

Phytochemical analysis of *Vicia sativa* L. leaves: Green synthesis of silver nanoparticles, quantitative analysis of phenolic compounds and antiproliferative activity

Vicia sativa L. yapraklarının fitokimyasal analizi: Gümüş nanopartiküllerinin yeşil sentezi, fenolik bileşiklerin kantitatif analizi ve antiproliferatif aktivite

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ABSTRACT

Medicinal plants are used as folk medicine by most of the world's population. The use of plants to produce commercially important natural or recombinant compounds has gained increasing interest in recent decades. Bioactive compounds derived from plants are effectively used in drug discovery and development. Plant-based nanoparticle synthesis is considered a green, reliable technique due to its environmentally friendly approach. In this study, a simple and eco-friendly approach for the synthesis of silver nanoparticles using *Vicia sativa* leaves as a reducing agent is reported. The change in colour of the leaves exposed to the AgNO₃ solution from yellow to brown confirmed the formation of silver nanoparticles (vs-AgNPs). The maximum absorption at 427 nm proved the desired product. X-ray diffraction (XRD) patterns confirming characteristic peaks indicate the crystal planes of the face-centered cubic structure. Transmission Electron Microscopy (TEM) analysis confirmed the presence of spherical nanoparticles with an average size of 18.4 nm. Quantitative analysis revealed that the *V. sativa* leaves include chlorogenic acid and rutin as major compounds. Antiproliferative activity of the extract and nanoparticles was evaluated using Caco-2 (Human colon adenocarcinoma) cell lines and MCF-7 (Breast cancer) cell lines. The viability of Caco-2 cells was reported to be 36.4 ± 0.7% and 48.1 ± 0.3%, after interaction with vs-AgNPs and the extract, respectively, at 0.5 µg/mL. Moreover, the viability of MCF-7 cells was determined to be 36.2 ± 1.1% and 43.0 ± 1.4%, respectively, upon treatment with vs-AgNPs and the extract at 0.5 µg/mL. Consequently, vs-AgNPs may be a promising agent for pharmaceutical applications.

Keywords: *Vicia sativa* L. leaves, quantitative analysis, silver nanoparticles, anticancer activity

ÖZET

Tıbbi bitkiler, dünya nüfusunun büyük bir kısmı tarafından halk hekimliğinde kullanılmaktadır. Ticari açıdan önemli doğal veya rekombinant bileşiklerin üretimi için bitkilerin kullanımı, son yıllarda giderek artan bir ilgi görmektedir. Bitkilerden elde edilen biyoaktif bileşikler, ilaç keşfi ve geliştirme süreçlerinde etkin bir şekilde kullanılmaktadır. Bitki bazlı nanopartikül sentezi, çevre dostu yaklaşımı nedeniyle yeşil ve güvenilir bir teknik olarak kabul edilmektedir. Bu çalışmada, indirgeyici ajan olarak *Vicia sativa* yaprakları kullanılarak gümüş nanopartiküllerinin sentezi için etkin ve çevre dostu bir yaklaşım sunulmuştur. AgNO₃ çözeltilisine maruz kalan yaprakların renginin sarıdan kahverengiye değişmesi, gümüş nanopartiküllerinin (vs-AgNPs) oluşumunu doğrulamıştır. 427 nm'de maksimum absorpsiyon, istenen ürünün elde edildiğini kanıtlamıştır. Karakteristik pikleri doğrulayan X-ışını difraksiyon (XRD) pikleri, yüzey merkezli kübik yapının kristal düzlemlerini göstermektedir. Geçirgenli Elektron Mikroskobu (TEM) analizi, ortalama boyutu 18,4 nm olan küresel nanopartiküllerin varlığını doğrulamıştır. Kantitatif analiz, *V. sativa* yapraklarının başlıca bileşenleri arasında klorojenik asit ve rutin bulunduğunu ortaya koydu. Ekstraktın ve nanopartiküllerin antiproliferatif aktivitesi, Caco-2 (İnsan kolon adenokarsinomu) hücre hatları ve MCF-7 (Meme kanseri) hücre hatları kullanılarak değerlendirilmiştir. Caco-2 hücrelerinin canlılığının, 0,5 µg/mL konsantrasyonda sırasıyla vs-AgNP'ler ve ekstrakt ile etkileşimden sonra %36,4 ± 0,7 ve %48,1 ± 0,3 olduğu bildirilmiştir. Ayrıca, 0,5 µg/mL konsantrasyonda vs-AgNPs ve ekstrakt ile etkileşen MCF-7 hücrelerinin canlılığı, sırasıyla %36,2 ± 1,1 ve %43,0 ± 1,4 olarak belirlenmiştir. Sonuç olarak, vs-AgNPs farmasötik uygulamalar için umut vaat eden bir ajan olabilmeye potansiyeline sahiptir.

Anahtar kelimeler: *Vicia sativa* L. yaprakları, kantitatif analiz, gümüş nanopartiküller, antikanser aktivite

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Plants have been consumed as folk medicine and food since ancient times (Topçu et al., 1999). The fantastic compounds found in plants exhibit significant biological activity, and intensive scientific research is underway to isolate them and use them in the pharmaceutical industry (Aksit et al., 2014; Demirtas et al., 2013; Elmastas et al., 2004; Sahin Yaglioglu et al., 2013). Natural compounds offer researchers a new perspective as templates for the synthetic modification of new drugs. Biological and chemical diversity of natural compounds has served as a source of inspiration for the development of therapeutic agents (Cakmak et al., 2006; Lu et al., 2014). Many bioactive compounds have been developed inspired by natural products. Natural compounds are sometimes synthesized, and in some cases, their effectiveness is enhanced by functionalization (Erenler & Biellmann, 2005; Erenler & Cakmak, 2004; Ökten et al., 2013). Quantitative analysis of phenolic compounds in plants is extremely important for drug development (Erenler, Demirtas, et al., 2018; Erenler et al., 2015). These analyses reveal the mechanism underlying the relationship between activity and the compound (Erenler, Telci, et al., 2018; Genç et al., 2020). This analysis also identifies the target molecule to be isolated. Thus, the selection of target compounds in drug development studies is based on scientific principles (Erenler, Geçer, et al., 2022; Yaman et al., 2022). Quantitative analyses are an effective method for standardizing plant extracts. Standardization is crucial for the safe, effective, and reproducible use of natural products. At the same time, quantitative analyses help to evaluate the effects of different extraction methods or growing conditions on compound amounts. This information contributes to the development of extracts that provide the highest biological activity (Atalar et al., 2023; Erenler, Atalar, et al., 2023).

Nanotechnology is a branch of science concerned with materials at the nanoscale (Erenler, Geçer, et al., 2021; Geetha et al., 2013). This field is rapidly developing and impacting every aspect of life. Nanotechnology involves the creation, characterization, and manipulation of materials with sizes ranging from 1 to 100 nm (Erenler, Temiz, et al., 2021; Geçer, 2021). Nanomaterials represent the main tools of nanotechnology (Genc et al., 2021). Nanomaterials possess properties such as biolabeling and biosensing (Dag, 2022). They are also widely used in catalysis, antibacterial and antiviral activity, drug delivery, antioxidant applications, DNA sequencing, and gene therapy (Erenler & Dag, 2022; Erenler & Geçer, 2022a).

Physical and chemical methods are commonly used to synthesize nanoparticles (Erenler & Geçer, 2022b). However, biological synthesis remains an important alternative (Erenler, Geçer, et al., 2022). The biological method in nanomaterial synthesis has led to the emergence of the green nanotechnology sub-discipline (Geçer & Erenler, 2022a). Green nanotechnology is the application of green chemistry principles to eliminate the production and use of hazardous materials (Geçer & Erenler, 2022b). The biological production of biocompatible, non-toxic materials has enabled their use in biomedicine (Geçer et al., 2022; Karan et al., 2022). Green-synthesized silver nanoparticles have been reported to exhibit efficient biological activities (Yaglioglu et al., 2025). Silver nanoparticles synthesized from *Dittrichia graveolens* revealed the antibiofilm activity (Tunç & Erenler, 2025). *Astragalus pennatulus*-mediated silver nanoparticle synthesis was achieved that revealed the high antioxidant effect (Geçer & Erenler, 2025).

Cancer is a deadly disease. It arises from the uncontrolled growth or division of cells in the body (Zerrouki et al., 2022). Cancer is the second most terrifying and serious disease in the world (Li et al., 2023). The limited success of currently used clinical methods in cancer treatment, such as radiation, chemotherapy, immunomodulation, and surgery, indicates an urgent need for a new cancer treatment approach (Erenler, Carlik, et al., 2023). Due to the cost of chemotherapeutic agents and the side effects of cancer drugs, the search for effective natural anticancer agents that will prevent and slow cancer development continues (Erenler, Yildiz, et al., 2022). Medicinal plants have a special place in cancer management. Estimates suggest that plant-derived compounds account for more than 50% of cancer-preventing drugs (Erenler et al., 2017; Yildiz et al., 2017).

In this study, phenolic compounds were determined by LC-MS/MS from *Vicia sativa* L. leaves, and silver nanoparticles were synthesized from the leaves of this plant. Antiproliferative activity of nanoparticles and extract was evaluated using the Caco-2 and MCF-7 cell lines.

Materials and methods

Quantitative analysis of phenolic compounds

Phenolic compounds in *Vicia sativa* L. leaves were determined by an LC-MS/MS instrument. Thermo Scientific Dionex Ultimate 3000 - TSQ Quantum with Thermo ODS Hypersil 250 × 4.6 mm, 5 μm column were used for quantitative analysis (Başar et al., 2024).

Synthesis of silver nanoparticles

Silver nanoparticles were synthesized using the *Vicia sativa* L. leaves. The leaves (2.0 g) were mixed with distilled water (80 mL), and the mixture was heated at 40 °C for 2 hours. After filtration, the mixture solution was reacted with silver nitrate (1.0 mM, 80 mL) at 55 °C for 1 hour. The reaction progress was monitored by UV-Vis analysis. Once the reaction was complete, the nanoparticles were centrifuged at 5000 rpm for 20 min, then dried by lyophilization (Gecer et al., 2022).

Characterization of silver nanoparticles

The green-synthesized silver nanoparticles were characterized by spectroscopic analyses and microscopy. The maximum absorption of vs-AgNPs was determined by UV-Vis. The FTIR spectrum presented the functional groups of the leaf extract responsible for reducing silver ions. XRD analysis presented the particle size and crystal structure of vs-AgNPs. The TEM image showed the morphology of nanoparticles (Karan et al., 2022).

Antiproliferative activity

Antiproliferative activity of the extract and nanoparticles was evaluated using Caco-2 (Human colon adenocarcinoma) and MCF-7 (Breast cancer) cell lines. The cells (5.0 μL) were mixed with trypan blue and transferred to a Thoma lam. The cytotoxic effects of the extract and nanoparticles were determined using a 96-well plate. Cells (100 μL, 10 × 10³ per well) were placed into a culture medium and incubated for 24 hours. Samples were added to the wells at various concentrations (1.0–0.125 μg/mL) and then incubated for a further 24 hours. After removing the culture medium, MTT solution (1.0 μg/mL, 50 μL) was added, and the solution was incubated at 37 °C for 3 hours. Cell viability was measured by ELISA at 570 nm (Sahin Yaglioglu et al., 2013).

Results and discussion

UV-Vis analysis is an important method for determining silver nanoparticles. Mostly, maximum absorption of silver nanoparticles was observed at 400-550 nm. In this study, the maximum absorption was observed at 427 nm, indicating the successful formation of silver nanoparticles (Figure 1).

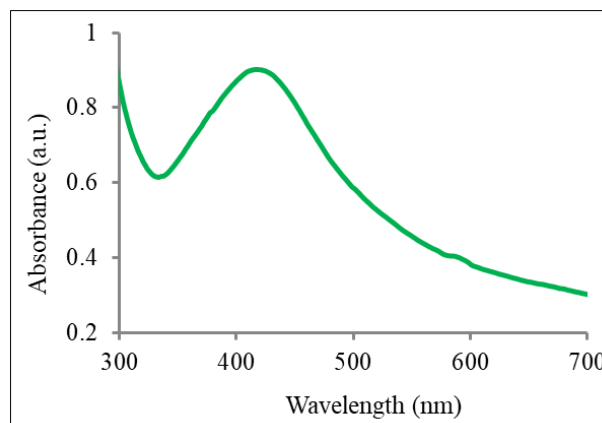


Figure 1. UV-Vis spectrum of vs-AgNPs

FTIR analysis determined the compound moiety acting as reducing agent. The signal observed at 3372 cm⁻¹ belonged to the hydroxyl group. The peaks at 2923 cm⁻¹ and 2847 cm⁻¹ may be attributed to the C-H stretching of an alkane and an aldehyde, respectively. The carbonyl signal of the compound responsible for reducing and capping agent appeared at 1681 cm⁻¹. Moreover, the peaks at 1250 cm⁻¹ and 1036 cm⁻¹ may be due to the stretching of C-O (Figure 2).

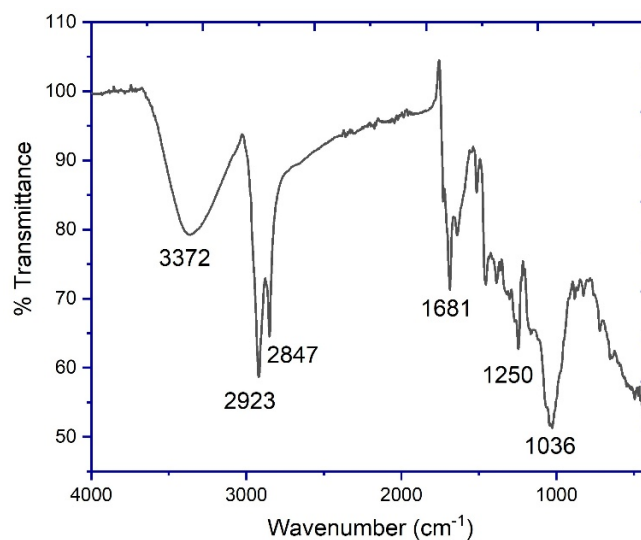


Figure 2. FTIR spectrum of vs-AgNPs

The TEM image provides the size, shape, and dispersion of AgNPs. The TEM image of AgNPs showed that the particles are spherical, with a size of 18.4 nm. It also showed the minimal aggregation (Figure 3).

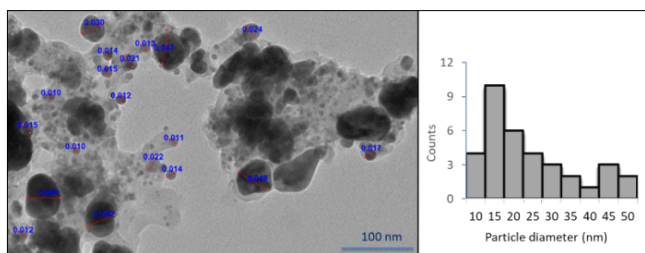


Figure 3. TEM image and particle size distribution of vs-AgNPs

XRD (X-ray diffraction) analysis is one of the most fundamental characterisation methods for silver nanoparticles. It provides information on the crystalline structure and size of the nanoparticles. The diffraction pattern exhibited signals (2θ) at 38.24° , 44.46° , 64.62° , and 77.57° , which can be indexed to the (111), (200), (220), and (311) lattice planes of the face-centred cubic (fcc) structure. The impurity peaks indicated the presence of residual organic compounds from the plant extract (Figure 4).

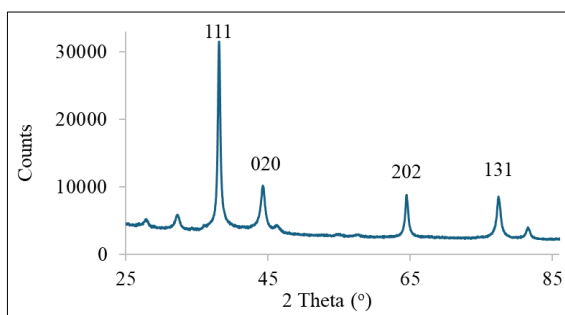


Figure 4. XRD spectrum of vs-AgNPs

Quantitative analysis showed that *Vicia sativa* leaves contain chlorogenic acid ($209.38 \mu\text{g/g}$ extract) and rutin ($1604.87 \mu\text{g/g}$ extract) as the major compounds. (Table 1).

The cytotoxic effects of the extract and nanoparticles were evaluated using MCF-7 and Caco-2 cells. The viability of Caco-2 cells was determined to be $32.9 \pm 0.6\%$ and $44.3 \pm 1.2\%$ after treatment with nanoparticles and the extract, respectively. In addition, the viability of Caco-2 was detected as $36.4 \pm 0.7\%$ and $48.1 \pm 0.3\%$ at $0.5 \mu\text{g/mL}$, respectively. At $0.25 \mu\text{g/mL}$, the viability of Caco-2 was detected to be $43.4 \pm 1.2\%$ and $56.3 \pm 1.0\%$ after the

treatment of nanoparticles and extract, respectively. The same trend was observed for the MCF-7 cell lines. At $1.0 \mu\text{g/mL}$ concentration, the viability of MCF-7 cells was determined to be $23.6 \pm 0.8\%$ and $34.4 \pm 1.1\%$, respectively. Viability increased with decreasing concentration (Figure 5).

Table 1. Quantitative analysis of phenolic compounds of *Vicia sativa* ($\mu\text{g/g}$ extract)

Compound	RT	Yield
Shikimic acid	1.41	
Gallic acid	3.23	3.25
Chlorogenic acid	7.11	209.38
Hydroxybenzaldehyde	7.60	1.66
Caffeic Acid	7.77	1.62
Syringic acid	8.41	26.87
Vanillin	8.66	2.21
o-coumaric acid	9.39	2.51
Salicylic Acid	9.54	16.12
Trans-ferulic acid	10.12	4.04
Sinapic acid	10.77	3.21
p-coumaric acid	11.54	1.23
Coumarin	11.57	29.14
Isoquercitrin	11.81	41.91
Rutin	12.39	1604.87
Kaempferol-3-glucoside	13.29	25.89
Fisetin	13.44	3.43
Naringenin	15.07	3.46
Hesperetin	15.87	3.039
Kaempferol	16.12	10.71

RT: Retention Time

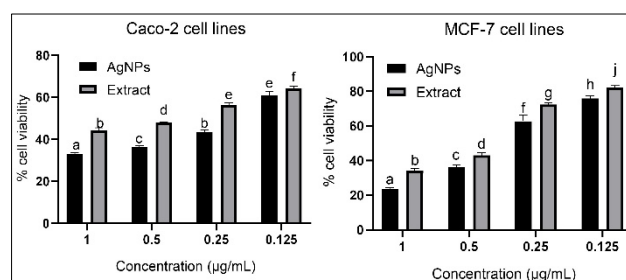


Figure 5. Antiproliferative activity of nanoparticles and extract

Conclusion

The phytochemistry of *Vicia sativa* L. leaves was determined. Quantitative analysis provided the major compounds responsible for activity. Silver nanoparticles synthesized from *V. sativa* leaves exhibited high

antiproliferative activity against Caco-2 and MCF-7 cell lines. The extract and nanoparticles could be promising agents for the development of anticancer drugs.

Author contributions

ENG: Supervision, conceptualization, methodology, validation;
İY: Data curation, validation, formal analysis, methodology; RE:
Writing-review, editing, validation.

Declaration of interests

The authors declare no conflict of interest.

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