biosynthesized ZnO NPs (Vijayakumar et al., 2018). The optical property of EA-ZnO NPs are shown in Figure 2. The peak corresponding to the surface plasmon resonance of ZnO NPs was measured at 350 nm. This peak has been reported in previous studies that ZnO NPs form peaks in the range of 310 nm to 360 nm (Gur et al., 2022; Jayachandran et al., 2021). The UV-vis result confirms that ZnO NPs were synthesized.

Figure 3 shows the FT-IR spectrum of EA-ZnO NPs. FT-IR is used to detect possible functional groups involved in the synthesis of ZnO NPs. The FT-IR spectrum was in the range of 547-3362 cm^{-1}. These spectra are due to the interaction of phenolics, flavonoids, terpenes and alkynes. These functional groups were responsible for the reduction of the NPs (Nava et al., 2017).

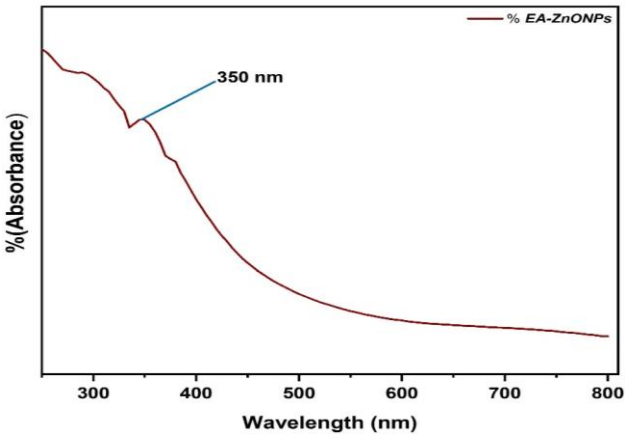

Figure 2. UV-vis analysis of zinc nanoparticles synthesized using *E. arborea* extract. EA-ZnO NPs; *E. arborea*-mediated biogenic zinc oxide nanoparticles

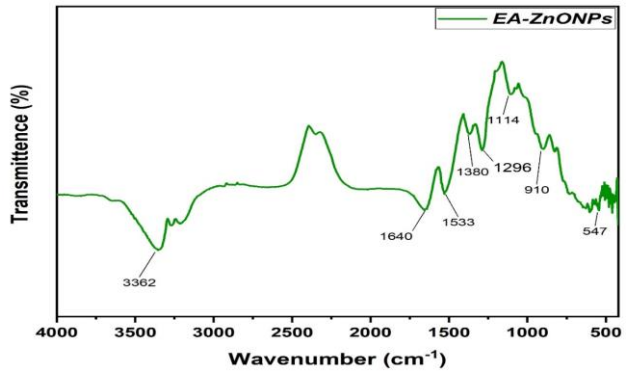

Figure 3. FT-IR analysis of zinc nanoparticles synthesized using *E. arborea*. EA-ZnO NPs; *E. arborea*-mediated biogenic zinc oxide nanoparticles.

Antimicrobial activity: Antimicrobial analysis of *E. arborea* aqueous extract-mediated synthesized EA-ZnONPs was performed by disc diffusion method. Leaf extract and ZnO NPs were compared with the antibiotic rifampin. The analysis showed that the leaf extract was less susceptible to pathogenic bacteria. However, ZnO NPs formed a zone diameter in all pathogenic strains of bacteria and fungi. Moreover, ZnO NPs were found to be more effective against *B. cereus*, *B. subtilis* and *C. albicans* compared to rifampin antibiotic (Table 1). The findings showed that ZnO NPs exhibited antibacterial and antifungal activity. Indeed, ZnO NPs obtained from *Thymbra spicata* plant extract were found to have an inhibitory effect against pathogenic microorganisms (Gur et al., 2022). Similarly, ZnO NPs were found to be effective against *S. aureus* bacteria, *C. albicans* fungus (Janaki et al., 2015). Furthermore, it is generally accepted that noble metals carry a positive charge while microorganisms carry a negative charge. Therefore, it was hypothesized to cause the formation of reactive oxygen species and increase the antimicrobial activity of NPs (Ahmad & Kalra, 2020). This study is consistent with the literature and suggests that it may exhibit possible antibacterial and antifungal activity with the above-mentioned mechanism of action.

Table 1. Inhibition zone diameters (mm) of *E. arborea* aqueous extract and EA-ZnO NPs against pathogenic microorganisms.

Test Microorganisms	Zone of Inhibition (mm)		
	E. arborea extract	EA-ZnO NP	Rifampin
Pseudomonas aeruginosa ATTC 27853	8.5 ± 1.8	9.5 ± 1.5	-
Bacillus cereus ATTC 10876	-	11.0 ± 2.3	8.0 ± 2.5
Bacillus subtilis ATCC 6633	-	12.0 ± 2.7	11.0 ± 1.9
Staphylococcus aureus ATTC 29213	9.0 ± 1.6	13.0 ± 2.2	16.0 ± 1.7
Candida albicans ATTC 90028	-	10.0 ± 1.2	8.5 ± 1.6

Analyses were performed with three replicate measurements. mean ± SD (n = 3). EA-ZnO NPs; *E. arborea*-mediated biogenic zinc oxide nanoparticles.

***Antioxidant and LPO activity*:** DPPH assay was performed to determine the antioxidant properties of ZnO NPs and the antioxidant activity of the test samples was determined by calculating the IC_{50} value. The IC_{50} value, when high, means that it exhibits a low antioxidant property. If the IC_{50} value is low, it indicates that the test samples

have strong radical scavenging activity. The findings of this study showed that the IC_{50} value decreased at different concentrations (2-10 mg/mL) of ZnO NPs. The IC_{50} value of ZnO NPs (9.21±0.10) showed high antioxidant capacity compared to EA aqua extract (18.71±0.35). Moreover, α-tocopherol, the reference control, had the lowest value (4.45±0.40). These results proved that ZnO NPs exhibited DPPH radical scavenging activity. Indeed, it was emphasized that *Artocarpus gomezianus* synthesized ZnO NPs showed antioxidant properties with an IC_{50} value of 10.8 mg/ml (Suresh et al., 2015). Mango plant extract-mediated ZnO NPs have been reported to exhibit radical scavenging activity at increasing concentrations (Rajeshkumar et al., 2018). These studies have shown that plant-doped ZnO NPs increase antioxidant activity. The element zinc has been suggested to exhibit antioxidant activity by preventing cellular damage caused by reactive oxygen species and activates some enzymes involved in oxidative processes (Zeghoud et al., 2022). In this context, it suggests that plant-mediated ZnO NPs may exhibit stronger antioxidant activity. These ZnO NPs may prevent possible mitochondrial damage that may occur in the cell due to oxidative stress.

Table 2. DPPH radical scavenging and lipid peroxidation (LPO) activity of EA-ZnO NPs.

Sample	DPPH (IC_{50}) mg/ml	LPO (IC_{50}) mg/ml
EA aqua extract	18,71±0.35^{a}	16,61±2.08^{a}
EA-ZnO NPs	9.21±0,10^{b}	5,33±0.09^{b}
α-Tocopherol	4.45±0,40^{c}	2.08±0.14^{c}

^{a,b,c} p: values with different letters are significant when compared with each other. Data are presented as mean ± SD (P < 0.05). EA-ZnO NPs; *E. arborea*-mediated biogenic zinc oxide nanoparticles.

LPO occurs as a result of excessive oxidation of lipids that provide the structural integrity of cells. Disruption of redox hemostasis in the organism triggers LPO (Gaschler & Stockwell, 2017). Increased LPO can lead to cell dysfunction and death and contribute to the development of various chronic diseases (Kocak et al., 2023; Meydan et al., 2022). This study used the TBA method for LPO analysis. Biosynthesized ZnO NPs, plant

extract and α-tocopherol were analyzed at different concentrations (2-10 mg/mL). The findings showed that ZnO NPs were more effective than the plant extract. The IC_{50} inhibition value of ZnO NPs was 5.33±0.09. For *E. arborea* leaf extract, this value was 16.61±2.08 (Table 2). Furthermore, α-tocopherol, the reference control, was found to strongly inhibit LPO. Plant-mediated ZnO NPs have been reported to have the potential to inhibit LPO in previous studies (Del Carmen Sánchez-Navarro et al., 2018; Meydan et al., 2022). ZnO NPs synthesized by *Rheum ribes* have been reported to inhibit LPO at increasing concentrations (Meydan et al., 2022). Similarly, the present study shows that it prevents LPO, in line with the literature. It is suggested that there is a synergistic interaction as a consequence of the bio conjugation of metabolites in the plant content with zinc metal and prevents LPO.

CONCLUSION

This study demonstrated that ZnO NPs can be produced using the *E. arborea* plant in an environmentally friendly, economical, non-toxic and simple method. Zinc metal was successfully characterized by reducing it through phytochemical components in the plant. SEM analysis revealed that the nanopatterns were spherically dispersed and the absorbance of the surface plasmon resonance by UV-vis was 350 nm. NPs were effective on pathogenic bacteria *P. aeruginosa*, *B. cereus*, *B. subtilis* and *S. aureus*. It also exhibited antifungal activity against *C. albicans*. EA-ZnO NPs were more effective in DPPH radical scavenging activity compared to the plant extract. Moreover, ZnO NPs may contribute to prevent oxidative stress-induced cellular damage by exhibiting LPO inhibitory activity. These results emphasize that NPs derived from natural sources can be potential therapeutic agents. It can also contribute to research on nano-based drug delivery systems. In this context, more detailed studies are needed to understand the mechanism of action of EA-ZnO NPs .

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CONFLİCTS OF İNTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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