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Silver nanoparticles generated by green synthesis from *Verbascum cymigerum*: *In vitro* bioactivities and *in silico* evaluations

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ABSTRACT

Verbascum cymigerum Hub.-Mor. is an endemic species of the *Verbascum* genus of the *Scrophulariaceae* family, popularly known as the oxbow. This study is the first to investigate the biological activities of *Verbascum cymigerum* (VC) and its silver nanoparticle formulations. In this study, we evaluated the antioxidant, antibacterial, DNA-protective, anti-inflammatory, and enzyme inhibitory activities of the aqueous (AQ) extract of VC and its synthesized silver nanoparticles (AgNPs). Furthermore, the phenolic profile and content of the VC-AQ extract were characterized using LC-ESI-MS/MS analysis. The peak shown at 440 nm in UV-vis showed that a nanoparticle was formed, and as seen in SEM and XRD, the particle size was 12.47 nm, and it had a face-centered cubic structure. It was determined that VC-AQ contained high amounts of rutin (815.40 µg/g). The DPPH˙ scavenging activity and urease inhibition potential of VC-AgNP (IC_{50}, 4.43±1.88 µg/mL and 1.54±0.40 µg/mL, respectively) were higher than the used standards. The urease enzyme was shown to be strongly inhibited by the VC-AgNP model in both *in vitro* and *in silico* experiments. It was found that the DNA protection activity of VC-AgNP was more effective than the extract, with 88.77%. In addition, it was observed that VC-AgNP showed better antibacterial activity than the extract against *B. cereus*, but lower antibacterial activity than the antibiotic. Based on these results, VC-AgNPs exhibit significant potential for use as free radical scavengers, urease inhibitors, and DNA protective agents. These findings suggest that VC-AQ and VC-AgNPs hold significant potential for diverse industrial applications, providing a foundation for future research in these fields.

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ÖZET

Verbascum cymigerum Hub.-Mor. halk arasında öküz yayı olarak bilinen Scrophulariaceae familyasından *Verbascum* cinsinin endemik bir türüdür. *Verbascum cymigerum* (VC) ve gümüş nanopartikülü ile ilgili biyolojik aktivite çalışmalarını ilk kez incelenmeyi amaçladık. Bu çalışmada, VC'nin sulu (AQ) ekstresinin ve sentezlenen gümüş nanopartiküllerinin (AgNPs) antioksidan, antibakteriyel, DNA koruyucu, antiinflamatuar ve enzim inhibitör aktiviteleri değerlendirilmiştir. Ayrıca, VC-AQ ekstresinin fenolik profili ve içeriği LC-ESI-MS/MS analizi kullanılarak karakterize edilmiştir. UV-görünür ışıkta 440 nm'de gösterilen pik, nanopartikülün oluştuğunu ve SEM ve XRD'de görüldüğü gibi partikül boyutunun 12.47 nm olduğunu ve yüzey merkezli kübik bir yapıya sahip olduğunu göstermiştir. VC-AQ'nun yüksek miktarda rutin (815.40 µg/g) içerdiği belirlenmiştir. VC-AgNP'nin DPPH˙ giderme aktivitesi ve üreaz inhibisyon potansiyeli (IC_{50}, 4.43±1.88 µg/mL ve 1.54±0.40

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µg/mL) kullanılan standartlardan daha yüksek olarak saptanmıştır. Üreaz enziminin hem *in vitro* hem de *in silico* deneylerde VC-AgNP modeli tarafından güçlü bir şekilde inhibe edildiği bulundu. VC-AgNP'nin DNA koruma aktivitesinin %88.77 ile ekstreden daha etkili olduğu görülmüştür. Ayrıca, VC-AgNP'nin *B. cereus*'a karşı ekstreden daha iyi antibakteriyel aktivite gösterdiği, ancak antibiyotikten daha düşük antibakteriyel aktivite gösterdiği belirlendi. Bu sonuçlara dayanarak, VC-AgNPs'nin serbest radikal temizleyici, üreaz inhibitörü ve DNA koruyucu madde olarak kullanım açısından önemli bir potansiyel gösterdiği görülmektedir. Bu bulgular, VC-AQ ve VC-AgNPs'nin çeşitli endüstriyel uygulamalar için önemli bir potansiyel taşıdığını ve bu alanlardaki gelecekteki araştırmalar için bir temel oluşturduğunu göstermektedir.

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INTRODUCTION

Scrophulariaceae is a cosmopolitan family and was first described by de Jussieu (1789). It is the largest family among the families within the order Lamiales (formerly Scrophulariales). The Scrophulariaceae family is divided into two groups: the "Scroph I" group includes *Celsia* L.*, Verbascum* L.*, Nicodemia* Ten.*, Selago* L.*, Buddleja* L.*,* and *Scrophularia* L. A second group, "Scroph II", includes *Digitalis L., Antirrhinum* L.*, Plantaginaceae* L.*, Veronica* L.*, Callitrichaceae* L.*,* and *Hippuridaceae* L. (Olmstead & Reeves, 1995). The genus Verbascum is one of the most species-rich genera in the Flora of Turkey. While it is represented by approximately 450 species worldwide, its diversity in Turkey has reached nearly 400 taxa, comprising 261 species and 132 natural hybrids. Notably, 201 of these species are endemic, representing a remarkably high endemism rate of approximately 77% within the country (Güner & Aslan, 2012). *Verbascum* is native to the European and Asian continents and grows wild in clearings, barren lands, stony ground, roadsides, and woodlands. *Verbascum* L. species are herbaceous plants that might be annual, biennial, or perennial; they are seldom tiny shrubs. They are mostly found in uncultivated areas and on roadsides. Their stems are upright, sometimes branched, and covered with woolly hairs. The fruits are round and have many seeds (Davis, 2019). Methanol extracts from selected *Verbascum* species were assessed for their phenolic content and biological activities, specifically their antioxidant and antibacterial properties. In particular, *Verbascum* species showed effective antimicrobial activity against *Mycobacterium smegmatis, Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus,* and *Micrococcus luteus* (Dulger et al., 2005).

Verbascum species display a diverse pharmacological profile, including expectorant, emollient, diuretic, antipyretic, sedative, antiseptic, antimalarial, and narcotic activities. Traditionally, these plants have been employed both as infusions and topical poultices. They have been documented in the management of respiratory disorders, such as bronchitis, asthma, migraine, and spasmodic coughs; inflammatory conditions, including rheumatism, hemorrhoids, and tumors; as well as superficial fungal infections, gastrointestinal spasms, pruritus, and ocular discomfort. Moreover, certain *Verbascum* species have demonstrated inhibitory effects against specific influenza viruses, highlighting their potential antiviral applications (Demirezen, 2019).

V. cymigerum Hub.-Mor.*, V. sinaiticum* Benth.*, V. gypsicola* Vural & Aydoğdu*,* and *V. sinuatum* L. were found to exhibit good antibacterial effects (Alper & Basaran, 2009; Dulger & Gonuz, 2004; Dulger et al., 2005; Tadeg et al., 2005). Güzelvardar (2021) investigated the antioxidant activities of *V. serratifolium* Hub.-Mor.*, V. bugulifolium* Lam.*, V. basivelatum* Hub.-Mor., *V. ovalifolium* Donn ex Sims*, V. bithynicum* Boiss.*, V. degenii* Halácsy, *V. yurtkuranianum* Kaynak, Daşkın & Yılmaz*, V. olympicum* Boiss.*, V. stenostachyum* Hub.-Mor.*, V. afyonense* Hub.-Mor.*, V. prusianum* Boiss.*, V. vacillans* Murb.*, V. gypsicola* Vural & Aydoğdu*, V. speciosum* Schrad.*, V. cheiranthifolium* Boiss. species.

In previous studies, there were no studies on antioxidant, anti-inflammatory, DNA protection, urease inhibition, and urease inhibition kinetics related to *V. cymigerum*. Consequently, this study represents the first comprehensive evaluation of these synergistic activities, filling a significant gap in the current phytochemical understanding of these taxa. In addition, the activities of AgNPs were investigated by forming nanoparticles (NP) with Ag-metal, and UV-vis, FTIR, SEM, and XRD were used to characterize the NP. LC-ESI-MS/MS analyzed the phenolic components of the VC-AQ. The interaction of Ag metal with the highest of the compounds determined in

LC-ESI-MS/MS and its effect on the urease enzyme were determined by computer calculations and docking. According to these results, the *V. cymigerum* extract and silver nanoparticles are understood to be promising.

MATERIAL and METHOD

Plant Collection

VC, popularly known as bunch mullein, is endemic and has herbarium number YY618 at the Department of Biology, Faculty of Arts and Sciences, Bingöl University, from locality number 52 of the Hiro plateau and its surroundings, located in Adaklı district in the north of Bingöl province. It was collected by Prof. Dr. Lutfi Behcet.

Extract Preparation

The aerial part of the VC is extracted using water by the maceration method. The plant extract was then filtered on filter paper and removed from the water with the help of a lyophilizer. The dry extract is collected and will be stored for use in chemical analysis and bioactivity tests at +4°C.

Silver nanoparticle (AgNP) Synthesis

5 g of plant, 100 mL of water, and the maceration process were applied. Then the plant extract was filtered through filter paper (No. 1). The pH of the obtained extract was adjusted to 7, and the extract was added to 100 mL of 0.01 M $AgNO_3$. The plant solution was added drop by drop to the heated AgNP solution (50 °C). The resulting reaction mixture was transferred to Falcon tubes and subjected to centrifugation to isolate the synthesized nanoparticles (4500 rpm, 30-40 min) (Erenler et al., 2021). As a negative control, a solution containing only 0.01 M $AgNO_3$ (without plant extract) was incubated under identical conditions (50°C, pH 7.0). No color change or SPR peak was observed, confirming that nanoparticle formation is solely mediated by VC-AQ. A color transformation was observed at the end of the reaction, indicating AgNP synthesis. After the procedure, the upper liquid is excreted, and the collapsed part is left to dry. The dry sample is crushed in the mortar. It is stored at 0°C for use in tests.

Characterization of AgNPs

VC-AgNP was centrifuged for 25 min at 10000×g in optimized conditions (Erenler et al., 2021). After centrifugation, the residue was lyophilized and dried. To determine the formation of VC-AgNP, scanning was performed in the 300-700 nm range by UV-Vis spectrophotometry (BIOTEK (Epoch2) microplate reader). Fourier transform infrared spectroscopy (Perkin Elmer, FTIR spectroscopy) analysis was performed to determine the chemical groups of the natural compounds in the VC-AgNP formation. The morphology and particle diameter of the nanoparticles were determined using scanning electron microscopy (SEM, JSM-7001F Schottky Emission Scanning Electron Microscope) with EDS analysis, and the crystal structure of the nanoparticles was also determined using X-ray diffraction (XRD, Rigaku Smart Lab X-Ray Diffractometer).

LC-ESI-MS/MS Analysis

We conducted the VC-AQ content analysis using Agilent 1260 Infinity II LC-ESI-MS/MS equipment. Chromatographic separation was performed using an Agilent Poroshell120 EC-C18 reversed-phase analytical column, measuring 100 mm × 3.0 mm and 2.7 µm (Basar et al., 2024). The method procedure was carried out under the same conditions as in the studies of Atalar et al. (2023) and Yildiz et al. (2024).

Antioxidant Assay

The antioxidant activities of VC-AQ and VC-AgNP were evaluated by phosphomolybdate reductase assay (PMRA) (Prieto et al., 1999) and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) scavenging activity (Blois, 1958). For PMRA, the activity was presented as the effective concentration of 0.5 absorbance (µg/mL), and for $DPPH^{\cdot}$, the IC_{50} value (µg/mL) was presented as the 50% half-maximum scavenging concentration. PMRA was measured at 695 nm, and $DPPH^{\cdot}$ scavenging activity was measured at 517 nm. Ascorbic acid was tested simultaneously with the samples, and the results obtained were compared with those of the standard compound.

Urease Inhibitory Potential

Urease inhibitory potentials of VC-AQ and VC-AgNP were studied (Zhang et al., 2006). This analysis used a microplate reader to calculate indophenol formation of VC-AQ and VC-AgNP as IC50 from absorbance values at 630 nm. The results were compared with the standard drug thiourea.

Antibacterial Assay

The antibacterial potential of VC-AQ and VC-AgNPs was evaluated against six bacterial strains using the disk diffusion method (Reller et al., 2009). *Pseudomonas aeruginosa* ATCC 15442, *Klebsiella pneumoniae* ATCC 10031, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Bacillus cereus* CCM 99, and *Staphylococcus aureus* ATCC 25213 bacteria were obtained from the Biochemistry Research Laboratory of the Department of Chemistry, the Faculty of Science at Ondokuz Mayis University. Before starting the activity, each bacterial culture was diluted to the McFarland turbidity standard of 0.5 (1.5×10^8 CFU/mL). Before being placed on agar, the samples (50 µL) were absorbed into discs. It was incubated at 37ºC for 16 to 18 hours. Millimeters are used to measure the inhibitory zone. Chloramphenicol was used as a positive control.

Anti-inflammatory Activity

A somewhat different approach was taken than that of Rahman et al. (2012) while investigating the effects of VC-AQ and VC-AgNP on the denaturation of bovine serum albumin (BSA) protein. 0.05 mL of different diclofenac sodium (DFS) concentrations (50, 100, and 250 µg/mL) or samples were combined with 0.45 mL BSA (0.5% w/v). The control solution comprises 0.45 mL of BSA and 0.05 mL of distilled water. After incubating the samples for 20 minutes at 37ºC and three minutes at 57ºC, 2.5 mL of phosphate buffer 0.1 M pH 6.3, was added. At 255 nm, absorbance values were determined with a UV-Vis spectrophotometer (Başar et al., 2025). IC_{50} values were provided for the results.

DNA Protection Activity

Agarose gel electrophoresis was used to assess the DNA-protecting properties of VC-AQ and VC-AgNP (Başar et al., 2023). After creating a 1% agarose gel (using 1% Tris-Borate-EDTA (TBE)), 2 µL of ethidium bromide was added. The DNA plasmid (3 µL) and ddH_2O (6 µL) served as the negative control (C1). The positive control (C2) consisted of a mixture containing 3 µL of plasmid DNA, 6 µL of ddH_2O, and 1 µL of H_2O_2, which was subsequently exposed to UV radiation for five minutes. The sample combination was 3 µL of plasmid DNA, sample (5 µL, 1000 µg/mL), H_2O_2 (1 µL), and UV for five minutes. Both the control and the sample received two microliters of blue loading dye. Wells were run for 60 minutes at 90 V. A UV transilluminator (320 nm, 8000 µW/cm) was used for imaging.

The percentage of supercoiled DNA (Form I) was calculated using ImageJ software by measuring the integrated density of each band and normalizing to the negative control (C1). DNA protection activity (%) was calculated as: [Form I value (sample) / Form I value (negative control)] × 100.

Statistical Analysis

Each variable in the biological activity was evaluated three times, yielding an average±standard deviation. The results were statistically examined using SPSS 20.0. The analytic mean would be regarded as homogeneous across more than two groups if the data distribution were regular. Given that Tukey's HSDa,b is a multiple comparison process, it was used. There were statistically significant differences ($p < .05$) between the values derived from each activity analysis and the samples and standards.

Preparation of Molecules and Docking Applications

The rutin was initially drawn using ChemDraw, and its energy minimization was performed with Chem3D. The 3D structures of all target ureases from Jack bean were obtained from the Protein Data Bank (PDB) (PDB ID: 4GY7 at 1.49 Å resolution), selecting crystal structures with high resolution (Berman et al., 2000). Each protein structure was pre-processed using the Molegro Virtual Docker 6.0.1 software; structural errors were corrected, and all ligands, non-protein molecules, and water molecules were removed before docking. The protein was defined as the receptor, and rutin as the ligand. Protein-ligand docking was done using the same software and the MolDock scoring function (Alyar et al., 2021; Thomsen & Christensen, 2006). The best binding interactions between the protein and rutin were visualized using BIOVIA Discovery Studio Visualizer. This program generated two-dimensional and three-dimensional interaction diagrams to clarify the ligand's position within the protein's active site and highlight the types of bonds formed (Almaz et al., 2021; Biovia, 2019).

RESULTS and DISCUSSION

LC-ESI-MS/MS

LC-ESI-MS/MS analyzed the phenolic components of VC-AQ. Twelve components were determined, and their amounts are shown in Table 1. The retention times are shown in the chromatogram in Fig. 1. According to these

results, the most abundant phenolic components were found to be rutin (815.40 µg/g extract), vanillic acid (497.43 µg/g extract), and hesperidin (143.08 µg/g extract).

The presence of key secondary metabolites, such as rutin, vanillic acid, and hesperidin, has been extensively documented in various *Verbascum* species. These compounds are well-recognized for their potent antioxidant, anti-inflammatory, and cardioprotective properties (Amini et al., 2022; Angeloni et al., 2021; El Gizawy et al., 2019; Gökmen et al., 2021; Hazman et al., 2024; Marian et al., 2018; Yagi et al., 2024; Zengin et al., 2021; Zengin et al., 2023).

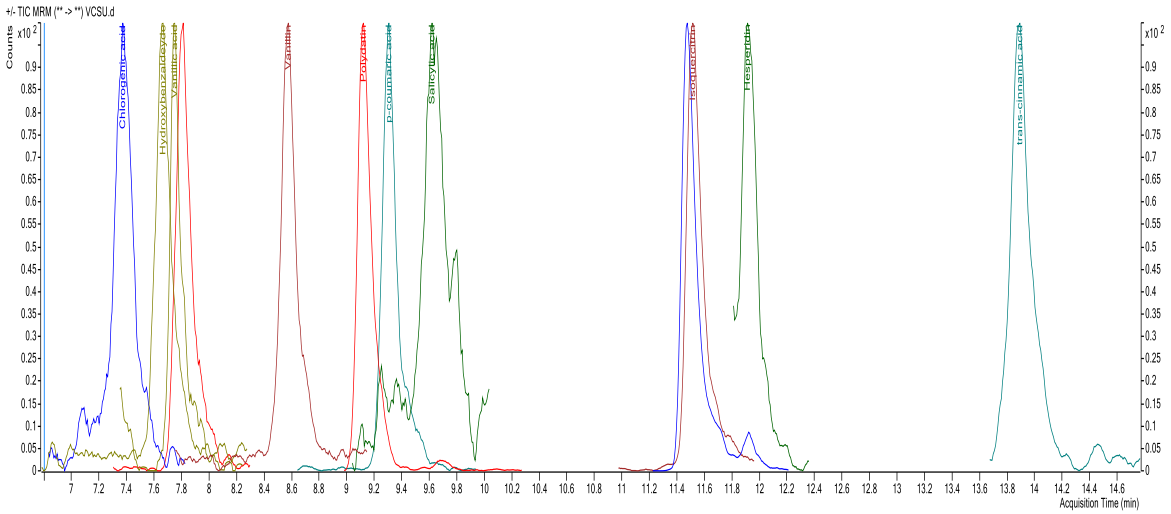


Figure 1. The LC-ESI-MS/MS chromatogram of VC-AQ extract
Şekil 1. VC-AQ'nun LC-ESI-MS/MS kromatogramı

Table 1. The LC-ESI-MS/MS analysis results of VC-AQ
Çizelge 1. VC-AQ'nun LC-ESI-MS/MS analiz sonuçları

No.	Components	R.T. (min.)	VC-AQ, µg/g extract
1	Chlorogenic acid	7.36	15.82
2	Hydroxybenzaldehyde	7.63	12.60
3	Vanillic acid	8.19	497.43
4	Caffeic acid	7.81	26.35
5	Vanillin	8.58	14.03
6	*p*-coumaric acid	9.33	33.95
7	Salicylic acid	9.61	23.22
8	Polydatin	9.74	18.72
9	Isoquercitrin	11.55	36.95
10	Rutin	11.81	815.40
11	Hesperidin	11.51	143.08
12	*trans*-cinnamic acid	13.90	39.93

R.T.; Retention time, **VC-AQ:** *Verbascum cymigerum* aqueous extract

UV–Vis Analysis

The $AgNO_3$ was converted from its aqueous ionic form into solid metal nanoparticles to complete the synthesis. The development of VC-AgNPs was indicated by the change in the color of the solution from green to black. UV-Vis spectroscopy was used to analyze these particles and confirm that the plant extract was used in the creation of the nanoparticles. UV-Vis spectra from VC-AQ are displayed in Fig. 2. In contrast to the VC-AQ, which lacked peaks, the VC-AgNPs had a distinct peak at 440 nm (Fig. 2).

Numerous scientists have used UV-Vis spectra to monitor the nanoparticles' creation; in the AgNP UV-Vis spectrum, a prominent peak is typically seen between 400 and 500 nm, as in our results (Başar & Erenler, 2024; Başar et al., 2024; Karan & Erenler, 2024; Nayel et al., 2024).

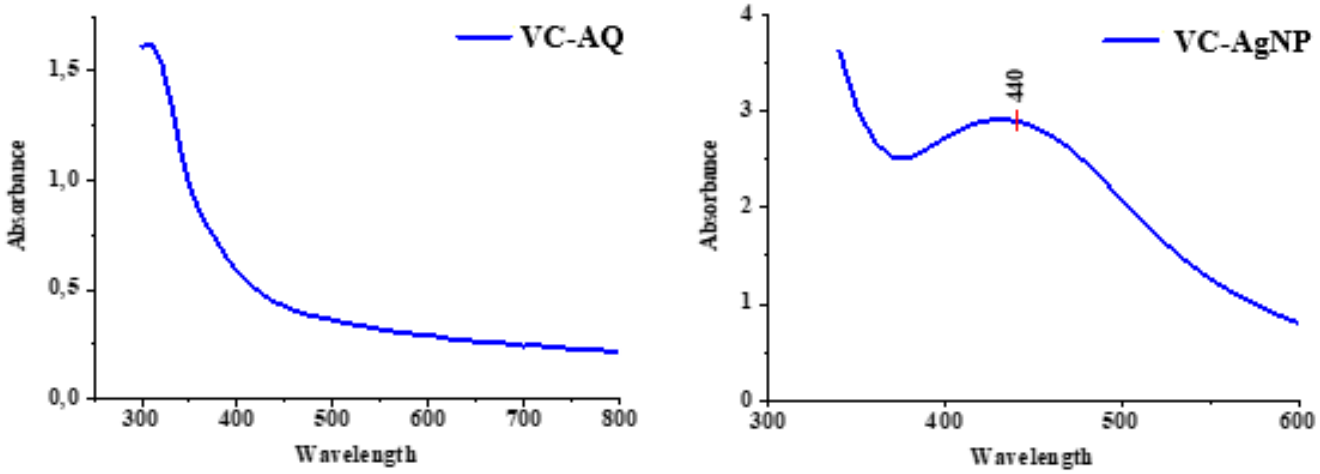


Figure 2. The UV–Vis spectra of VC-AQ and VC-AgNP
Şekil 2. VC-AQ ve VC-AgNP'nin UV-Vis spektrumları

FTIR Analysis

FTIR spectroscopy was applied to determine the chemical composition of AgNPs' active groups. The recorded spectra were obtained across the wavelength range of 400–4000 cm^{-1}, as shown in Fig. 3. The FTIR spectra show that the following causes are responsible for the observed changes: 3309 cm^{-1} (due to N-H stretching and amides), 2904 cm^{-1} (caused by C-H stretching and alkanes), 1110 cm^{-1} (phenotypic hydroxyl groups) and 1039 cm^{-1} (caused by C-stretching and ether groups). As can be seen in the accompanying images, these results suggest that the molecular functional groups may be responsible for lowering Ag ions. Examples of phytochemical compounds found in biomass include ether or epoxy groups, aldehydes, alkanes, and alcohols. These compounds are believed to have a role in the nucleation process that transforms Ag+ into Ag (Daoudi et al., 2022). Alkaloids, pigments, proteins, carbonyl groups, amides, flavanones, amines, phenolics, terpenoids, and reduction factors present in biological extracts are among the many compounds that might initiate the production of nanoparticles (Hamouda et al., 2023).

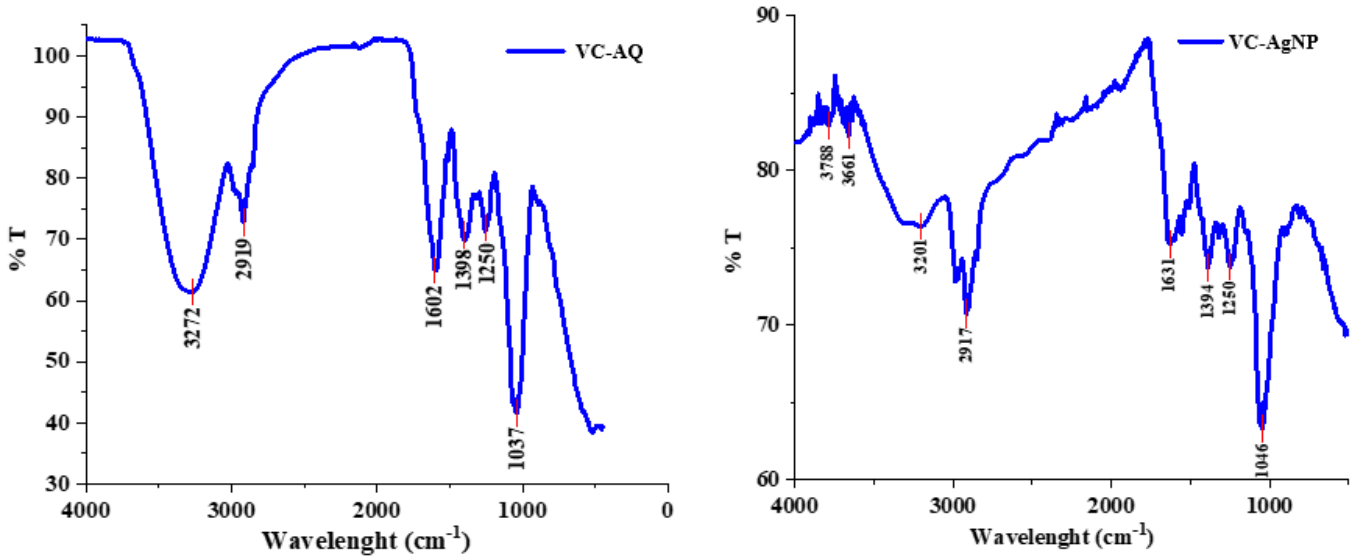


Figure 3. The FTIR spectra for VC-AQ and VC-AgNP
Şekil 3. VC-AQ ve VC-AgNP için FTIR spektrumları

SEM and XRD Analysis of AgNP

AgNP created the SEM image seen in Figure 4 on a carbon-coated copper scanning electron microscopy (SEM) grid. This micrograph shows spherical AgNPs with a modest density dispersion and a VC-AgNP size of 12.47 nm. Sondi

and Salopek-Sondi (2004) demonstrated using TEM to characterize the nanoparticles. According to our literature study, the EM technique has been extensively employed in the characterization of nanoparticles, yielding crucial details regarding the particles' stability and production in addition to their size and shape (Fetyan et al., 2024). The X-ray diffraction (XRD) scan's distinct peaks offered more evidence and confirmation that the produced AgNPs were accurate (Fig. 4). According to the XRD spectra, similar peaks are observed in the diffraction peaks of the extract nanoparticles (2θ= 27.86°, 32.27°, 38.18°, 44.38°, 46.28°, 54.89°, 57.54°, 64.57°, 77.28°). They were assigned to the Ag (111), (200), (220), and (311) planes, and the nanoparticle structure was found to be face-centered cubic (fcc). Additionally, Debye-Scherrer's equation (Eq. 1), developed by Ajitha et al. (2014), was used to estimate the average crystalline size of the silver nanoparticles:

$$D = 0.9\lambda/\beta \cos\theta \quad (1)$$

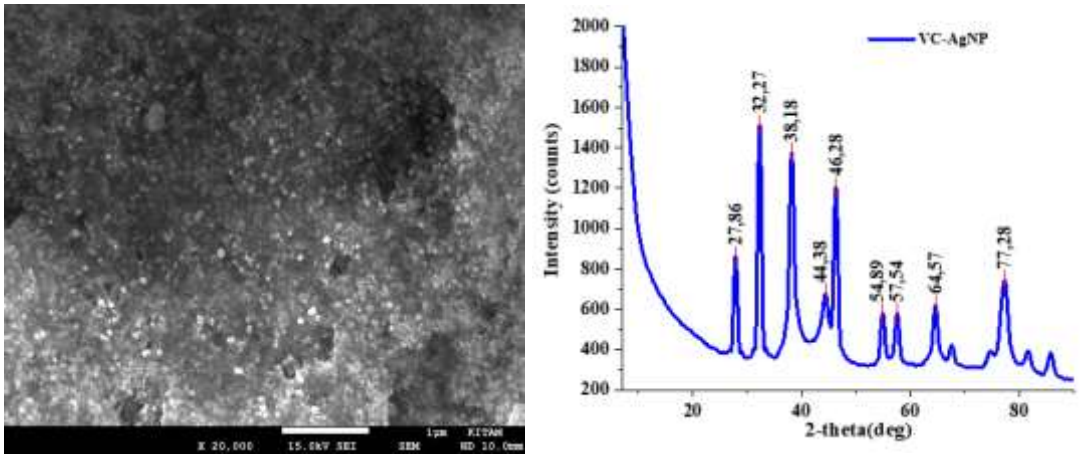


Figure 4. The SEM images and XRD pattern of VC-AgNP
Şekil 4. VC-AgNP'nin SEM görüntüsü ve XRD deseni

Antioxidant Activity

Free radicals in living things are closely related to degenerative processes in molecular biology. The imbalance between oxidants and free radicals in organisms leads to oxidative stress, which is exacerbated by these processes and causes numerous issues in the human body (Munteanu & Apetrei, 2021; Shahidi & Zhong, 2015). Furthermore, this oxidative stress has been linked to many health issues over time, including rheumatoid arthritis, pulmonary and ophthalmic disorders, cardiovascular problems, neurological and respiratory issues, Parkinson's disease, Alzheimer's disease, cancer, and fertility issues (Pham-Huy et al., 2008; Silva et al., 2024). The body can be shielded from these oxidative reactions by the presence of chemical substances called antioxidants from several sources (Munteanu & Apetrei, 2021). The process of cancer begins with chromosomal abnormalities and oncogene activation, which are aided by the organism's free radicals (Valko et al., 2004).

By neutralizing free radicals in biological cells, these antioxidants can prevent the harmful effects of free radicals on the body (Munteanu & Apetrei, 2021). As a result, many researchers are seeking novel compounds that can provide antioxidant activity in organisms, in addition to those naturally found in fruits and vegetables. Furthermore, issues exacerbated by professional relationships, the burden of household duties on top of professional work, social injustices, family obligations, the environment, free time, food, and other facets of life connected to work, housing, and income have an important impact on human health and encourage oxidative stress in the body (do Amparo Madureira et al., 2024; Silva et al., 2024).

The antioxidant activities of the extract and nanoparticles were determined by phosphomolybdate reducing assay (PMRA) and radical scavenging methods, and the results were compared with the standards. In PMRA activity, VC-AQ ($A_{0.5}$, 75.28±0.24 µg/mL) showed higher activity than VC-AgNP ($A_{0.5}$, 129.06±0.55 µg/mL). It showed lower activity than the standard. It was noted that VC-AgNP (IC_{50} 4.43±1.88 µg/mL) had the best radical scavenging effect (Table 2).

Phosphomolybdenum activity findings of *V. diversifolium* Hochst. extracts prepared from n-hexane, dichloromethane, ethyl acetate, ethanol, 70% ethanol, and water ranged from 0.97 to 2.58 mmol TE/g, with the water extract exhibiting lower activity (Yagi et al., 2024). Although we used a different *Verbascum* species in our study, the water extract exhibited lower activity, which is consistent with the literature.

Table 2. Antioxidant activity, urease inhibition potential, and anti-inflammatory activity results
Çizelge 2. Antioksidan aktivite, üreaz inhibisyon potansiyeli ve anti-inflamatuar aktivite sonuçları

Samples/ Standards	PMRA,	DPPH˙ scavenging	Urease	Anti-inflammatory
	$A_{0.5}$, µg/mL	IC_{50}, µg/mL		
VC-AQ	75.28±0.24^{b}	29.55±10.40^{b}	20.87±4.50^{c}	75.36±2.77^{b}
VC-AgNP	129.06±0.55^{c}	4.43±1.88^{a}	1.54±0.40^{a}	73.18±0.74^{b}
Ascorbic acid	20.93±0.02^{a}	5.88±2.96^{a}	*nt*	*nt*
Thiourea	*nt*	*nt*	4.04±0.01^{b}	*nt*
DFS	*nt*	*nt*	*nt*	8.88±0.37^{a}
F (p value)	3.82 (p = .02)	3.82 (p = .02)	3.82 (p = .02)	3.82 (p = .02)

VC-AQ: *Verbascum cymigerum* aqueous extract, **VC-AgNP:** *Verbascum cymigerum* silver nanoparticles, **DFS:** Diclofenac sodium, **DPPH˙**: 2,2-Diphenyl-1-picrylhydrazyl, **PMRA:** Phosphomolybdate reductase assay, n: 3, *nt*: not tested, $p < .05$

In a previous study, the IC_{50} values of leaf methanol extracts of sixteen *Verbascum* species for DPPH˙ scavenging activity were calculated as between 10 and 400 µg/mL (Güzelvardar, 2021). The DPPH˙ radical scavenging activity of *V. diversifolium* Hochst. extracts followed a polarity-dependent trend, with the highest capacities observed in ethanolic solvents (46.86 and 46.33 mg TE/g) (Yagi et al., 2024). This suggests that the radical-scavenging potential is primarily driven by polar secondary metabolites, whereas non-polar fractions (hexane and dichloromethane) contribute negligibly to the overall antioxidant profile. The literature reports that the water extract exhibited a moderate radical-scavenging effect. Still, in our study, it was observed that the water extract had a low radical scavenging effect. In prior research, when compared to vitamin C, which was used as a reference, the DPPH˙ scavenging activity of both (*V. insulare*) Boiss. & Heldr. VIE aqueous extract and VIE-AgNPs nanoparticles were shown to be poor (Hazman et al., 2024). Contrary to the literature, our study showed high radical scavenging activity of AgNPs. The IC_{50} value of DPPH˙ scavenging activity of the methanol extract of *V. pinetorum* Boiss. & Heldr. was determined as 13.04±0.21 mg/mL (Ozcan et al., 2011). DPPH˙ scavenging activity of lyophilized extract of *V. flos-cossonis* Hub.-Mor. was found to be 86.36±13.67% (Marian et al., 2018). DPPH˙ scavenging activity of *V. pseudoholotrichum* IC50 values were obtained as 0.775, 1.254, and 0.539 mg/mL for methanol, ethanolic, and water extracts (Yabalak et al., 2022). Although the water extract of *V. pseudoholotrichum* Hub.-Mor. showed high radical scavenging activity in the study, the water extract of *V. cymigerum* in our study showed low activity. The IC_{50} values of DPPH˙ scavenging activity of *V. sinaiticum* Benth. leaf extract and silver nanoparticles were determined as 143 µg/mL and 216 µg/mL (Geyesa et al., 2024). Contrary to the literature, in our study, it was determined that the radical scavenging activity of AgNPs was high. In a previous study, the IC_{50} values of six *Verbascum* species for DPPH˙ scavenging activities were calculated as between 160 and 257 µg/mL (Mihailović et al., 2016). The IC_{50} values of hexane, ethyl acetate, and water extracts of ten *Verbascum* species for DPPH˙ scavenging activities were calculated as between 9.81 and 199.62 µg/mL (Selseleh et al., 2020).

Urease Inhibition Potential

Gastrointestinal ulcer is one of the most important gastrointestinal diseases (Cryer & Mahaffey, 2014). The development of stomach ulcers and chronic gastritis is significantly influenced by *Helicobacter pylori* (Cover & Blaser, 2009). The mucosal layer contains this bacterium, situated close to epithelial cells, and results in inflammation, tissue lesions, cellular abnormalities, and mucosal alterations (Blaser, 1993; Goodwin et al., 1997). This bacterium produces large amounts of urease, a multimeric protein containing nickel that hydrolyzes urea to produce ammonium and carbon dioxide (Qin & Cabral, 2002; Zerner, 1991). Because the urease enzyme alkalizes the surrounding pH, it may be a significant virulence factor contributing to gastric mucosal colonization and mucosal injury. Urease inhibitors prevent stomach ulcers and tissue injury by reducing enzyme activity(Hassan & Šudomová, 2017). Gastrointestinal disorders, such as ulcers, cause serious health issues all over the world. The bacteria's antibiotic resistance over the past ten years has prevented the development of a viable treatment, despite the availability of numerous medications intended to control these illnesses (Boyanova et al., 2023; Tshibangu-Kabamba & Yamaoka, 2021). There is no prior research on VC's inhibitory action against urease.

The enzyme inhibition potential of the extract and nanoparticles was determined using urease, and the calculated IC_{50} values were compared with those of thiourea. VC-AgNP exhibited the highest urease inhibition with an IC_{50} value of 1.54±0.40 µg/mL (Table 2).

Although no studies have been reported on the urease inhibitory activity of *Verbascum* species, several investigations have demonstrated that urease inhibition is widely distributed among plant taxa and is mainly associated with specific secondary metabolites rather than taxonomic position. For example, plant extracts from different species such as *Rumex* spp. and *Pistacia atlantica* have shown significant urease inhibitory activity with notable IC_{50} values (Ahmed et al., 2013; Uddin et al., 2016). In addition, studies on isolated plant phenolics,

including flavonoids and related compounds, confirmed their ability to inhibit urease enzyme activity *in vitro* (Rauf et al., 2017).

Moreover, broader screening studies revealed that numerous plant extracts from unrelated families exhibit strong urease inhibition, highlighting the widespread nature of this activity across the plant kingdom (Amin et al., 2013; Rana et al., 2021). Since *Verbascum* species are known to be rich in phenolic compounds, flavonoids, and iridoid glycosides, it is reasonable to suggest that they may possess urease inhibitory potential, although this has not yet been experimentally verified.

Therefore, the lack of studies on *Verbascum* species represents a significant gap in the literature rather than an indication of the absence of activity.

Anti-inflammatory Activity

AgNPs' antibacterial activity and anti-inflammatory activity can hasten wound healing and lessen or completely eradicate inflammation (Mani et al., 2015). Reduced physiological functions, redness, swelling, heat, increased protein denaturation, altered membranes, and increased vascular permeability are all signs of inflammation. Inflammation is the body's immune system's defense against infection, injury, or damage caused by microorganisms, harmful chemicals, or physical trauma (Ginwala et al., 2019; Ogbomade et al., 2019). The release of blood components, clotting factors, and platelets at the wound or injury site starts the processes of inflammation and hemostasis (Giri et al., 2019). Red blood cell (RBC) hemolysis is typically evaluated *in vitro* to check for anti-inflammatory properties of medications that maintain RBC plasma membranes, preventing heat- or hypotonicity-induced hemolysis (Ogbomade et al., 2019). Inflammation is commonly treated with nonsteroidal drugs, but these drugs have many adverse effects, including gastrointestinal irritation, which can lead to the development of stomach ulcers (Ratnayake et al., 2019). Therefore, AgNPs with anti-inflammatory properties could be a promising therapeutic option for inflammatory illnesses. Because of their angiogenic and anti-inflammatory qualities, AgNPs stop microbial infection in wounds, which speeds up the healing process (Giri et al., 2019).

Denaturation of BSA was investigated for anti-inflammatory activity. The activities of VC-AQ and VC-AgNP were observed to be lower than those of DFS. However, the activities of VC-AQ and VC-AgNP were very close (Table 2). The anti-inflammatory activity of VC-AQ and VC-AgNP was evaluated by their ability to prevent heat-induced denaturation of BSA, a well-established in vitro model that correlates with *in vivo* membrane stabilization effects.

Although no studies were found regarding the anti-inflammatory activity of *Verbascum* species, several genera within the same or closely related families have been extensively investigated. For instance, *Scrophularia* species have been reported to exhibit significant anti-inflammatory effects, mainly attributed to iridoid glycosides and phenylpropanoid derivatives that inhibit pro-inflammatory mediators (Pasdaran & Hamedi, 2017). Similarly, *Plantago* species are well known for their anti-inflammatory activity, which has been linked to compounds such as aucubin and various flavonoids (Samuelsen, 2000).

These findings indicate that anti-inflammatory activity is relatively common among phylogenetically related genera and is largely associated with the presence of phenolic compounds and iridoid glycosides. Since *Verbascum* species are also rich in these classes of secondary metabolites, the absence of studies on their anti-inflammatory activity likely represents a gap in the literature rather than a lack of pharmacological potential.

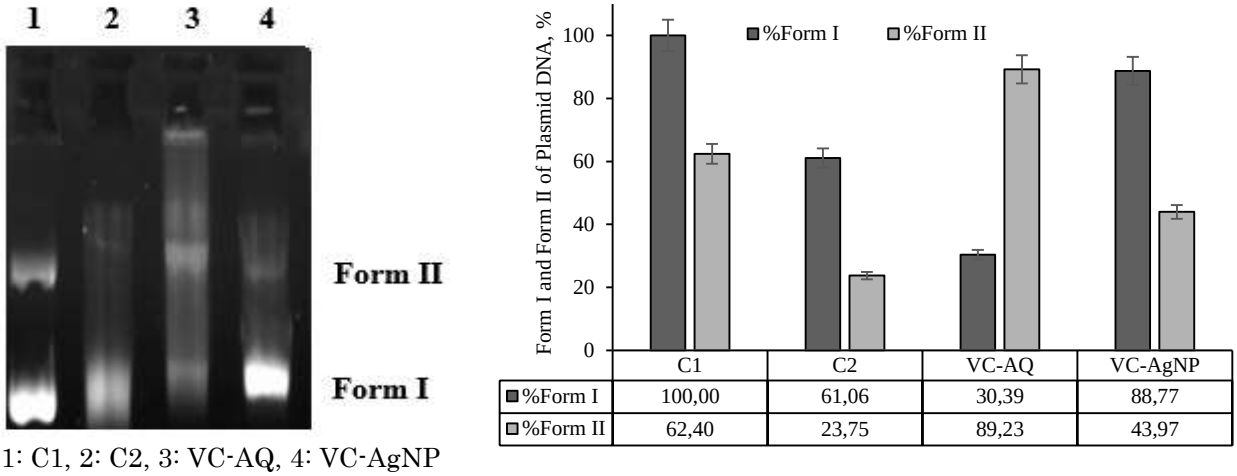
DNA Protection Activity

DNA protection activity was obtained by agarose gel electrophoresis, and percentage form values were calculated with the ImageJ program. It was observed that VC-AQ did not protect DNA but instead degraded it. VC-AgNP was observed to protect DNA at a high level with 88.77% (Form I) (Fig. 5).

Interestingly, VC-AQ itself caused DNA degradation, whereas VC-AgNP showed strong protection (88.77%). This may be attributed to the presence of redox-active phenolic compounds (e.g., vanillic acid, caffeic acid) in VC-AQ, which can generate reactive oxygen species under UV light. The encapsulation of these compounds within AgNPs may neutralize their pro-oxidant effects, converting them into radical scavengers.

Enzyme Kinetic

Urease enzyme inhibition of the samples was examined. Both Michaelis-Menten and Lineweaver-Burk graphs were plotted for inhibition (Fig. 6). VC-AQ and VC-AgNP showed mixed half-competitive inhibition, and their inhibition constants were determined as 36.99 and 65.49 µg/mL, respectively (Table 3). When the literature was searched, no studies were found on the urease inhibition kinetics of *Verbascum* species.



	C1	C2	VC-AQ	VC-AgNP
%Form I	100,00	61,06	30,39	88,77
%Form II	62,40	23,75	89,23	43,97

1: C1, 2: C2, 3: VC-AQ, 4: VC-AgNP

VC-AQ: *Verbascum cymigerum* aqueous extract, VC-AgNP: *Verbascum cymigerum* silver nanoparticles

Figure 5. Agarose gel images and percent DNA protection activity

Şekil 5. Agaroz jel görüntüleri ve DNA koruma aktivitesinin yüzdesi

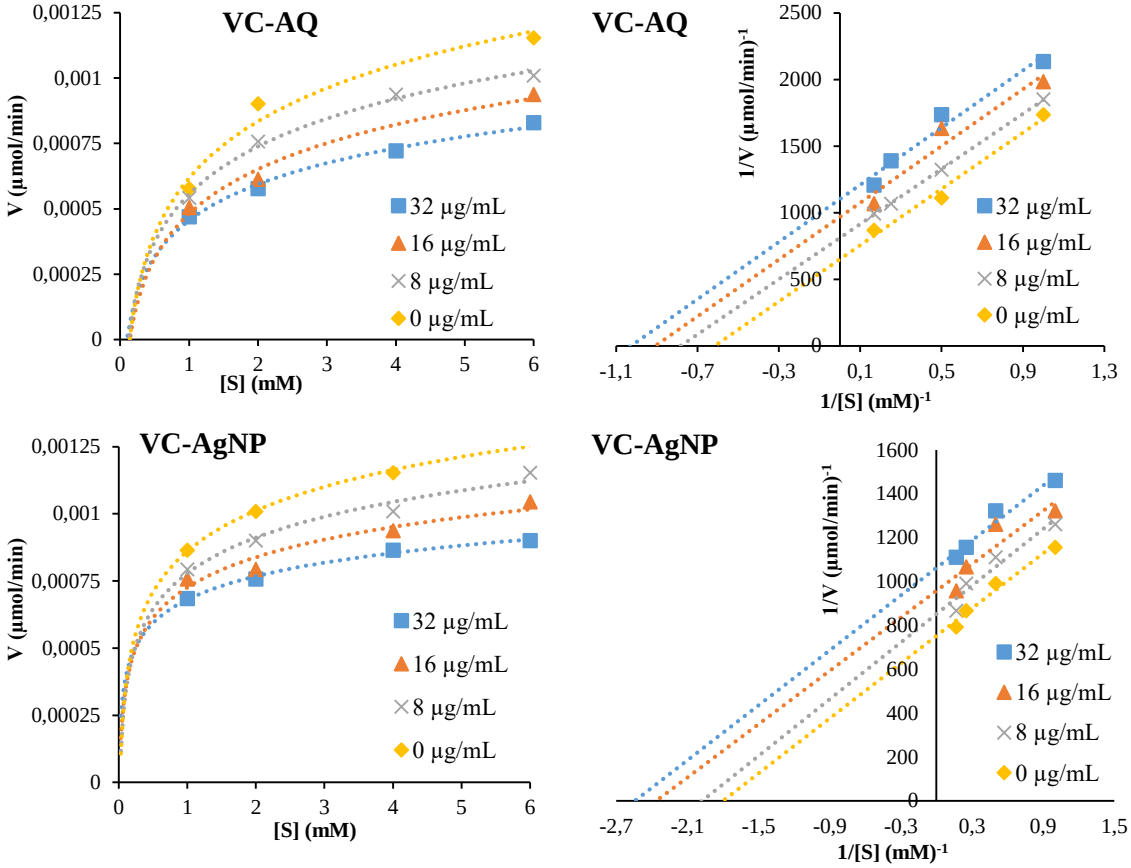


Figure 6. Michaelis-Menten and Lineweaver-Burk graphs of samples

Şekil 6. Örneklerin Michaelis-Menten ve Lineweaver-Burk grafikleri

Antimicrobial Activity

In terms of antibacterial activity, both samples and antibiotics were found to showed no effect against *P. aeruginosa*. While no effect was observed against *K. pneumoniae*, an effect was observed with the antibiotic. Although both samples showed the same effect against *E. coli* and *E. faecalis*, they showed a lower effect than the antibiotic. VC-AgNP has a higher antibacterial effect than VC-AQ on *B. cereus*. On *S. aureus*, the effect is the opposite of that on *B. cereus*. CM is known to have limited activity against *P. aeruginosa*, which explains the lack of an inhibition zone for the antibiotic control in this study. The antibacterial activity of the samples is lower than that of the antibiotic on both bacteria (Table 4).

Table 3. Inhibition types, inhibition constant, V_{max} and K_m values of samples
Çizelge 3. Örneklerin inhibisyon tipleri, inhibisyon sabiti, V_{max} ve K_m değerleri

Samples	Conct. (µg/mL)	V_{max} (µmol/min)	K_m (mM)	K_i (µg/mL)	Inhibition type
VC-AQ	0	0.0015	1.626	36.99	mixed-uncompetitive
	8.00	0.0012	1.276		
	16.00	0.0010	1.101		
	32.00	0.0009	0.975		
VC-AgNP	0	0.0013	0.556	65.49	mixed-uncompetitive
	8.00	0.0012	0.507		
	16.00	0.0010	0.423		
	32.00	0.0009	0.394		

VC-AQ: *Verbascum cymigerum* aqueous extract, **VC-AgNP:** *Verbascum cymigerum* silver nanoparticles

Table 4. Inhibition zone (mm) of antimicrobial activities of the samples
Çizelge 4. Numunelerin antimikrobiyal aktivitelerinin inhibisyon bölgesi (mm)

Sample	*S. aureus*	*B. cereus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*E. faecalis*
VC-AQ	10.00±0.00	8.50±0.71	7.00±0.00	-	-	8.00±0.00
VC-AgNP	9.50±0.71	10.00±1.41	7.00±0.00	-	-	8.00±1.41
CM	14.0±1.41	23.0±0.0	22.5±2.12	9.5±0.71	-	-

VC-AQ: *Verbascum cymigerum* aqueous extract, **VC-AgNP:** *Verbascum cymigerum* silver nanoparticles, **CM:** Chloramphenicol, n: 3

In a previous study, the antibacterial properties of different Verbascum species were investigated. It was observed that these species dexhibitedno antibacterial activity against *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. However, their antibacterial activity against *S. aureus* and *B. cereus* was recorded with inhibition zones ranging from 14 to 19 mm (Dulger et al., 2005). Our current study observed lower efficacy against *S. aureus* and *B. cereus* than in the literature results. At the same time, no efficacy was observed against *K. pneumoniae* and *P. aeruginosa,* as in the literature. Its antibacterial activity against *E. coli* was found to be contrary to what is reported in the literature, although it was low.

Meurer-Grimes et al. (1996) have previously documented the antibacterial activity of nine species of *Verbascum* (Meurer-Grimes et al., 1996). Previous studies demonstrated that extracts derived from the flowers, seeds, leaves, and roots of various *Verbascum* species exerted significant growth inhibition against a broad spectrum of gram-positive and gram-negative bacteria.

Tadeg et al. (2005) found that the *V. sinaiticum* Benth. they studied had lower antibacterial activity than VC against *S. aureus* and *P. aeruginosa*. Dulger and Gonuz (2004) observed lower antibacterial activity of the *V. gypsicola* plant against *S. aureus* and *B. cereus* than VC. Alper and Basaran (2009) reported that extracts of *V. sinuatum* L.exhibited relatively low antibacterial activity against a panel of pathogens, including *E. faecalis, E. coli, K. pneumoniae,* and *P. aeruginosa.*

According to another investigation, VIE had antibacterial effectiveness against isolates of *S. aureus* and *E. coli*. Compared to the antibiotic employed in the trial (penicillin, 5 IU), VIE had a stronger antibacterial impact against *S. aureus* and *E. coli*. Likewise, VIE-AgNPs exhibited more antibacterial activity (Hazman et al., 2024). According to research employing AgNPs and the microorganisms employed in the study, particle size has no bearing on antibacterial effectiveness.

Contrary to some earlier assumptions, it is widely recognized that smaller-sized nanoparticles exhibit significantly higher antibacterial efficiency than their larger counterparts. It is possible that unsaturated structures or thiol groups in the bacterial membrane cause Ag ions to be released from AgNPs into the environment when they are discovered in microorganisms' surroundings. By interacting with membrane-bound thiol groups, these Ag ions discharged into the environment are said to impact membrane permeability. Accordingly, it has been demonstrated that blocking bacterial enzymes and DNA replication can have an antibacterial impact (Ginjupalli et al., 2018).

The *V. sinaiticum* leaf extract showed a 21.2 mm zone of inhibition at the maximum concentration of 2 mg/ml. In contrast, the AgNPs' zone of inhibition against *S. aureus* was greater, measuring 35.1 mm. Additionally, a zone of inhibition of 15.6 mm for the extract and 17.5 mm for the AgNP was seen in *P. aeruginosa* (Geyesa et al., 2024). Contrary to the literature, in our study, AgNP and water extract showed similar effects against *S. aureus*, while both extracts and antibiotics did not show any effect against *P. auregonisa*. Additionally, since the size of the AgNP in our study (12.47 nm) is smaller than the nanoparticle size in the literature (14 nm), the observed effect against bacteria is less.

Molecular Docking

The docking score was found to be -121 for the rutin complex and -132 for the AgNP-rutin complex. *In silico* studies revealed that the AgNP–rutin complex exhibits a higher inhibitory potential than rutin alone. Although pure rutin was not tested *in vitro*, our in silico findings suggest that the AgNP-rutin complex (docking score: -132) has a stronger binding affinity to urease than rutin alone (-121), indicating a synergistic effect of the nanoparticle matrix. This enhanced effect is primarily attributed to the formation of 24 distinct interactions between the AgNP–rutin complex and the urease enzyme. Among these, ten were identified as strong hydrogen bonds, which are believed to contribute to the formation of a more stable complex structure. As a result of this stability, the inhibition of urease was observed to be more effective (Fig 7).

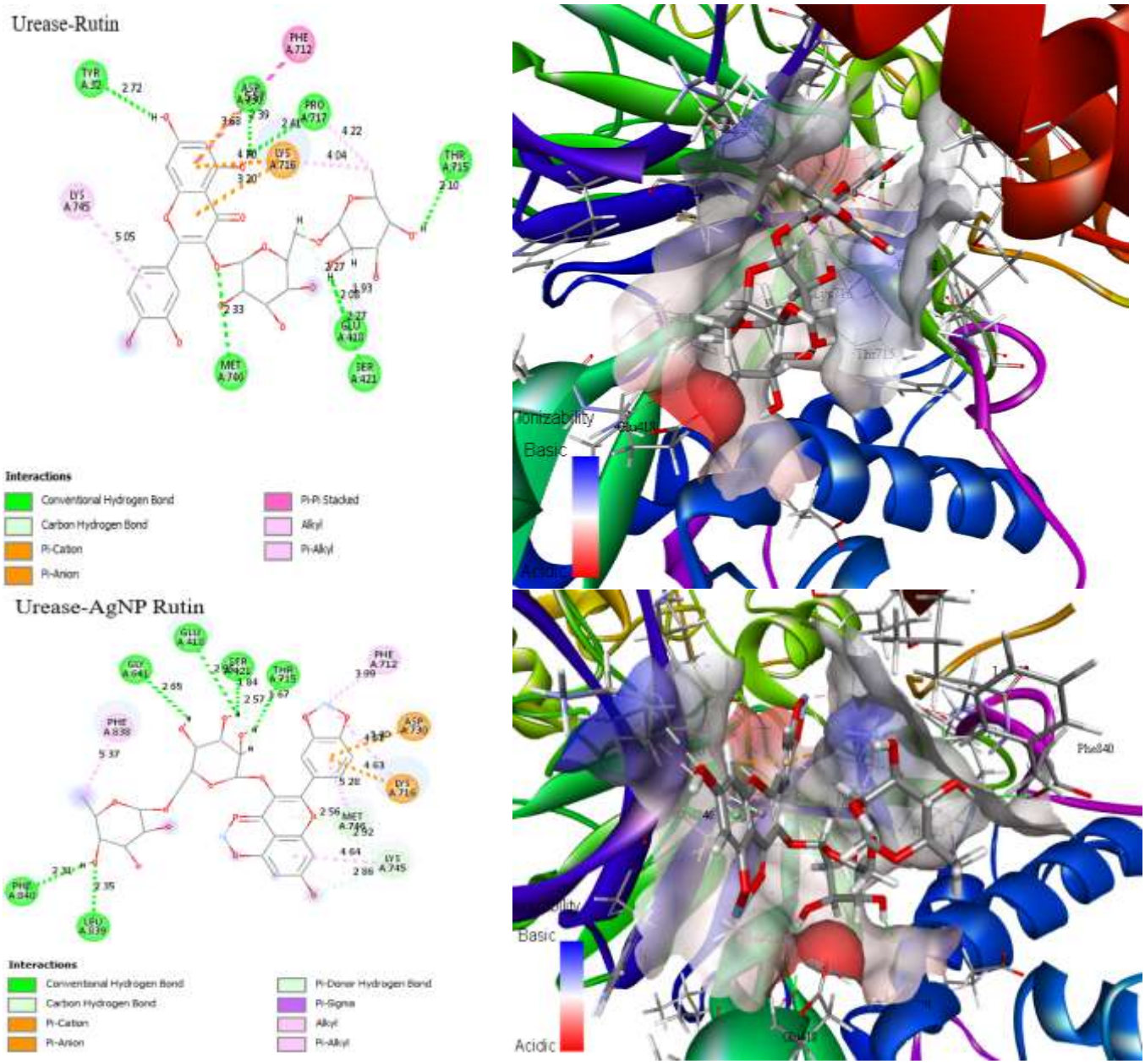


Figure 7. Urease-rutin and urease-AgNP Rutin 2D interaction, 3D ionizability interaction
Şekil 7. Üreaz-rutin ve üreaz-AgNP Rutin 2D etkileşimi, 3D iyonlaşabilirlik etkileşimi

The rutin complex was stabilized through a total of seventeen interactions, including:

- Seven conventional hydrogen bonds (CHB) involving residues MET746, PRO717, ASP730, GLU418, SER421, THR715, and TYR32,
- Two carbon–hydrogen bonds with the GLU418 residue,
- A π–cation electrostatic interaction with LYS716,

- A π–cation/π-donor hydrogen bond with GLU418,
- A π–anion electrostatic interaction with ASP730,
- A π–π stacked hydrophobic interaction (HI) with PHE712,
- An alkyl HI with LYS716,
- And three π–alkyl HI with LYS716.

The AgNP-rutin complex was formed through a total of twenty-four interactions, comprising:

- Six CHB involving LEU839, THR715, GLU418, SER421, GLY641, and PHE840,
- Three carbon–hydrogen bonds with LYS745 and SER421,
- Two π–cation electrostatic interactions with LYS716,
- Three π–anion electrostatic interactions with ASP730 (two interactions) and GLU418,
- A π-donor hydrogen bond with MET746,
- A π–sigma HI with MET746,
- A π–π stacked HI with PHE712,
- An alkyl HI with LYS716,
- And six π–alkyl HI with PHE712, LYS716, PHE838, LYS745, LYS716 (repeated), and MET746.

CONCLUSIONS

Species belonging to the genus *Verbascum* have long been utilized in traditional medicine to treat conditions such as stomach spasms, superficial fungal infections, inflammation, wounds, rheumatic pain, and diarrhea. Furthermore, recent studies have characterized the antibacterial and antioxidant activities, as well as the total phenolic content, of methanolic extracts from several *Verbascum* taxa. This study investigated antioxidant, anti-inflammatory, DNA protection, enzyme inhibition, and enzyme inhibition kinetics of *V. cymigerum* aqueous extracts and AgNPs. According to the test results, VC-AgNP was characterized, and the particle size was calculated as 12.47 nm. LC-ESI-MS/MS analysis of VC-AQ, which was determined to contain high amounts of rutin, vanillic acid, and hesperidin. VC-AgNP exhibited the best activity in $DPPH^{\cdot}$ scavenging and urease inhibition. *In silico* studies demonstrated that the AgNP–rutin complex exhibited stronger urease inhibition than rutin alone. In the phosphomolybdate reducing assay and anti-inflammatory activities, VC-AQ and VC-AgNP showed lower activity than the standards. In the DNA protection activity, it was determined that VC-AgNP could protect DNA, but VC-AQ damaged DNA. VC-AQ was recorded to bind to the enzyme more strongly in the urease enzyme inhibition kinetics. The effect of both the extract and AgNP was observed in antibacterial activity. This study on *V. cymigerum* contributes to the literature by demonstrating that AgNP prepared from the aqueous extract can be used as a DNA protective agent, antibacterial agent, and urease inhibitor.

Furthermore, because the plant is rich in rutin, it is thought that the AgNP proposed with this compound may be an effective inhibitor for use in diseases caused by the urease enzyme. These results may lead to further studies. Future studies can be conducted on this plant, including various anticancer and in vivo studies.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

The authors of the article declare that they have no conflict of interest.

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