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Comparison of Antioxidant and Antimicrobial Properties of Zinc oxide and Selenium oxide Nanoparticles using *Verbascum kotschyi* Boiss. & Hohen.

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ABSTRACT
Nanoparticle applications have been studied in many fields in recent years. Among these studies, the synthesis of nature-friendly and health-friendly nanoparticles through green synthesis attracts much attention. These investigations also highlight the significance of several plant species, many of whose worth and traits remain unknown. The goal of this work is to create zinc oxide and selenium nanoparticles from *Verbascum kotschyi* Boiss. & Hohen., a plant species that hasn't received much attention, and to ascertain the antioxidant and antibacterial qualities of these nanoparticles. To accomplish this, three distinct techniques (DPPH, CUPRAC, and FRAP) were used to assess the produced nanoparticles' *in vitro* antioxidant capabilities after SEM, EDX, and FTIR analyses. Furthermore, the disk diffusion technique was utilized to ascertain the antibacterial efficacy of these nanoparticles against both gram-positive and gram-negative bacteria and fungus. In conclusion, *V. kotschyi*-derived zinc oxide nanoparticles outperformed selenium nanoparticles in terms of antibacterial activity. But when it came to antioxidant activity, selenium nanoparticles outperformed zinc oxide nanoparticles. Thus, it was determined that the products created by nanoparticle synthesis from *Verbascum kotschyi* have properties that can be used in different fields.

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Verbascum kotschyi Boiss. & Hohen. Kullanılarak Sentezlenen Çinko Oksit ve Selenyum Oksit Nanopartiküllerinin Antioksidan ve Antimikrobiyal Özelliklerinin Karşılaştırılması

ÖZET
Son yıllarda nanopartiküllerin birbirinden farklı alanlarda kullanımı araştırılmaktadır. Bu çalışmalar içinde özellikle yeşil sentez ile doğa dostu ve sağlığa faydalı nanopartiküllerin sentezi oldukça ilgi çekmektedir. Aynı zamanda bu çalışmalar ile değeri ve pek çok özelliği bilinmeyen çok sayıda bitki türünün önemi de açığa çıkarılmaktadır. Bu nedenle bu bitki türlerinden biri olan ve üzerinde nanopartikül çalışmaları yapılmayan bir tür olan *Verbascum kotschyi*'den çinko oksit ve selenium nanopartiküller sentezleyip bu nanopartiküllerin antimikrobiyal ve antioksidan özelliklerini belirlemek amaçlanmıştır. *Verbascum kotschyi*'den sentezlenen nanopartiküllerin SEM, EDX, FTIR analizleri yapıldıktan sonra üç farklı yöntemle (DPPH, CUPRAC ve FRAP) *in vitro* antioksidan kapasiteleri belirlenmiştir. Ayrıca disk difüzyon yöntemi ile gram-pozitif, gram-negatif bakteriler ve mantar üzerine antimikrobiyal etkisi belirlenmiştir. Sonuç olarak *Verbascum kotschyi*'den sentezlenen çinko oksit nanaopartiküller selenyum nanopartiküllere kıyasla daha fazla antimikrobiyal özellik sergilemiştir. Ancak selenyum nanaopartiküller çinko oksit nanaopartiküllerden daha etkin antioksidan özellik sergilemiştir. Böylece *Verbascum kotschyi*'nin nanopartikül sentezi ile oluşturulabilecek ürünleri sayesinde farklı alanlarda kullanılabilecek özelliklere sahip olduğu tespit edildi.

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Selenyum nanopartikül
Verbascum kotschyi

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INTRODUCTION

Because they differ from their bulk form in industrial domains by having specific electrical and optical properties, nanoparticles are useful across several domains, such as the agricultural, environmental, and biomedical (Nayak et al., 2021). In many areas, nanoparticles have been widely applied. Physical and chemical techniques are the primary means of identifying the nanoparticle synthesis processes; nevertheless, these techniques can be costly, hazardous, and adsorb on the nanoparticle surface (Mosalam & Marzouk, 2013; Kalishwaralal et al., 2016). Because of its advantages, including cost-effectiveness, ease of use and speed in manufacturing, friendliness with the environment, and other advantages, plant-mediated creation of nanoparticles has garnered an abundance of attention lately (Singh et al., 2014).

It is possible to create selenium nanoparticles using chemical, biological, and physical techniques. High temperatures, dangerous substances, and an acidic pH are required for chemical and physical procedures; these conditions are very hazardous and unfit for biological use (Iranifam et al., 2013). However, the biological production of SeNPs (Selenium Nanoparticles) is nontoxic, affordable, environmentally benign, and safe (Wadhwani et al., 2016). Furthermore, the organic materials that naturally coat the surface of biologically produced SeNPs make them more stable because they prevent nanoparticles from aggregating over time (Park et al., 2011).

The scientific community worldwide has been captivated by zinc oxide nanoparticles due to their exceptional catalytic activity, semiconducting nature, and ultraviolet filtration capabilities (Nagajyothi et al., 2013). Furthermore, reports indicate that these nanoparticles are biocompatible, non-toxic, and safe for biological systems (Anjum et al.,2021; Agarwal & Shanmugam, 2020; Bhuyan et al., 2015). Because zinc oxide nanoparticles can take in dangerous radiation like UV-A and UV-B, they are also used in sunscreen lotions and cosmetics (Ramesh et al., 2014). Zinc oxide is safe and can be utilized as a medicine, according to the US Food and Drug Administration (Lopez De Romaña et al., 2002). To eliminate infectious microorganisms, zinc oxide nanoparticles can be employed as an antibacterial substance. Shape, particle size, concentration, and duration of being in contact with the bacterial cell all affect how they inflict harm to the cell wall, seep inside, build up in the cell membrane, and eventually cause death by interfering with metabolic activities (Siddiqi et al., 2018).

Crucial trace elements in living things, zinc and selenium are crucial for immune system function, antioxidant defense, and antitumor activity in humans. Because biosynthesized zinc and selenium nanoparticles are more biodegradable, less toxic, and easily removed from the body, they are very advantageous (Zhuang et al., 2007; Schomburg, 2017).

Around 360 species of *Verbascum* L. (Scrophulariaceae) are known to exist in the world. There are roughly 249 species in Türkiye, among these, 191 are endemic (Huber-Morath, 1978; Georgiev et al., 2011; Güner et al., 2012). *Verbascum* species, also referred to as "sigirkuyrugu," have been utilized as a sedative, expectorant, mucolytic, anti-diarrheic, diuretic, and wound healer in traditional Turkish folk medicine (Baytop, 1999; Tuzlaci & Erol, 1999; Sezik et al., 2001).

The purpose of the selection of the *V. kotschyi* species in this study is to show the species' widespread spread in the Mardin province, the plant is being used for medical purposes by the public; and no previous study has been done (Mungan Kılıç & Kılıç, 2022; Eksik, 2020).

MATERIALS and METHODS

The aerial sections of *Verbascum kotschyi* were gathered from Mardin (Türkiye) - Mardin - Ortaköy highway, roadside, 37°17'07"N 40°46'32"E, 742 m altitude, on 11 May 2022. Flora of Türkiye (Huber-Morath, 1978) and Flora of Türkiye checklist (Güner et al., 2012) were used to identify plant species. An expert in botany verified the plants (Dr. Fatma Mungan Kılıç, Mardin Artuklu University), and one voucher specimen (Voucher No: M. Kılıç 264) was kept at Mardin Artuklu University in Türkiye.

60 g of *V. kotschyi* sample, which had been dried in the shade, was combined with 900 ml of distilled water and stirred with a magnetic stirrer for four hours at 60 °C. After passing through filter paper, the plant extraction was obtained. The extract was split into two halves. For 20 minutes at 60 °C, 250 ml of plant extract and 200 ml of 0.1 M $Zn(CH_3COO)_2 2H_2O$ were mixed in the first section. This solution appeared to be lightening in color. When the second portion of the extract (250 ml of plant extract) was mixed with 50 mM Na_2SeO_3 and heated to 60 °C for 20 minutes, a reddish color shift was noticed. The absorbance of both extracts was then recorded in the 200–800 nm

range using the UV-VIS spectrophotometer following a 24-hour incubation period at room temperature. Following a 30-minute centrifugation at 5000 rpm for both solutions, the precipitates were incubated for three hours at 100 °C in etuve.

By measuring the DPPH radical reduction potential of zinc nanoparticles (VkZnO) and selenium oxide nanoparticles (VkSe) produced from *V. kotschyi* using 60 µM DPPH radical solution, an antioxidant capacity analysis test was carried out. Trolox and the sample (VkZnO, VkSe) produced at several doses (10, 20, 30 µg ml^{-1}) were measured for their absorbance at 517 nm to determine their DPPH reduction capacities (Makhlouf-Gafsi et al., 2018).

The FRAP technique was employed to analyze the antioxidant capacity of VkZnO and VkSe solutions at varying concentrations. The solutions were mixed with 20 mM $FeCl_3$ solution and FRAP reagent (10 volumes of 0.3 M acetate buffer (pH:3.6), 1 volume of 10 mM TPTZ solution, and 1 volume of 20 mM $FeCl_3.6H_2O$ solution), and their total absorbance at 593 nm was noted (Gülçin, 2012).

The CUPRAC technique was used to perform antioxidant testing. VkZnO, VkSe, and Trolox solutions were made at varied concentrations. 0.01 M $CuCl_2$, $7.5x10^{-3}$ M neocuprin solution, and 1 M ammonium acetate buffer (pH: 6.5) were included, and their absorbances were measured at 450 nm (Apak et al., 2007).

The molecular structure of the *V. kotschyi*-derived nanoparticles was determined by SEM, EDX, and FTIR analyses. Zinc oxide nanoparticles (VkZnO) at a concentration of 100 mg ml^{-1} and selenium oxide nanoparticles (VkSe) at a concentration of 60 mg ml^{-1}, which were produced from *V. kotschyi*, were used for antimicrobial activity testing. Water acted as the negative control in these tests, while the antibiotic rifampin (5 µg) acted as the positive control. Using the disk diffusion technique, antimicrobial activity tests were conducted on gram (-) bacteria *(P. aeruginosa, E. coli, K. pneumoniae)*, gram (+) bacterium *(S. aureus)*, and fungus *(C. albicans)* (Wayne, 1997).

Statistical Analysis

In this investigation, every experiment was carried out in triplicate. The findings are presented as the mean value ± standard deviation. To ascertain if the antioxidant and antimicrobial properties of VkZnO and VkSe varied substantially from one another, data were analyzed using a one-way ANOVA for multiple comparisons of means. The threshold for statistical significance was $p<0.050$. Statistical analyses were performed separately for each method in antioxidant analyses. This statistical study revealed that VkZnO had antibacterial activity. VkSe and VkZnO showed antioxidant capacities, especially VkSe, in CUPRAC, FRAP, and DPPH methods. $p<0.050$ was found in the antibacterial and antioxidant tests with nanoparticles. The intergroup significance Fp: 0.027 in the FRAP method, significance Cp: 0.030 in the CUPRAC method, and intergroup significance Dp: 0.026 in the DPPH method were found. Significance p:0.000 was found in antimicrobial analyses.

RESULTS and DISCUSSION

UV-VIS, SEM-EDX, and FTIR analyses were carried out to elucidate whether the nanoparticles synthesized from *Verbascum kotschyi* were synthesized chemically and the molecular structure of the synthesized nanoparticles. Figure 1 displays the UV-VIS data of zinc oxide and selenium oxide nanoparticles that were made from *V. kotschyi*.

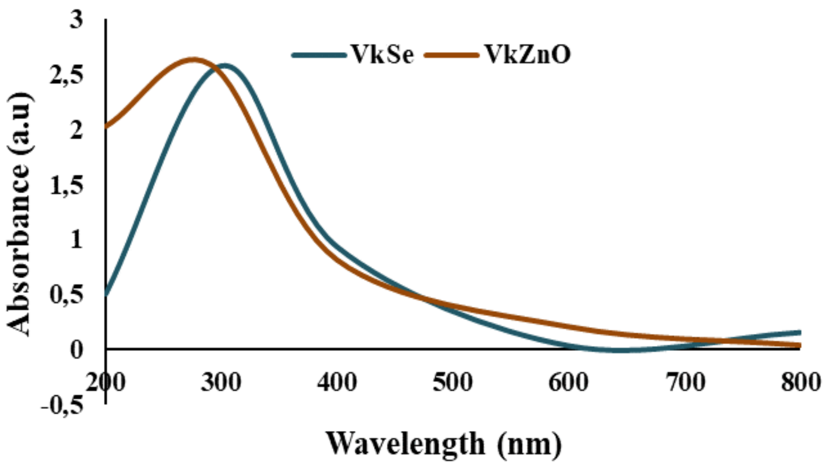


Figure 1. UV-VIS spectrophotometer results of VkZnO and VkSe nanoparticles
Şekil 1. VkZnO ve VkSe nanoparçacıklarının UV-VIS spektrofotometre sonuçları

The highest absorbance of VkZnO was measured at 290 nm. The synthesis of the zinc oxide nanoparticle is shown by this peak. SPR (surface plasmon resonance) happens when light interacts with the movable surface electrons of VkZnO nanoparticles (Akintelu & Folorunso, 2020; Al-Dhabi & Arasu, 2018). The synthesis of zinc oxide nanoparticles was demonstrated by the appearance of the peak at 295 nm wavelength, which is where the SPR of VkZnO nanoparticles is produced (Rajakumar et al., 2018; Akintelu & Folorunso, 2020).

The surface plasmon vibration peak at 300 nm in the UV-VIS spectrum seen in Figure 1 verified the production of VkSe nanoparticles (Yang et al., 2008; R. R. Mishra et al., 2011; Mishra et al., 2013; Srivastava & Mukhopadhyay, 2015). SEM images of zinc oxide nanoparticles produced from *V. kotschyi* are displayed in Figure 2.

VkZnO was visible in SEM pictures as stones and pieces of broken rock. It failed to take on a regular, symmetrical form. Figure 3 shows VkSe nanoparticle SEM images.

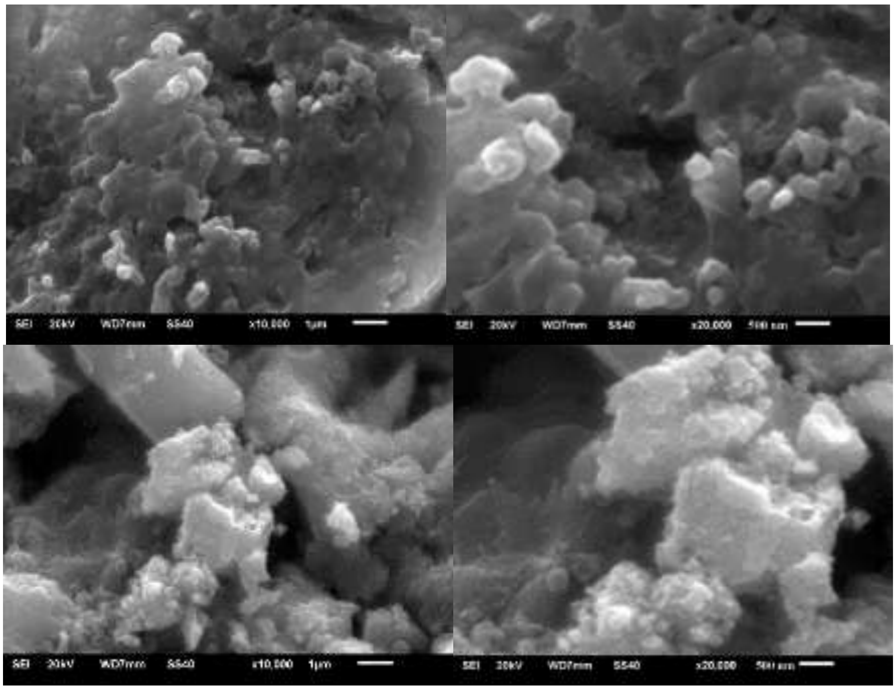


Figure 2. VkZnO nanoparticles SEM images
Şekil 2. VkZnO nanoparçacıklarının SEM görüntüleri

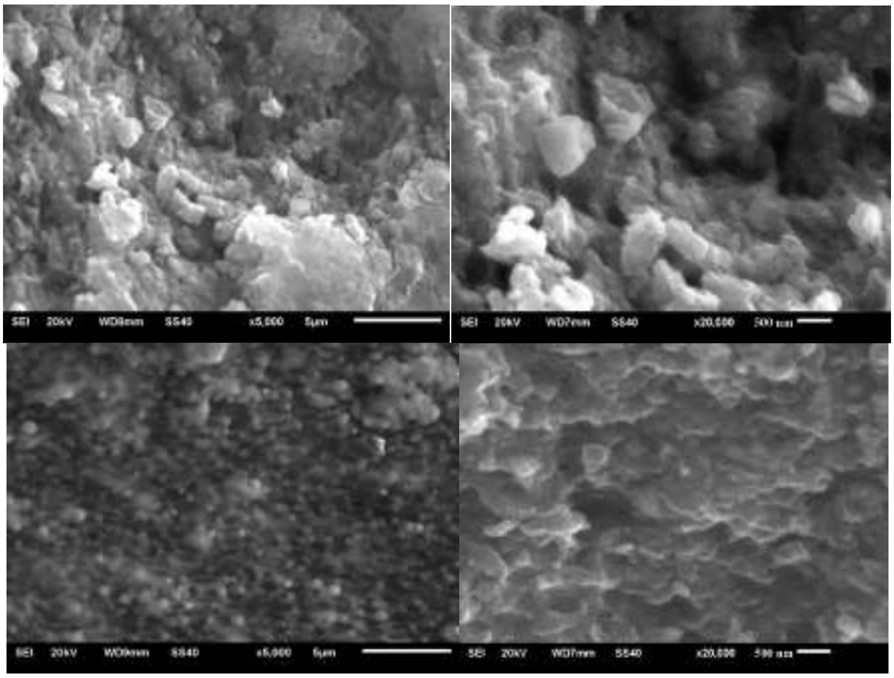
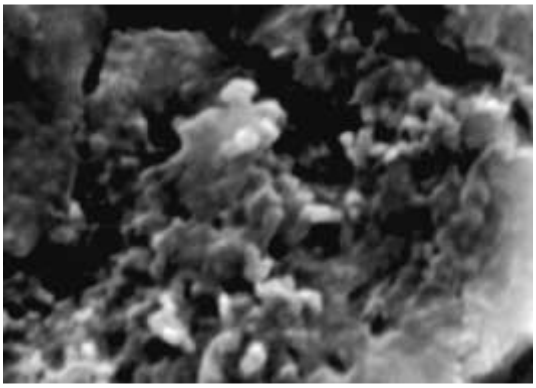


Figure 3. VkSe nanoparticles SEM images
Şekil 3. VkSe nanoparçacıklarının SEM görüntüleri

In the SEM images, VkSe produced tiny spherical spots and patterns that resembled reliefs on karst cave ceilings. Figure 4 displays the VkZnO nanoparticle EDX (Energy Dispersive X-Ray Spectroscopy) data.



Elt.	Line	Intensity ($c\ s^{-1}$)	Error 2-sig	Conc	Units
O	Ka	48.71	2.176	3.091	wt.%
Al	Ka	16.11	3.204	0.483	wt.%
Si	Ka	43.04	3.655	1.006	wt.%
P	Ka	887.12	8.433	18.451	wt.%
K	Ka	13.53	3.438	0.228	wt.%
Ca	Ka	91.11	3.889	1.494	wt.%
Fe	Ka	25.45	2.912	0.571	wt.%
	Ka	1,317.08	9.681	74.675	wt.%
			Total	100.000	wt.%

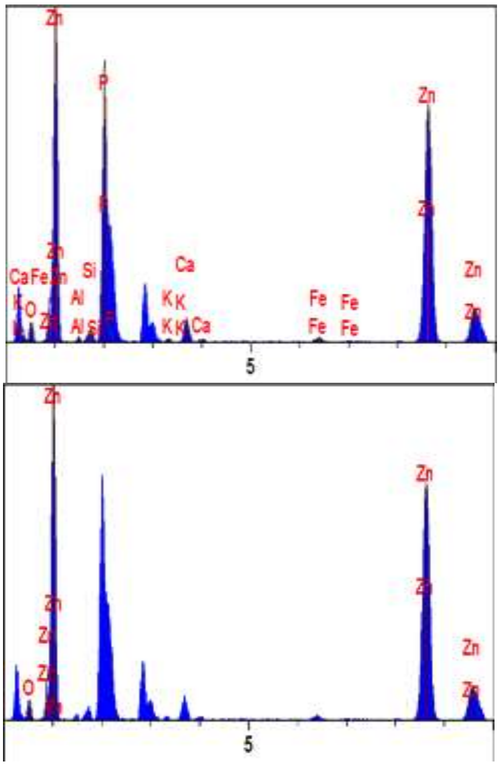
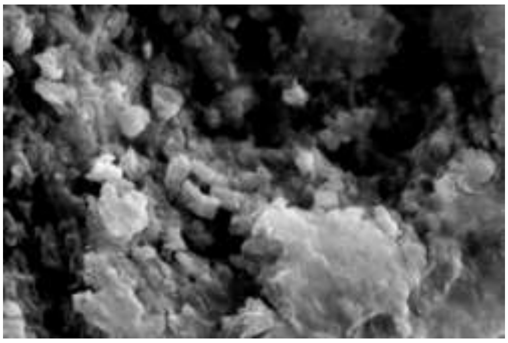


Figure 4. EDX spectroscopy results of VkZnO nanoparticles
Şekil 4. VkZnO nanoparçacıklarının EDX spektroskopisi sonuçları

VkZnO nanoparticles' elemental composition was ascertained by EDX analysis. In addition to Zn and O, the data show that VkZnO also contains elements like P and Ca.

Figure 5 shows the VkSe nanoparticles EDX data.



Elt.	Line	Intensity ($c\ s^{-1}$)	Error 2-sig	Conc	Units
C	Ka	1,002.42	8.442	68.494	wt.%
O	Ka	84.65	2.883	9.540	wt.%
Si	Ka	223.96	5.510	2.412	wt.%
P	Ka	568.21	7.261	6.111	wt.%
K	Ka	100.17	4.214	1.102	wt.%
Ca	Ka	218.75	4.841	2.492	wt.%
Fe	Ka	43.30	2.873	0.954	wt.%
Se	Ka	65.69	2.691	8.895	wt.%
			Total	100.000	wt.%

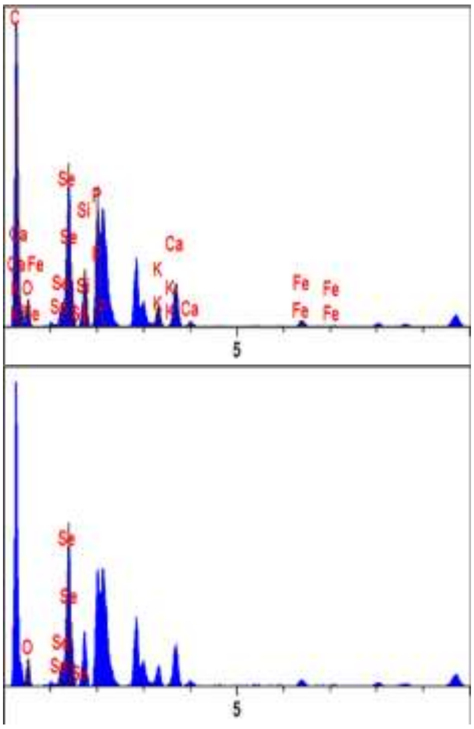


Figure 5. EDX spectroscopy results of VkSe nanoparticles
Şekil 5. VkSe nanoparçacıklarının EDX spektroskopisi sonuçları

Selenium oxide nanoparticles were produced from *V. kotschyi*, as demonstrated by the results of the EDX study. Figure 6 shows the VkZnO nanoparticles' FTIR (Fourier Transform Infrared Spectroscopy) findings.

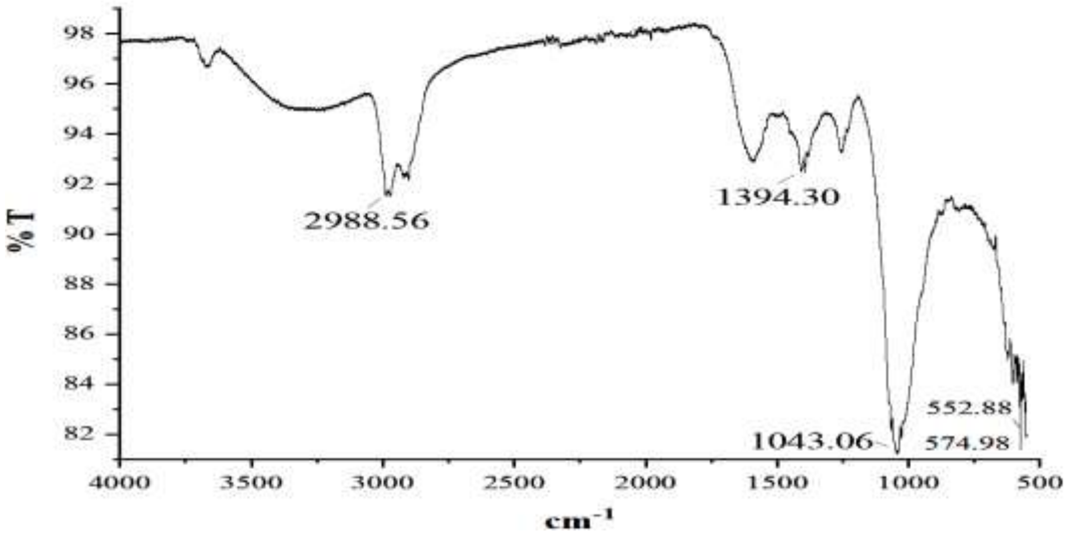


Figure 6. FTIR analysis results of VkZnO nanoparticles
Şekil 6. VkZnO nanoparçacıklarının FTIR analiz sonuçları

The intergroup vibration peaks in the 2988, 1394, 1043, and 574 cm^{-1} bands were produced by VkZnO nanoparticles. A variety of bioactive compounds, including amines, carboxylic acids, phenols, and alcohols, may help to stabilize zinc oxide nanoparticles (Fakhari et al., 2019). The aromatic C=C bond is shown by the stretching of 1394 cm^{-1}, the C-O stretching is demonstrated by the observed peak of 1043 cm^{-1}, and the Zn-O stretching is indicated by the bands of 574 cm^{-1} (Dole et al., 2011; Sangeetha et al., 2011; Kumar et al., 2014; Fakhari et al., 2019).

Figure 7 displays the VkSe nanoparticles FTIR analysis findings.

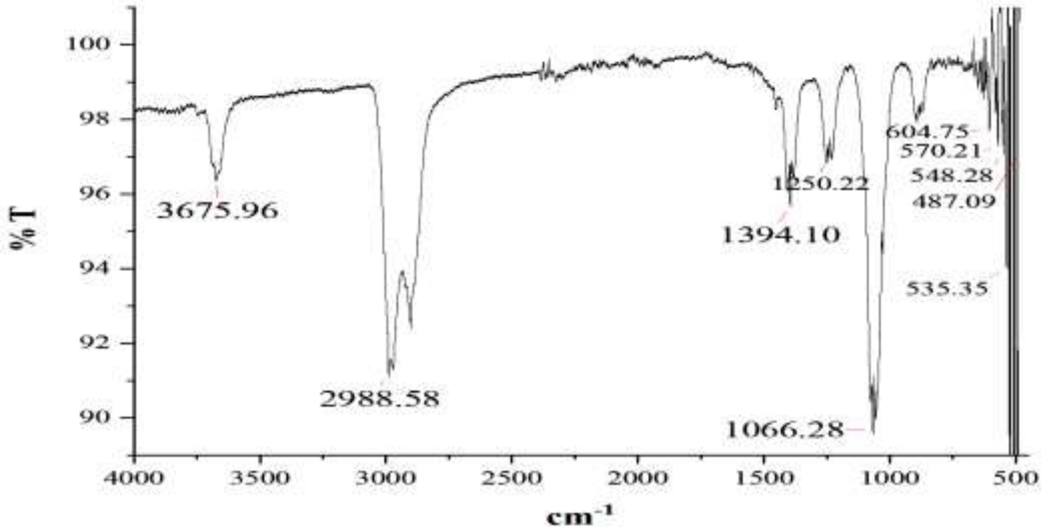


Figure 7. FTIR analysis results of VkSe
Şekil 7. VkSe'nin FTIR analiz sonuçları

The molecular bonds of VkSe nanoparticles gave vibration peaks in the 3675, 2988, 1394, 1250, 1066, 604, 570, 548, 535, and 487 cm^{-1} bands. The FTIR spectrum's stretching vibration bands, which range from 604 to 487 cm, could show how Se-NPs attach to (OH) groups as Se-O (Salem et al., 2021). FTIR analysis results of VkSe and VkZnO are presented in Table 1.

Table 2 displays the findings of the antioxidant investigation performed with VkZnO and VkSe nanoparticles.

Table 1. FTIR analysis results of VkSe and VkZnO
Çizelge 1. VkSe veVkZnO'nun FTIR analiz sonuçları

Samples	Intergroup vibration peaks	Molecular group	References
VkZnO, VkSe	2988 cm^{-1}	Methyl, methylene, and methoxy groups	(Kora & Rastogi, 2016; Kumar et al., 2014)
VkZnO, VkSe	1394 cm^{-1}	Aromatic C=C bond	(Dole et al., 2011; Kumar et al., 2014; Pillai et al., 2020)
VkZnO	1043 cm^{-1}	C-O	(Sangeetha et al., 2011; Kumar et al., 2014)
VkZnO	574 cm^{-1}	Zn-O stretching	(Kumar et al., 2014; Fakhari et al., 2019).
VkSe	3675 cm^{-1}	O-H	(Pillai et al., 2020)
VkSe	1066 cm^{-1}	C-O vibration	(Kumar et al., 2014)
VkSe	604 - 487 cm^{-1}	Se-O	(Salem et al., 2021)

Table 2. Antioxidant capacity test results of VkZnO and VkSe nanoparticles
Çizelge 2. VkZnO ve VkSe nanoparçacıklarının antioksidan kapasitesi test sonuçları

Samples	DPPH		CUPRAC		FRAP	
	IC_{50} (µg ml^{-1})	Trolox equivalent (µg ml^{-1})	($A_{0,5}$)	Trolox equivalent (µg ml^{-1})	($A_{0,5}$)	Trolox equivalent (µg ml^{-1})
VkZnO	37.63 ±0.02	0.643	195.8±0.020	0.0958	166.55±0.01	0.0811
VkSe	16.71±0.01	1.448	100.88±0.05	0.186	83.77±0.03	0.161
Trolox	24.21±0.05	-	18.75±0.5	-	13.5±0.05	-

$A_{0,5}$: Concentration corresponding to 0.5 absorbance, IC_{50}: Concentration that inhibits 50% of the radical.

In the DPPH method, IC_{50} values were calculated with the help of the graph and equation created with the % inhibition values corresponding to different concentration values of nanoparticles and are presented in Table 2. In CUPRAC and FRAP methods, the increase in absorbance is directly proportional to the amount of antioxidants. In these methods, concentrations($A_{0.5}$) corresponding to 0.5 absorbances were calculated for both nanoparticles and Trolox with the help of the linear graph created using the absorbance values corresponding to the concentration values. IC_{50} and $A_{0.5}$ values of both nanoparticles and standard (Trolox) were calculated (Ercan et al., 2024). Thus, they were compared in terms of antioxidant capacity. Additionally, in Table 2, the antioxidant capacities of both nanoparticles in the three methods are given as Torolox equivalents. The antioxidant properties of both nanoparticles were less active compared to Trolox. According to the DPPH, CUPRAC, and FRAP techniques, VkSe nanoparticles showed greater antioxidant capabilities than VkZnO nanoparticles.

Table 3 presents the antimicrobial analysis findings for VkZnO and VkSe nanoparticles.

Table 3. Inhibition zone diameters (mm) for VkZnO and VkSe nanoparticles in antimicrobial tests
Çizelge 3. Antimikrobiyal testlerde VkZnO ve VkSe nanoparçacıklarının inhibisyon bölgesi çapları (mm)

Samples	*P. aeruginosa*	*K. pneumoniae*	*E. coli*	*S. aureus*	*C. albicans*
VkZnO	8.0±0.0	10.0±1.40	12.0±0.05	11.3±1.15	-
VkSe	-	7.0±0.0	-	-	-
Rifampin	16.0±3.6	12.3±0.6	11.3±1.15	20.0±0.0	12.6±1.50

When it came to antibacterial characteristics, VkZnO nanoparticles outperformed VkSe nanoparticles. In comparison to zinc nanoparticles, selenium nanoparticles had a very slight inhibitory impact on *K. pneumoniae*. However, VkZnO showed an inhibitory effect on *P. aeruginosa, K. pneumoniae, E. coli,* and *S. aureus* bacteria. VkZnO exhibited an inhibitory effect of 50% on *P. aeruginosa*, 81.3% on *K. pneumoniae*, 106.2% on *E. coli*, and 56.5% on *S. aureus* compared to the rifampin antibiotic. Zinc oxide nanoparticles react in both acidic and alkaline environments and release free Zn^{2+} ions as a result of their amphoteric nature. When these free Zn^{2+} ions interact with biomolecules like proteins and carbohydrates, they may disrupt the essential processes of bacteria (Siddiqi et al., 2018). Zinc oxide nanoparticles may have an advantage over selenium nanoparticles in antibacterial activity testing due to this characteristic. Antioxidant characteristics were demonstrated by both *V. kotschyi* nanoparticles. One of the generated nanoparticles, VkSe, had a higher antioxidant activity.

There are few studies on *V. kotschyi* in the literature. If we talk about some studies conducted with Verbascum species; Silver nanoparticles were synthesized with *Verbascum thapsus* and their photocatalytic activities were examined (Elemike et al., 2016). The antimicrobial activity of *Verbascum olympicum* Boiss., *Verbascum prusianum* Boiss., and *Verbascum bombyciferum* Boiss species was examined and it was reported that *Verbascum L.* species demonstrated antibacterial efficacy against gram (+) bacteria, and yeasts, but did not show activity against gram (-) bacteria (Dülger et al., 2002). According to different research, *V. sinuatum*'s beneficial bioactive components have anti-inflammatory, anti-cancer, cardiovascular, antibacterial, antidiabetic, and neuroprotective properties (Donn et al., 2023). Because of its antioxidant and antibacterial properties, *Verbascum pseudoholotrichum* has also been proposed as a food, chemically based, and pharmacology agent (Yabalak et al., 2022). *Verbascum thapsus* was used to create gold nanoparticles, which were then shown to have antiproliferative properties in a different study (Soto et al., 2022). *Verbascum thapsus* was used to create zero-valent iron nanoparticles once more, and the activity of Cr (VI) reduction was measured (Saleh et al., 2021).

V. sinaiticum showed antibacterial, antifungal, and antioxidant properties and this plant was highly suitable for the synthesis of Zinc-ferric bimetallic nanoparticles (Dinakarkumar et al., 2024). ZnONPs can be used in many biomedical applications such as anticancer, wound healing, drug delivery, antibacterial and diabetes treatment, anti-inflammation, and biological imaging (Emsen et al., 2023; Jiang et al., 2018; Mishra et al., 2017; Xiong, 2013; Zhang & Xiong, 2015). SeNPs are nanomaterials that attract attention due to their various therapeutic benefits such as anticancer, antioxidant, anti-inflammatory, and anti-diabetic effects (Khurana et al., 2019). *V. kotschyi* has not been the subject of any nanoparticle research reported yet. Unknown plants must be discovered and various species with antibacterial and antioxidant capabilities must be introduced via the manufacturing of nanoparticles. This study will introduce *V. kotschyi* and close what is lacking in the literature concerning *V. kotschyi*.

CONCLUSIONS and SUGGESTIONS

Because of their antioxidant and antibacterial qualities, *Verbascum kotschyi*-derived zinc and selenium oxide nanoparticles are good candidates for application in a range of product development fields, encompassing food preservatives, pharmaceuticals, and cosmetics. The durable and advantageous properties of *V. kotschyi* nanoparticles, a little-known species, may make them useful in product development.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Disclosure Statement

The authors report no conflicts of interest.

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