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ORIGINAL ARTICLE / ÖZGÜN MAKALE

GREEN SYNTHESIS OF GOLD NANOPARTICLES USING *PRUNUS CERASUS L.* LEAVES EXTRACT AND STUDY OF THEIR CYTOTOXIC EFFECT ON LIVER CELL LINE

PRUNUS CERASUS L. YAPRAK EKSTRAKTINI KULLANARAK ALTIN NANOPARTİKÜLLERİNİN YEŞİL SENTEZİ VE KARACİĞER KANSERİ HÜCRE HATTI ÜZERİNDE ANTİPROLİFERATİF ETKİLERİNİN ARAŞTIRILMASI

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ABSTRACT

Objective: *Gold nanoparticles (AuNPs) possess unique chemical, physical and biological properties that make them valuable in nanotechnology applications such as drug delivery, biosensor and photothermal therapy. This study aims to synthesize AuNPs using Prunus cerasus (P. cerasus) leaves extract as a green reducing and stabilizing agent and to evaluate their structural and biological properties.*
Material and Method: *Green synthesis of AuNPs using P. cerasus leaves extract. The obtained AuNPs were characterized using UV-Vis spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM) and zeta potential analysis to evaluate their structural, morphological and stability properties.*
Result and Discussion: *TEM analysis confirmed that the synthesized AuNPs were predominantly spherical and had an average size of 33±13 nm. Furthermore, the synthesized AuNPs were determined to have high stability by zeta potential analysis. Biological evaluations showed that AuNPs exhibited selective cytotoxic effects, approximately three times higher antiproliferative activity against liver cancer (HepG2) cells compared to P. cerasus leaves extract. These findings emphasize the potential of P. cerasus extract-mediated AuNPs for cancer treatment, supporting their applications in biomedical nanotechnology.*
Keywords: *Antiproliferative activity, AuNPs, biosynthesis, Prunus cerasus L.*

ÖZ

Amaç: *Altın nanopartiküller (AuNP'ler), ilaç dağıtımı, biyosensör ve fototermal terapi gibi nanoteknoloji uygulamalarında değerli olmalarını sağlayan benzersiz kimyasal, fiziksel ve biyolojik özelliklere sahiptir. Bu çalışma, yeşil indirgeyici ve stabilize edici ajan olarak Prunus cerasus (P. cerasus) yaprak özütü kullanılarak AuNP'leri sentezlemeyi ve yapısal ve biyolojik özelliklerini değerlendirmeyi amaçlamaktadır.*
Gereç ve Yöntem: *AuNP'ler, çevre dostu koşullar altında P cerasus yaprak özütü kullanılarak sentezlendi. Elde edilen nanopartiküller, yapısal, morfolojik ve kararlılık özelliklerini değerlendirmek için X-ışını kırınımı (XRD), Fourier dönüşümlü kızılötesi spektroskopisi (FTIR),*

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transmisyon elektron mikroskobu (TEM), UV-Vis spektroskopisi ve zeta potansiyel analizi kullanılarak karakterize edildi.
Sonuç ve Tartışma: *TEM analizi, sentezlenen AuNP'lerin ağırlıklı olarak küresel olduğunu ve ortalama boyutunun 33±13 nm olduğunu doğruladı. XRD sonuçları yüz merkezli kübik kafes yapıda olduğunu kanıtladı. Ayrıca sentezlenen AuNP'lerin yüksek stabiliteye sahip olduğu zeta potansiyel analizi ile belirlenmiştir. Biyolojik değerlendirmeler AuNP'lerin seçici sitotoksik etkiler sergilediğini, P. cerasus yaprak özütüne kıyasla karaciğer kanseri (HepG2) hücrelerine karşı yaklaşık üç kat daha yüksek antiproliferatif aktivite gösterdiğini gösterdi. Bu bulgular P. cerasus özütü aracılı AuNP'lerin kanser tedavisi için potansiyelini vurgulayarak biyomedikal nanoteknolojideki uygulamalarını desteklemektedir.*
Anahtar Kelimeler: *Antiproliferatif aktivite, AuNP'ler, biyosentez, Prunus cerasus L.*

INTRODUCTION

Nanotechnology has led to groundbreaking advancements in cancer treatment by introducing innovative approaches within the field of biomedicine. Despite progress in conventional treatments such as chemotherapy and radiotherapy, challenges like low specificity, systemic toxicity, and drug resistance continue to limit their effectiveness [1]. The ability of nanoscale materials to interact precisely with biological systems enables more targeted diagnostics and therapies. In this context, gold nanoparticles (AuNPs) have emerged as highly promising nanomaterials due to their chemical stability, surface functionalization potential, and distinctive optical properties. AuNPs are widely applied in targeted drug delivery systems, photothermal and photodynamic therapies, and advanced imaging techniques [2-6]. These features help overcome the limitations of traditional approaches, offering a more efficient and safer strategy for cancer diagnosis and treatment.

Traditional methods of synthesizing AuNPs, such as chemical reduction and physical deposition, often rely on hazardous reagents, require high energy inputs, and produce toxic by-products [7]. These drawbacks emphasize the need for green synthesis aproach. Green synthesis, which utilizes biological resources such as plant extracts, microorganisms, and biomolecules, has emerged as an appealing alternative for the eco-friendly production of nanoparticles [8,9]. Among these, plant-based synthesis has gained considerable attention due to its cost-effectiveness, accessibility, and compatibility with green chemistry principles. Plants are rich in bioactive compounds such as phenolic acids, flavonoids, tannins, and alkaloids, which serve as natural reducing and stabilizing agents, enabling the formation of nanoparticles.

Numerous plant species have been successfully employed for the green synthesis of AuNPs, including *Diospyros kaki*, *Magnolia kobus* [10], *Terminalia catappa* [11], *Cacumen platycladi* [12], *Gymnocladus assamicus* [13], *Musa acuminata colla* [14], *Prunus domestica* [15] and *monogyna* [16]. However, the biosynthesis of AuNPs using *P. cerasus* leaves extract has not been previously reported. This study aims to fill this gap by synthesizing AuNPs using *P. cerasus* leaves extract, which is rich in phenolic compounds, and evaluating their biological properties, including their anticancer potential.

MATERIAL AND METHOD

Preparation of Plant Extract

The leaves of *P. cerasus* were collected from the Eğirdir region of Isparta, and the plant species was taxonomically identified. To prepare the leaves extract, five grams of air-dried *P. cerasus* leaves were placed in a flask containing 100 ml of 80% ethanol. The maceration process was carried out over 24 hours to ensure efficient extraction of bioactive compounds. After the initial maceration, the extract was filtered, and the residual plant material was subjected to re-extraction using fresh ethanol. This process was repeated three times to maximize the yield of the extract [17]. The obtained filtrate was stored at +4°C for subsequent experimental use.

Synthesis of Au NPs

Sodium chloroaurate ($NaAuCl_4.2H_2O$) was supplied by Sigma Aldrich. To synthesize AuNPs, 1 mL of 0.01 M $HAuCl_4$ was added to 10 ml of the *P. cerasus* extract and stirred at room temperature in

a dark for 24 hours. The change in color of the solution from dark yellow to purple confirmed the formation of AuNPs by reduction reactions [18]. The synthesized AuNPs were centrifuged and washed with distilled water.

Cytotoxic Assay (MTT Assay) of Synthesized AuNPs

The liver cancer cell line (HepG2, ATCC® HB-8065™) and the human normal fibroblast cell line (L929) were procured from the American Type Culture Collection. Cells were cultured in Dulbecco's Modified Eagle's Medium-high-glucose (DMEM) supplemented with 10% fetal bovine serum (FBS), and %1 glutamax. The cell seeding was done at a density of $4.5x10^3$ (for L929), $5x10^3$ (for HepG2) cells/well into sterile 96-well plates. After seeding, the cells were exposed to AuNPs at varying concentrations: 5, 2.5, 1.25, 0.625, and 0.3125 µg Au /ml for L929 and 5, 2.5, 1.25, and 0.625 µg Au/ml for HepG2. The exposure duration was 48 hours. Following the exposure period, an MTT solution was added to each well to evaluate cell viability, and the plates were incubated for an additional 2 hours. Absorbance readings were obtained using an Epoch 2 ELISA plate reader at 590 nm. The half-maximal inhibitory concentration (IC_{50}) values were determined using GraphPad Prism Software 5.

Characterization of Synthesized AuNPs

The characterization of the synthesized AuNPs was carried out using UV-Vis, FTIR, XRD, TEM and zeta potential analysis. UV-Vis spectra were obtained using a JASCO V-770 UV/Vis spectrometer with a resolution of 1 nm, scanning wavelengths from 350 to 800 nm. FTIR spectroscopy was performed to identify functional groups in the plant extract and AuNPs, with spectra recorded at room temperature in the 400–4000 cm^{-1} range at a resolution of 4 cm^{-1}. XRD analysis was conducted utilizing monochromatic Cu-Kα radiation (wavelength: 0.154056 nm) to determine the crystalline structure of AuNPs, with data collected over a 2θ range of 20° to 90°. The size and morphology of the nanoparticles were examined using TEM on a JEOL JEM-1020 instrument. Zeta potential measurements were also conducted to evaluate the stability of the synthesized AuNPs.

RESULT AND DISCUSSION

AuNPs are well-known for their characteristic surface plasmon resonance (SPR) band, which is observed in the UV-Vis spectrum. This SPR band enables qualitative analysis of AuNPs, providing insight into their presence and stability. For spherical AuNPs, this band typically appears within the wavelength range of 500–550 nm, depending on factors such as particle size, shape, and the surrounding medium. The UV-Vis spectrum of the *P. cerasus* leaves extract and synthesized AuNPs by using *P. cerasus* leaves extract is shown in Figure 1. Synthesized AuNPs exhibit a characteristic SPR band at 545 nm in the UV-Vis spectrum. The obtained UV-Vis spectrum is consistent with findings reported in the literature [19-21].

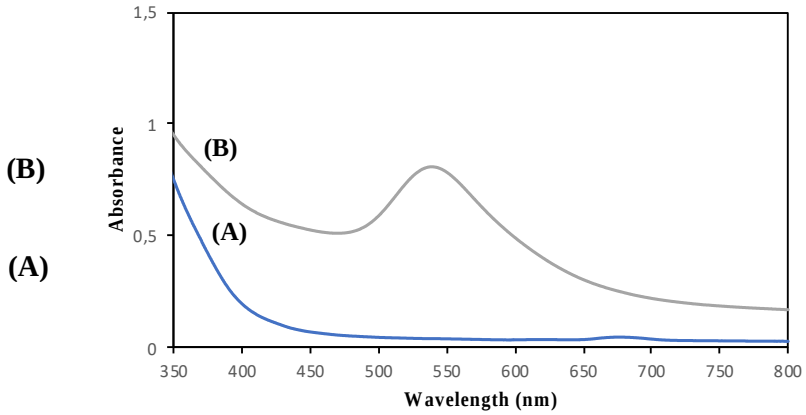

Figure 1. UV-Vis spectrum of (A) *P. cerasus* leaves extract, (B) synthesized AuNPs in the *P. cerasus* leaves extract

FTIR analysis was performed to identify the bioactive components in *P. cerasus* leaves extract that contribute to the synthesis and stabilization of AuNPs. The FTIR spectra, as shown in Figure 2, revealed prominent absorption bands at 3291, 2923, 1600, 1375, and 1029 cm^{-1} for both the leaves extract and synthesized AuNPs using the extract. The broad peak at 3291 cm^{-1} corresponds to O–H and N–H stretching vibrations, indicative of the presence of phenolic compounds and proteins. The peak at 2923 cm^{-1} is attributed to C–H stretching vibrations. The band observed at 1600 cm^{-1} represents C=O stretching vibrations, suggesting the presence of key flavonoids and terpenoids. The band at 1375 cm^{-1} corresponds to C=C stretching vibrations, associated with alkenes and aromatic compounds. Lastly, the peak at 1029 cm^{-1} is linked to C–N stretching vibrations, which are characteristic of aliphatic amines, alcohols, or polyphenols [22]. Notably, reductions in intensity or slight shifts in these peaks were observed in the spectra of AuNPs compared to the extract alone, as shown in **Fig. 2.** These changes imply that functional groups in the *P. cerasus* leaves extract, such as phenolic and amine groups, play an active role as reducing and stabilizing agents during the biosynthesis of AuNPs.

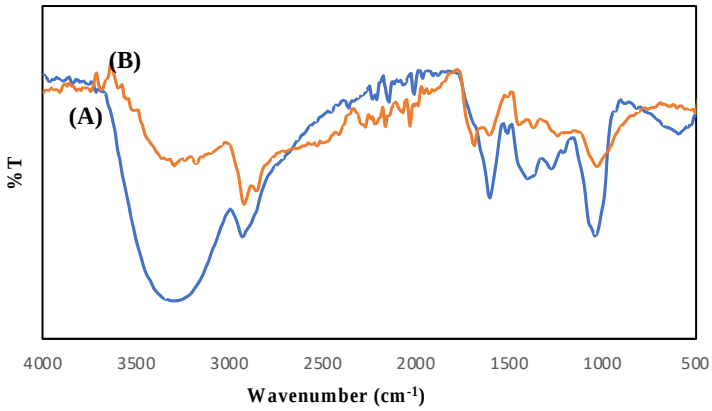

Figure 2. FTIR spectrum of (A) *P. cerasus* leaves extract (B) synthesized AuNPs

The XRD analysis of the AuNPs synthesized using *P. cerasus* leaves extract, shown in Figure 3, was conducted over a 2θ range of 20° to 90°. The diffraction pattern revealed five prominent peaks at 38.1°, 44.4°, 64.6°, 77.8°, and 81.8°, corresponding to the (111), (200), (220), (311), and (222) crystalline planes, respectively. These results align with the Powder Diffraction Standards Joint Committee data (card number 04-0784). The intense diffraction peak at 38.1° is particularly notable, confirming the presence of pure crystalline gold [23]. Additionally, the XRD analysis clearly indicates that the AuNPs formed by the reduction of $AuCl_4^-$ ions by the bioactive components present in the using *P. cerasus* leaves extract possess a face-centered cubic (fcc) lattice structure [23]. This analysis confirms the crystalline nature of the nanoparticles and highlights the role of the plant extract in their synthesis.

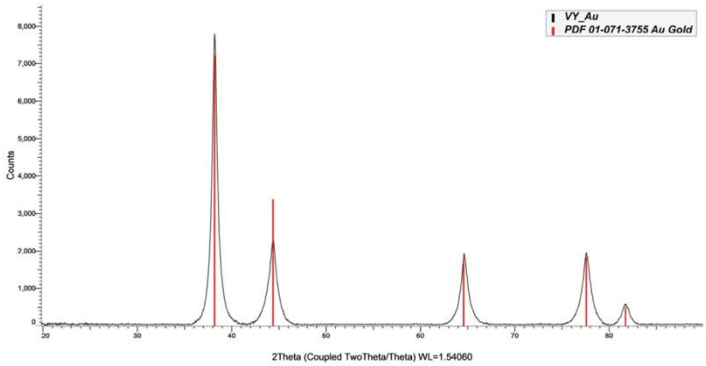

Figure 3. XRD spectrum of synthesized AuNPs

The TEM analysis provides information on the morphology and size of the synthesized AuNPs. As shown in Figure 4, the TEM results indicate that the synthesized AuNPs are approximately spherical, with an average diameter of 33±13 nm.

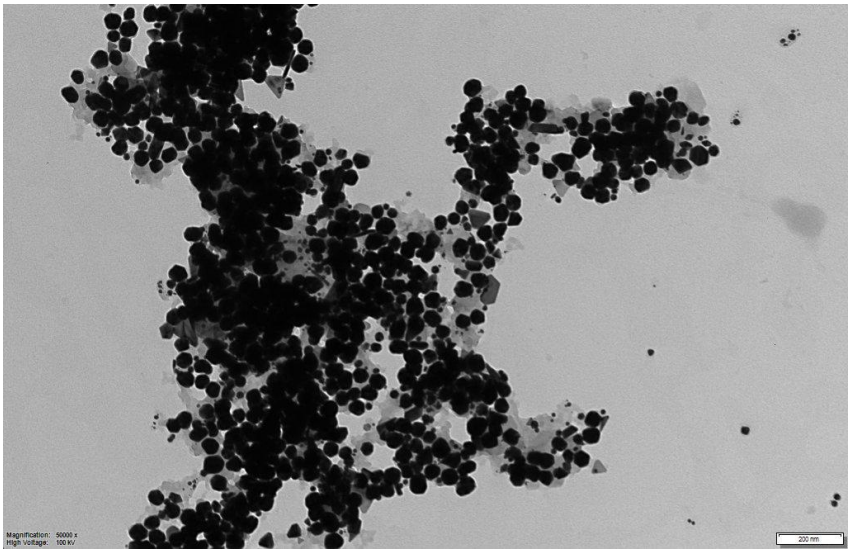

Figure 4. TEM images of synthesized AuNPs (Scale bar: 200 nm)

The size distribution of the synthesized AuNPs was determined by analyzing TEM images using Adobe Photoshop 7. Measurements were taken for 100 individual nanoparticles, and the resulting size distribution is illustrated in Figure 5. According to the analysis, the nanoparticles ranged in size from 10 nm to 59 nm.

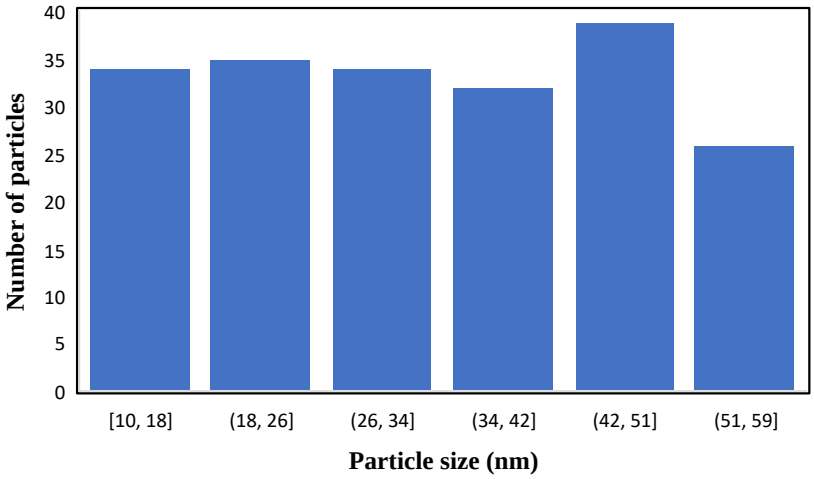

Figure 5. Particles size distribution of synthesized AuNPs

Zeta potential is a widely used parameter for characterizing the surface charge properties of nanoparticles. It provides insights into the electrical potential at the particle surface and is influenced by both the composition of the nanoparticles and the characteristics of the dispersing medium. Nanoparticles with a zeta potential above ±30 mV are considered stable in suspension due to the surface charge preventing particle aggregation [24]. The zeta potential of synthesized AuNPs using *P. cerasus* extract was found to be –54.4 mV (Figure 6), indicating that the synthesized AuNPs are stable.

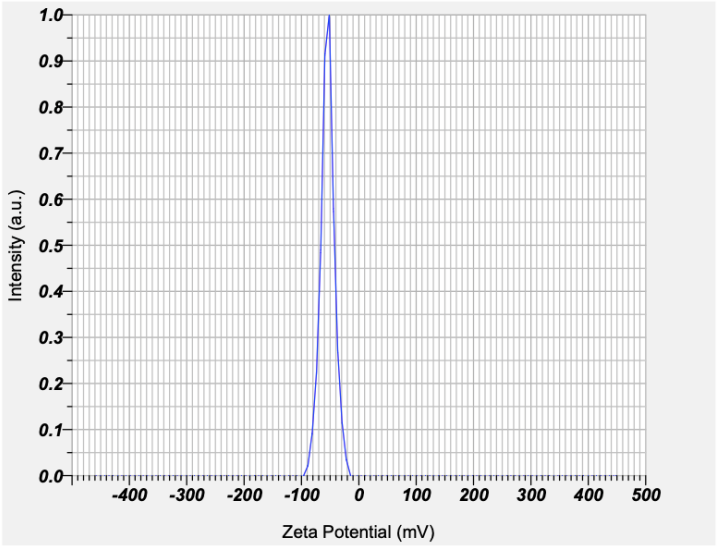

Figure 5. Zeta potential of synthesized AuNPs

The anticancer potential of the synthesized AuNPs was assessed against liver cancer cell lines *in vitro*. Both human liver cancer cells and normal human cell lines were used for the evaluation, with tests performed in duplicate, and the results averaged. As presented in Table 1, the AuNPs demonstrated approximately threefold greater cytotoxicity in inhibiting liver cancer cell growth compared to the plant extract alone. Moreover, the AuNPs exhibited higher cytotoxic activity against human liver cancer cells (HepG2) compared to normal human cell lines (L929), clearly demonstrating their selective activity.

Table 1. IC_{50} results for AuNPs against liver cancer and the human normal fibroblast cell line

Compounds	**IC_{50} (µl/ml)**	
	HepG2	**L929**
P. cerasus extract	11.09	19.42
Synthesized AuNPs	4.133	9.451

Several studies have similarly explored the cytotoxic properties of AuNPs synthesized from various plant extracts against different human cancer cell lines [25-28]. In one study, AuNPs synthesized using *Marsdenia tenacissima* extract with an approximate size of 50 nm were evaluated for their cytotoxic effects on lung cancer A549 cell lines. The findings indicated that the cytotoxicity of the nanoparticles was dependent on both their size and concentration [29]. For example, Al-Khedhair and wahap studied the cytotoxic effects of AuNPs of different sizes (10-15 nm, 20-30 nm, 45 nm) against Liver Cancer Cells. They found that among three different sizes of AuNPs, small-sized nanoparticles were the most effective in controlling cancer cells. They also reported that small size facilitates easy entry into cells and reaction with cell organelles [30]. In another study, AuNPs synthesized using *Curcumae kwangsiensis folium* leaves extract with an average diameter of 16.6 nm demonstrated significant activity against ovarian cancer cell lines. It was also reported that the cytotoxic effects of the synthesized AuNPs increased in a dose-dependent manner [31].

Conclusion

This study highlights the significant potential of green synthesis methods in medical nanotechnology by demonstrating the successful synthesis of AuNPs using *P. cerasus* leaves extract and evaluating their structural properties and anticancer activity. The characterization techniques, including UV-Vis, FTIR, XRD, TEM and zeta potential analyses, confirmed the formation of stable,

crystalline, and morphologically desirable AuNPs. Biological assays further revealed that these nanoparticles exhibit approximately threefold higher antiproliferative activity against HepG2 liver cancer cells compared to the leaf extract alone, while also showing selective cytotoxicity over normal cells. These findings underscore the unique physicochemical and therapeutic potential of green-synthesized AuNPs, positioning them as strong candidates for targeted cancer therapy. Moreover, the use of P. cerasus leaves as a natural and sustainable reducing agent reinforces the feasibility of eco-friendly approaches in nanoparticle production. Overall, this research provides valuable insights into sustainable nanomaterial development and opens new avenues for future applications of green nanotechnology in biomedical sciences.

CONFLICT OF INTEREST

The author declares that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The author declares that the ethics committee approval is not required for this study.

AUTHOR CONTRIBUTION

Concept: F.M.S.; Design: F.M.S.; Control: F.M.S.; Sources: F.M.S.; Materials: F.M.S.; Data Collection and/or Processing: F.M.S.; Analysis and/or Interpretation: F.M.S.; Literature Review: F.M.S.; Manuscript Writing: F.M.S.; Critical Review: F.M.S.; Other: -

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