

Research Article

Synthesis and Antimicrobial Evaluation of Silver Nanoparticles Mediated by *Alchemilla erzincanensis*

Hüseyin Akşit* and Ömer Gergin

Department of Analytical Chemistry, Faculty of Pharmacy, University of Erzincan Binali Yildirim, 24002 Erzincan, Türkiye

*Corresponding author: huseyin.aksit@erzincan.edu.tr

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ABSTRACT

This study aimed to synthesize and characterize silver nanoparticles using *Alchemilla erzincanensis* extract and to evaluate their antimicrobial potential against selected bacterial strains. Silver nanoparticles (AgNPs) were successfully synthesized using an environmentally friendly green synthesis approach based on the extract of *A. erzincanensis*, an endemic plant species in Türkiye. The synthesized nanoparticles were characterized using Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD) and Energy-Dispersive X-ray Spectroscopy (EDX) techniques. SEM analysis revealed that the AgNPs were spherical in shape with sizes ranging from 34.24 to 48.43 nm. XRD results confirmed the face-centered cubic (FCC) crystalline structure of the particles. EDX spectra indicated a high silver content. Antibacterial activity tests showed that the AgNPs exhibited notable inhibitory effects particularly against Gram-positive bacteria such as *Bacillus cereus* and *Staphylococcus aureus* and also demonstrated activity against resistant Gram-negative bacteria like *Pseudomonas aeruginosa*. This study is the first to report the use of *A. erzincanensis* for AgNP synthesis and highlights its potential contribution to the development of novel antimicrobial agents against antibiotic-resistant pathogens.

Keywords: silver nanoparticles; *Alchemilla erzincanensis*; antimicrobial activity

1. INTRODUCTION

In recent years, rapid advances in science and technology have placed nanotechnology at the forefront of many fields. Defined as a multidisciplinary area focused on the synthesis, characterization, and use of materials at the nanoscale (1-100 nm), nanotechnology shows great potential in sectors such as biomedicine, drug delivery, environmental cleanup, food safety, and agriculture (Iravani et al., 2014). Of the many nanomaterials, metal nanoparticles have gained considerable attention because of their high surface area, chemical reactivity, and special optical features. Specifically, silver nanoparticles (AgNPs) are known for their strong antimicrobial, antifungal, antiviral, and antioxidant effects (Rai et al., 2009). These nanoparticles have potential applications in wound dressings, antibacterial surface coatings, and targeted drug delivery systems, with possible therapeutic use in managing chronic wounds, skin infections, and multidrug-resistant bacterial diseases. The chemical synthesis process using traditional

chemical methods often involves toxic reagents and harsh conditions, raising concerns about environmental safety and sustainability. In contrast, eco-friendly and biocompatible methods have gained increasing attention, with green synthesis techniques, especially those employing plant extracts, emerging as a promising alternative (Ahmed et al., 2016). In such processes, phytochemicals including phenolic compounds, flavonoids, and alkaloids function both as reducing agents and stabilizers, thereby offering ecological and economic advantages over conventional synthesis routes.

Türkiye hosts a remarkable diversity of endemic plant species due to its varied topography and climatic conditions, making it a valuable source for natural product-based research (Savcı et al., 2018). This exceptional biodiversity is largely attributed to the unique position of country at the intersection of three major phytogeographical regions, Euro-Siberian, Iran-Turanian, and Mediterranean, which results in a mosaic of habitats and microclimates. In addition, significant altitudinal gradients (from 0 to 4500 m), geological heterogeneity, and the presence of both continental and Mediterranean climate regimes further contribute to the rich floristic composition (Kandemir et al., 2022). These factors collectively create ideal conditions for the evolution and preservation of endemic plant taxa. One local endemic species is *Alchemilla erzincanensis* (Rosaceae), distributed in the Erzincan region of Eastern Anatolia (Kandemir et al., 2015). The *Alchemilla* genus has been traditionally used for its antiseptic, anti-inflammatory, diuretic, and wound-healing properties (Kanak et al., 2022). Phytochemical investigations have revealed that species within this genus are notably rich in polyphenolic compounds (Kaya et al., 2012). However, to date, limited pharmacological and biotechnological studies have been conducted on *A. erzincanensis*, indicating a significant knowledge gap regarding its potential applications. Unlike many plants used in green synthesis, *A. erzincanensis* is an endemic species with a rich polyphenolic profile, yet remains unexplored for nanoparticle synthesis.

This study aims to develop an eco-friendly synthesis protocol for silver nanoparticles using the extract of *A. erzincanensis*, a rare and local endemic plant species. The synthesized nanoparticles were characterized in terms of their morphological and structural properties, and their antimicrobial activity was thoroughly evaluated to assess their potential biomedical applications.

2. MATERIALS AND METHODS

2.1. Plant Materials

The aerial parts of *A. erzincanensis* were collected in three independent batches from their natural habitat in Ahmediye Village (Erzincan, Türkiye; 39° 52.1815' N, 39° 20.2613' E) in June 2024. The plant material was taxonomically identified by Prof. Dr. Ali Kandemir, and a voucher specimen was deposited in the Herbarium of Erzincan Binali Yıldırım University (EBYU) under the accession number EBYU0000037. After collection, samples were stored in airtight paper bags at room temperature for up to two weeks, dried in a shaded and well-ventilated area, and ground into a fine, homogeneous powder for extraction and nanoparticle synthesis.

2.2. Extraction Procedure

25 g of fine-grounded plant material were mixed with 500 mL of distilled water. The mixture was then boiled on a hot plate for 2 hours to ensure the dissolution of active constituents. After boiling, the solution was allowed to cool to room temperature and

subsequently filtered using Whatman No.1 filter paper. The resulting clear filtrate was collected as the aqueous extract and stored at +4°C for use in the synthesis of silver nanoparticles. The remaining plant residue was discarded after filtration. Although boiling may lead to partial degradation of thermolabile compounds, previous studies have demonstrated that polyphenolic compounds, which play a key role in nanoparticle synthesis, largely retain their reducing capacity after decoction-based extraction (Erenler et al., 2021).

2.3. Preparation of Silver Nanoparticles

To synthesize silver nanoparticles, 300 mL of *A. erzincanensis* aqueous extract was mixed with 300 mL of the AgNO₃ solution (0.024 M) in a round-bottom flask. The mixture was continuously stirred and to promote nanoparticle formation, the reaction mixture was heated and maintained at a boiling temperature for approximately 30 minutes. The progression of silver nanoparticle synthesis was visually monitored through a characteristic colour change of the solution, from yellow to dark brown, eventually approaching black. The gradual darkening of the reaction mixture during the process was considered a preliminary visual indication of successful silver nanoparticle formation. The solution was transferred to 50 mL Falcon tubes and centrifuged at 7000 rpm for 10 minutes. After centrifugation, the supernatant was discarded, and the nanoparticle pellet was washed twice with distilled water to eliminate unbound plant-derived residues. The resulting pellets were collected and stored for further characterization analyses (Genc et al., 2020).

2.4. Characterisation of AgNPs

The biosynthesized AgNPs were characterized using various analytical techniques to determine their structural, morphological, elemental, and functional properties.

X-ray Diffraction (XRD): The crystallographic structure of the synthesized AgNPs was determined using XRD analysis. Measurements were performed with a PANalytical Empyrean diffractometer equipped with a Cu K α radiation source ($\lambda = 1.5406 \text{ \AA}$), operated at 45 kV and 40 mA. The scanning was conducted in continuous mode over a 2θ range of 10° to 90°, with a step size of 0.053° and a scan time of 293.5 seconds per step. The instrument was configured with a fixed divergence slit of 0.4354°, and the goniometer was set to the theta-theta configuration. All measurements were carried out at room temperature (25°C) without sample spinning. The diffraction patterns obtained were used to evaluate the crystalline phase and estimate the average particle size of the synthesized AgNPs (Buniyamin et al., 2024).

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX): The morphological characteristics of the synthesized silver nanoparticles were examined using a field emission scanning electron microscope (FE-SEM) (FEI Quanta FEG 450). Prior to imaging, the dried nanoparticle samples were gently ground in an agate mortar to achieve homogeneity. A thin layer of the powdered sample was mounted onto aluminium stubs using a carbon adhesive tape. To ensure conductivity, the samples were subsequently coated with a thin layer of gold under vacuum conditions using a sputter coater. SEM imaging was conducted in secondary electron (SE) mode under high-vacuum conditions. Accelerating voltage was adjusted as needed to obtain optimal image contrast and particle resolution, with a maximum voltage of 30 kV applied where necessary. Elemental composition and distribution were analysed using an EDAX EDX detector integrated into the Quanta FEG 450 system. Both surface and spot analyses were performed to identify elemental signatures and confirm the presence of silver and associated elements (Genc, 2021).

2.5. Antimicrobial Activity of AgNPs

The antimicrobial activity of the *A. erzincanensis* extract (1 mg/mL in DMSO) and biosynthesized AgNPs (1 mg/mL in DMSO) was evaluated using the standard disk diffusion method. The test was performed against five bacterial strains: *Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus cereus*. The bacterial strains tested include clinically relevant Gram-positive and resistant Gram-negative pathogens that pose major public health challenges due to their growing antibiotic resistance. Each bacterial strain was cultured in nutrient broth and adjusted to a 0.5 McFarland standard turbidity ($\sim 1.5 \times 10^8$ CFU/mL). Mueller-Hinton agar plates were uniformly inoculated with the bacterial suspensions using sterile cotton swabs. Sterile 6 mm paper disks were individually impregnated with 20 μ L of the plant extract and AgNP solutions and placed on the inoculated agar surfaces. Imipenem (10 μ g/disk) was used as a positive control, while disks containing only DMSO served as the negative control. The plates were incubated at 37°C for 24 hours. Following incubation, the zones of inhibition around each disk were measured in millimeters. All tests were performed in triplicate, and the results were expressed as mean \pm standard deviation (Akşit et al., 2024; Latib et al., 2022). All bacterial strains were obtained from the American Type Culture Collection (ATCC). Discs were separately impregnated with AgNPs or plant extract. Imipenem was used as the positive control, and DMSO as the negative control. The bacterial strains were selected due to their clinical relevance, prevalence in nosocomial infections, and increasing resistance to conventional antibiotics.

3. RESULTS AND DISCUSSION

The surface morphology of the biosynthesized AgNPs was examined using SEM. Morphological analysis was performed using images obtained at 120,000 \times magnification, and size estimations were made with reference to the 500 nm scale bar. As shown in Figure 1, the nanoparticles predominantly exhibited spherical to semi-spherical morphology with relatively smooth surfaces. The particles were generally well-dispersed, with diameters ranging from 34.24 to 48.43 nm.

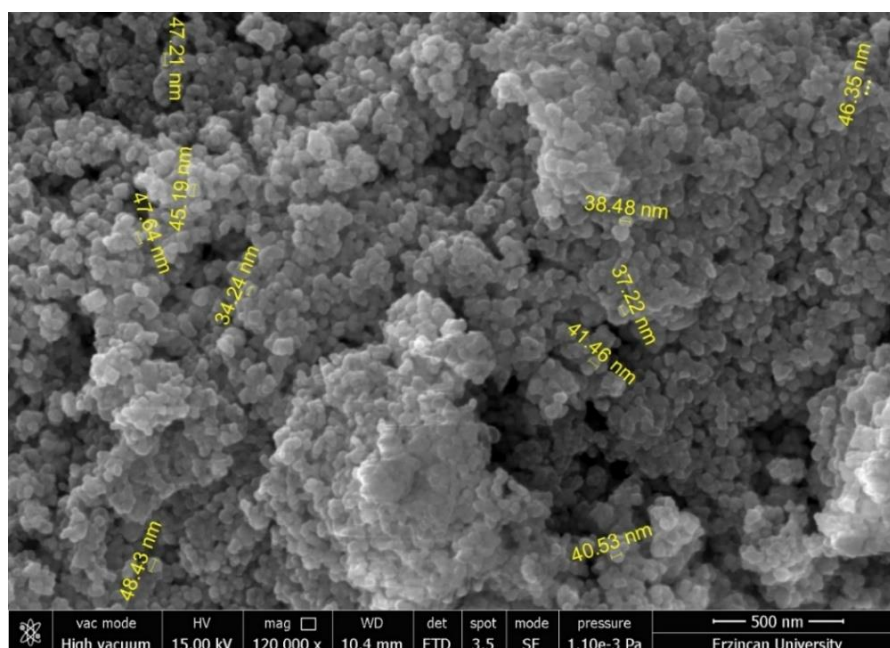


Figure 1. SEM image of biosynthesized AgNPs synthesized using *A. erzincanensis* extract

These morphological characteristics suggest that phytochemicals present in the *A. erzincanensis* extract, particularly phenolic compounds and flavonoids, acted effectively as both reducing and stabilizing agents during the nanoparticle synthesis process. Their capping function likely contributed to the formation of uniformly shaped nanoparticles with smooth surfaces and minimized agglomeration. Although a limited degree of aggregation was observed in some areas of the SEM images, this is commonly encountered in biologically synthesized nanoparticles and is generally influenced by the concentration and distribution of stabilizing agents. Overall, the SEM findings confirm the successful biosynthesis of AgNPs with controlled size and morphology, supporting the efficiency of the green synthesis approach employed in this study. These well-defined nanostructures, characterized by their high surface area, bioactive surfaces, and nanometric dimensions, are considered suitable candidates for various applications, including antimicrobial, anticancer, and catalytic technologies. Hence, the synthesized AgNPs show promising potential for use in advanced biomedical and environmental systems.

The EDX spectrum of the synthesized AgNPs is presented in Figure 2. The analysis revealed that silver (Ag) was the predominant element in the sample, with the highest signal intensity observed at characteristic peaks such as Ag L α , Ag L β , and Ag M α . The calculated silver content was approximately 78.54% by weight, strongly indicating that the nanoparticles primarily consist of metallic silver. This finding is consistent with the XRD results, which confirmed a high degree of crystallinity for the synthesized AgNPs. In addition to silver, the spectrum also showed signals for carbon (C), oxygen (O), and nitrogen (N) at lower intensities. These elements are likely associated with bioorganic residues such as phenolic compounds, flavonoids, and proteins originating from the *Alchemilla erzincanensis* plant extract used during synthesis. These biomolecules may have been adsorbed onto the nanoparticle surfaces, acting as natural capping and stabilizing agents. This supports previous reports suggesting the dual role of plant metabolites as both reducing and stabilizing agents in green nanoparticle synthesis (Iravani, 2011; Ahmed et al., 2016).

Moreover, minor chlorine (Cl) peaks were detected, which may originate from residual AgNO₃ precursor or the formation of AgCl during the reaction. The presence of gold (Au) signals in the spectrum is attributed to the gold sputter coating applied to the sample surface to enhance conductivity during SEM-EDX measurements. The EDX analysis confirms the successful biosynthesis of silver-rich nanoparticles, while also highlighting the presence of organic and elemental residues from the plant extract and sample preparation. These results emphasize the role of *A. erzincanensis* extract not only in reducing silver ions but also in providing structural stabilization through surface-bound biomolecules.

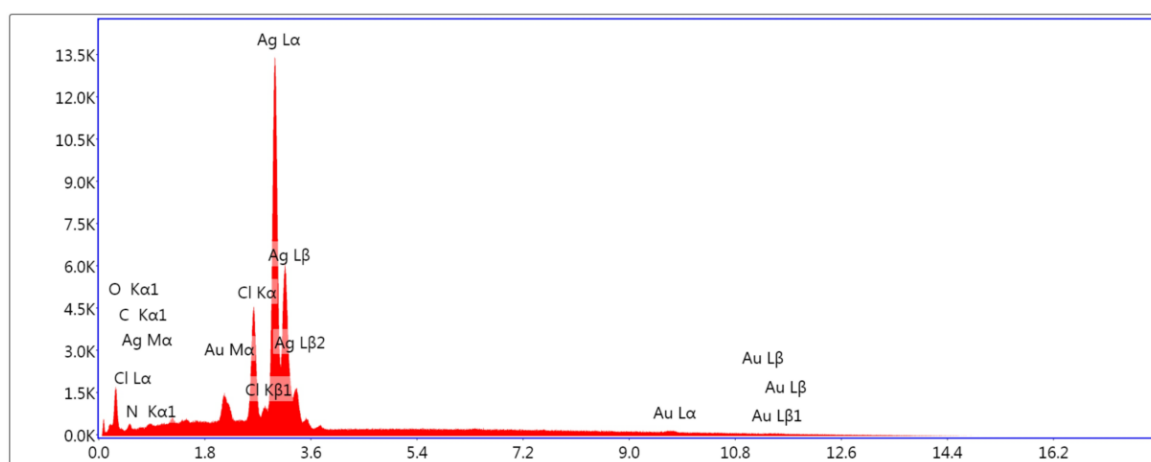


Figure 2. EDX spectrum of AgNPs biosynthesized using *A. erzincanensis* extract

The crystalline structure of the AgNPs synthesized using *A. erzincanensis* extract was analysed by XRD. The XRD pattern of the biosynthesized AgNPs (Figure 3) displayed distinct diffraction peaks at 2θ values of approximately 38.3° , 44.5° , 64.6° , and 77.5° , corresponding to the (111), (200), (220), and (311) lattice planes of face-centered cubic (fcc) silver, respectively. The most intense peak observed at 38.3° indicates that the (111) plane is the dominant orientation, a common feature among biologically synthesized AgNPs due to its thermodynamic stability (Shankar et al., 2004). The sharp and narrow nature of the diffraction peaks confirms the crystalline nature of the synthesized nanoparticles and suggests a relatively small crystallite size in the nm range. These findings are consistent with the SEM analysis, which revealed spherical particles predominantly ranging from 34.24 to 48.43 nm in diameter. The average crystallite size of the synthesized silver nanoparticles was estimated using the Scherrer equation, based on the full width at half maximum (FWHM) of the most intense diffraction peak at $2\theta = 38.3^\circ$, corresponding to the (111) plane. The calculated crystallite size was approximately 30.07 nm, which is in good agreement with the particle sizes observed in SEM analysis.

In addition to the characteristic silver peaks, a few minor and broad signals were also detected in the diffraction pattern. These are likely attributable to amorphous organic residues from the plant extract, such as phenolic compounds, which may have contributed structurally to the nanoparticle matrix. However, their low intensity suggests that the synthesized product consists primarily of highly crystalline and pure AgNPs. The XRD results confirm the successful biosynthesis of FCC-phase AgNPs with high structural regularity and purity. These attributes highlight the potential applicability of the AgNPs in biological, environmental, and technological fields where crystalline integrity plays a crucial role. While the synthesized AgNPs were predominantly spherical, some degree of aggregation was observed in SEM images, which may affect dispersion stability. EDX spectra also indicated minor elemental signals (e.g., Cl, N), potentially from residual plant metabolites or precursors, which could influence surface chemistry. These observations, although not uncommon in green synthesis, should be considered in future optimization studies.

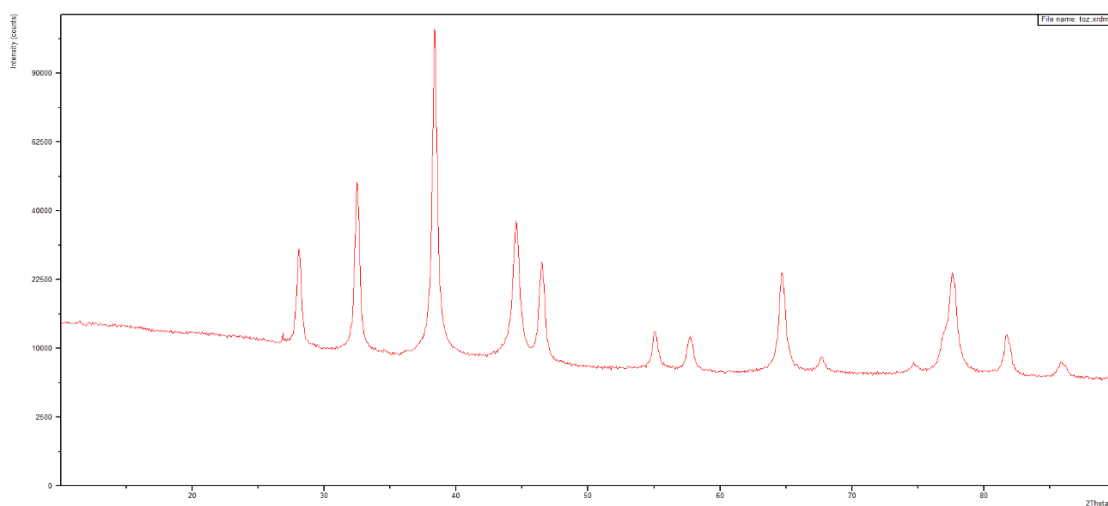


Figure 3. XRD pattern of synthesized AgNPs using *A. erzincanensis* extract

According to the Table 1, *A. erzincanensis* crude extract exhibited modest antibacterial activity with inhibition zones ranging from 11.2 ± 0.1 to 13.4 ± 0.3 mm, indicating the presence of bioactive compounds with limited antimicrobial potential. In contrast, the synthesized AgNPs demonstrated enhanced antibacterial activity against all tested strains. Notably, inhibition zones of 18.5 ± 0.5 mm for *B. cereus* and 15.3 ± 0.4 mm for *S. aureus* were recorded,

suggesting that synthesized AgNPs possess higher efficacy particularly against Gram-positive bacteria. Similar findings have been reported in earlier studies, indicating greater susceptibility of Gram-positive bacteria to AgNPs (Lara et al., 2010; Sondi and Salopek-Sondi, 2004). Compared to previous reports on AgNPs synthesized using *Handelia trichophylla* (Yazdi et al., 2019) and *Lycium shawii* (Mohammed et al., 2022) extracts which exhibited strong antimicrobial activity, our green-synthesized AgNPs displayed comparable or slightly superior activity, particularly against *B. cereus*.

Table 1. Antimicrobial activities of crude extract and synthesized silver nanoparticles*

Microorganism	Extract	Ag-NP	Imipenem
<i>Escherichia coli</i>	13.0±0.3	15.3±0.4	40.3±1.0
<i>Salmonella enterica</i>	11.9±0.5	12.1±0.2	32.0±0.9
<i>Pseudomonas aeruginosa</i>	-	14.0±0.1	25.2±0.4
<i>Staphylococcus aureus</i>	11.2±0.1	15.3±0.4	44.9±1.2
<i>Bacillus cereus</i>	13.4±0.4	18.5±0.5	23.1±0.8

*inhibition zones in mm (values are presented as mean ±SD of three independent experiments); - : no inhibition

A remarkable finding was observed for *P. aeruginosa*, a strain generally resistant to the crude plant extract. While the extract has no inhibition, AgNPs produced a clear inhibition zone measuring 14 mm, highlighting their potential broad-spectrum antimicrobial capability. This result is particularly important given the inherent resistance of *P. aeruginosa* to many conventional antimicrobials. These results support earlier findings that AgNPs can disrupt bacterial membranes, generate reactive oxygen species (ROS), and interfere with DNA replication, thus being effective even against resistant strains (Franci et al., 2015; Marambio-Jones and Hoek, 2010). Imipenem, as expected, showed the most potent antibacterial activity, with inhibition zones ranging between 23.1±0.8 and 44.9±1.2 mm against all tested strains. However, in some cases, the inhibition zones formed by AgNPs approached those of the reference antibiotic. This finding underscores the potential of biosynthesized AgNPs as alternative or complementary antimicrobial agents, particularly in the context of rising antibiotic resistance (Durán et al., 2015).

4. CONCLUSION

The present study demonstrates the potential of *A. erzincanensis* extract as a sustainable and environmentally friendly source for the green synthesis of biologically active silver nanoparticles. It also reinforces that plant-based nanotechnology represents a promising alternative to conventional chemical synthesis methods. In particular, the enhanced antimicrobial activity of phytochemical-mediated nanoparticles supports existing findings in the literature. Furthermore, the antimicrobial efficacy of the synthesized AgNPs highlights their potential in addressing the growing challenge of antibiotic resistance. This approach opens new avenues for the development of plant-mediated nanomaterials with potential applications in wound dressings, surgical coatings, disinfectant formulations, and broader uses in medicine, environmental protection, and biotechnological innovation. Future research will focus on cytotoxicity assays, stability studies, comparative evaluations with chemically synthesized AgNPs, and *in vivo* assessments to further determine their biomedical potential.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contribution Statement

Hüseyin Akşit: Data curation, writing original draft, reviewing and editing, and supervision. Ömer Gergin: Visualization and investigation.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

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