

Green Synthesis of Apricot Kernel Silver Nanoparticles and Their Biological Activity

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Abstract: Silver nanoparticles (AgNPs) exhibit cytotoxicity in various cancer cells, as well as high conductivity, chemical stability, catalytic, and antibacterial activity. A range of biological sources, including plants, fungi, and bacteria, are utilized for the synthesis of silver, gold, and other nanoparticles. The aim of this study is to synthesize environmentally friendly and cost-effective AgNPs from apricot kernel methanol extract and to investigate their antimicrobial and cytotoxic activities. The synthesized AgNPs were characterized using UV-vis spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR). The UV-vis spectra revealed a surface plasmon resonance peak at 420 nm, confirming the formation of apricot kernel silver nanoparticles. Additionally, FTIR spectra indicated the involvement of biological compounds in the synthesis of AgNPs. The synthesized AgNPs inhibited the growth of both Gram-negative and Gram-positive bacteria, including *E. aerogenes* CCM 2531, *B. subtilis* IM 622, *S. aureus* 6538 P, *S. aureus* ATCC 29213, and *L. monocytogenes* NCTL 53448. Furthermore, AgNPs reduced the viability of SH-SY5Y neuroblastoma cells. Based on these findings, it can be suggested that the environmentally friendly synthesized AgNPs exhibit multifunctional properties with potential applications against infectious bacterial strains and cancer.

Kayısı Çekirdeği Gümüş Nanopartiküllerinin Yeşil Sentezi ve Biyolojik Aktiviteleri

Anahtar Kelimeler

Kayısı çekirdeği,
Gümüş nanopartikül,
Sitotoksosite,
Kanser,
Antimikrobiyal

Öz: Gümüş nanopartiküllerin (AgNP'ler), çeşitli kanser hücrelerinde sitotoksositeye, yüksek iletkenliğe, kimyasal stabiliteye, katalitik ve antibakteriyel aktiviteye sahiptir. Gümüş, altın ve diğer nanoparçacıkların sentezi için bitkiler, mantarlar ve bakteriler dahil olmak üzere birçok biyolojik kaynak kullanılmaktadır. Bu çalışmanın amacı, çevre dostu ve uygun maliyetli AgNP'lerin kayısı çekirdeği metanol ekstraktından sentezlenmesi ile antimikrobiyal ve sitotoksik aktivitelerini araştırmaktır. Sentezlenen AgNP'ler UV-vis, X-ışını kırınımı (XRD), Taramalı Elektron Mikroskobu (SEM) ve Fourier dönüşümlü kızılötesi spektroskopisi (FTIR) ile karakterize edildi. UV-vis spektrumları, kayısı çekirdeği gümüş nanoparçacıklarının oluşumunu doğrulayan 420nm'de yüzey plazmon rezonans pikini ortaya çıkardı. FTIR spektrumları ayrıca biyolojik bileşiklerin AgNP'lerin sentezine katılımını göstermiştir. Sentezlenen AgNP'ler *E. aerogenes* ccm 2531, *B. subtilis* IM 622, *S. Aureus*

6538 p., *S. Aureus ATCC 29213* ve *L. monocytogenes NCTL 53448* olmak üzere gram negatif ve gram pozitif bakterilerin büyümesini engelledi. AgNP'ler ayrıca, SHSY5Y nöroblastom hücrelerinin canlılığını azalttı. Bu bulgulara dayanarak, çevre dostu sentezlenen AgNP'lerin çok işlevli özellikler gösterdiği, bulaşıcı bakteri türlerine ve kansere karşı kullanılabilme potansiyeli sahip olduğu söylenebilir.

1. INTRODUCTION

Neuroblastoma (NB) is a type of pediatric tumor that arises from neural tissues within the central nervous system (CNS) [1, 2]. It is one of the most common cancers found in infants and children, following leukemia and brain tumors. Additionally, NB serves as a model in neuroprotection research, aiding in the development of new therapeutic and preventive strategies for CNS-related disorders. Treatment approaches for NB are influenced by the severity of the disease, the patient's age, and underlying biological factors [3, 4]. Given these complexities, there is a pressing need for research focused on discovering novel biochemical compounds and innovative treatment strategies for NB.

For many years herbal medicines have been used as the primary source of medical treatment. The apricot has been used in folk medicine as a remedy for various diseases attributed to its rich antioxidant, antimicrobial and anticancer content [5,6]. The apricot fruit constitutes carbohydrates, vitamins, beta carotene and thiamine. Apricot kernels (Ak) contain a significant amount of protein, oil and fibre. Apricot kernels also contain the toxic cyanogenic glycoside amygdalin [5,7-9].

The eco-friendly synthesis of silver nanoparticles (AgNPs) by biological materials has considerable attention in view of therapeutic aspects. Environmentally friendly synthesis of nanoparticles by biological resources extract is currently under investigation. The AgNPs are reported to have numerous different biological applications. The study of antimicrobial and anticancer activity of these biosynthesized nanoparticles gives an idea of the activity and association of these nanoparticles with biomolecules of living systems [10-12].

Numerous research regarding the physico-chemical features of Ak are presented in the literature Gupta et al. [8], but not much knowledge is found regarding the in vitro antioxidant activities of the Ak. Likewise, the anticancer and antimicrobial activities of Ak have been less studied and the lack of research related to the antibacterial and anticancer activity of the kernels along with their nanoparticles have motivated the present study. In the present study, single step synthesis of the AgNPs by the reduction of silver nitrate (AgNO₃) at room temperature by Ak methanol extracts are presented. The synthesis of NPs has been monitored by UV-Visible spectroscopy (UV-Vis), Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). The synthesised AgNPs were evaluated for their anticancer and antimicrobial activity.

2. MATERIAL AND METHOD

2.1. Preparation of Ak Extracts

The extract was prepared from 200 gr Ak by granulating in a mortar and was extracted with 1 liter methanol. The mixture was sealed with aluminium foil and stirred for 24 hours. Following that, the extract was filtered with Whatman No.1 filter paper and the pellet was discarded while supernatant was stored at 4 °C.

2.2. Synthesis of Ak AgNPs (AkAgNPs)

100 ml of methanol extract was mixed with 900 ml of 1 mM AgNO₃ under magnetic stirring at room temperature. The greyish colour of the extract-AgNO₃ mixture at 0 min of reaction time changed to a dark brown in about 10 minutes after justifying pH of the mixture to alkali with NaOH. After observing the colour change, the absorbance of the dark brown mixture was measured at 430 nm by UV-vis spectrophotometer to observe the formation of AkAgNPs. Following that the mixture was centrifuged at 15000 rpm for 15 min and subsequently washed in pure water for 5 times at 15000 rpm to discard waste biological material.

2.3. Characterization of AkAgNPs

Initial characterisation of AkAgNPs were carried out by UV-Vis (Shimadzu UV-3600 UV-VIS-NIR Spectrophotometer) to determine the absorption spectra of the AkAgNPs between 200–800 nm.

The FTIR spectra were measured by the dried pellet method at resolution of between 4,000 to 400 cm⁻¹ (Perkin Elmer Spectrum 100) for the Ak extract powders and AkAgNPs.

The nanoparticles were further characterized by XRD using a Rigaku Optima IV diffractometer with Cu K α radiation ($\lambda = 0.1542$ nm).

The morphology of the AkAgNPs was characterised by SEM (JEOL). Samples were examined on a graphite-coated surface to obtain a uniform image by providing conductivity.

2.4. Anti-microbial Activity Test

The antimicrobial activity of synthesised AkAgNPs were investigated against *Enterobacter aerogenes ccm 2531*, *Bacillus subtilis IM 622*, *Staphylococcus aureus 6538 p.*, *Staphylococcus aureus ATCC 29213* and *Listeria monocytogenes NCTL 5348*. The antimicrobial activity was tested using the spread plate agar method, in which 6 mm discs placed on plates based. Distilled water was

used as negative control, penicillin and streptomycin mixture was used as positive control, Ak extract, AgNO₃ and different doses of AkAgNPs were absorbed into the disks. After incubating the plates at 37°C for 24 h, the diameter of the inhibition zone was measured with image J [13].

2.5. Cell Culture

Human NB cells (SH-SY5Y; American Type Culture Collection (ATCC, USA)) were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS and 1% penicillin-streptomycin solution (Biological Industries, Israel). The cells were cultured as described in [14].

2.6. Cell Proliferation Assay

WST-1 cell proliferation assay kit (Clontech, USA) was used to analyse cell proliferation and viability. Briefly, 15×10^3 NB cells/well in a 100- μ l medium were cultured in 96 well plates. 24 hour later, different concentrations of Ak extracts and AkAgNPs (8 mg/ml and 4 mg/ml) were added into the wells. The assay was performed according to the instructions supplied with kit.

2.7. Statistical Analyses

All experiments were repeated at least 3 times, and biostatistical analysis was carried out with GraphPad Prism 5.0. Data sets were compared and the analyses were performed with by one-way ANOVA Newman-Keuls Post-Hoc Test

3. RESULTS

3.1. Characterisation of Nanoparticles

Green synthesis AkAgNPs from 1 mM aqueous solution of AgNO₃ (pH 11,2) with Ak extract was initially confirmed by UV-Vis spectral analysis.

The initial greyish colour of the reaction mixture of the extract with AgNO₃ solution changed to dark brown colour as a result of excitation of surface plasmon resonance vibration of AkAgNPs. The surface plasmon of AkAgNPs occurred at about 420 nm for Ak extract reacted with AgNO₃ (Fig. 1). It is worth noting the reduction of Ag⁺ ions by the extract was occurred in about 10 minutes at room temperature.

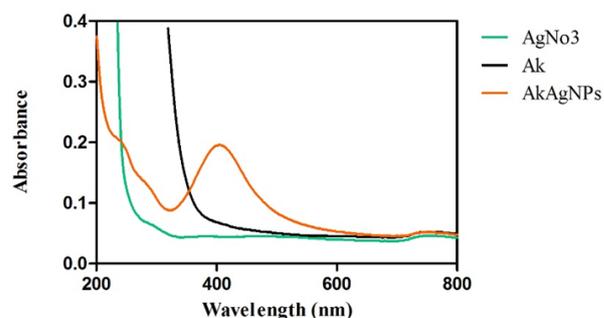


Figure 1. UV-Vis absorption spectra of AgNO₃, Ak extract, AkAgNPs (AgNO₃; silver nitrate, Ak Extract; apricot kernel extract; AkAgNPs; synthesized AgNPs with apricot kernel extract)

The FTIR measurements of biosynthesized AkAgNPs were performed to understand the interaction between biomolecules of the Ak extract and Ag⁺ ions. FTIR spectra of AkAgNPs showed absorption peak situated at about 3336, 2925, 2853, 1742, 1638 and 1016 cm⁻¹. The very intense broad band positioned at about 3336 cm⁻¹ position in the spectra of AkAgNPs could be attributed to O-H stretching. The peak placed at about 2925 and 2853 cm⁻¹ in the spectra could represent C-H stretching of alkyl group while stretching vibration at 2853 cm⁻¹ could be attributed to C-H stretching of aldehydes group. The absorption peaks located at 1,638 cm⁻¹ in both spectra represents N-H stretch of amines. The peak 1016 cm⁻¹ represents C-N stretching of aliphatic amines (Fig. 2).

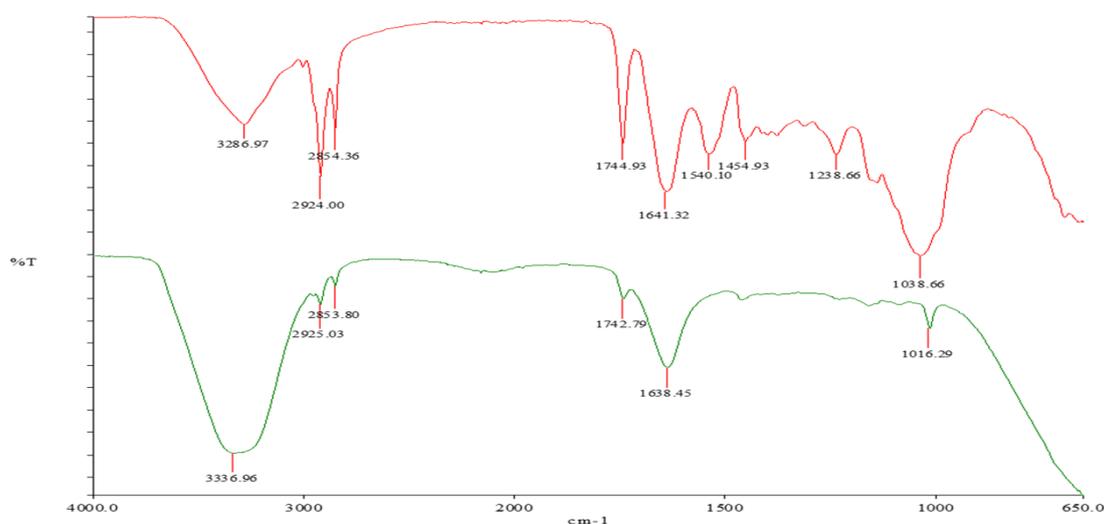


Figure 2. FTIR spectra of synthesized AkAgNPs (Green) and Ak Extract (Red)

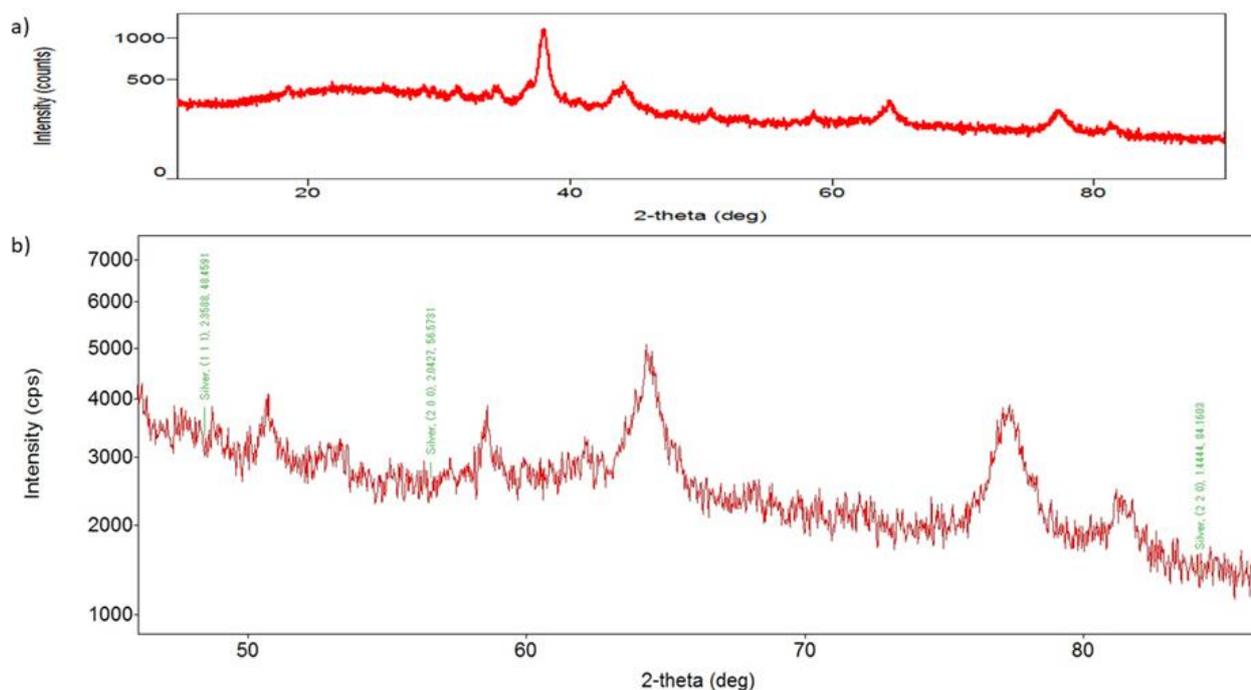


Figure 3. XRD spectrum of the nanoparticles. a) The nanoparticles revealed the presence of six distinct diffraction peaks at $2\theta = 34.36^\circ, 37.885^\circ, 43.98^\circ, 64.42^\circ, 77.02^\circ, 81.65^\circ$. b) The cubic silver indices of AkAgNPs crystals; 111, 200, 220, and 311

The presence of these vibrations at FTIR spectra suggests that some of the bio-organic molecules such as carbohydrates, proteins, oils, enzymes and amino acids are responsible for the reduction of Ag^+ ions and hence nanoparticle formation.

The crystalline structure of the AkAgNPs were confirmed by XRD analyses. XRD spectrum of the nanoparticles revealed the presence of six distinct diffraction peaks at $2\theta = 34.36^\circ, 37.885^\circ, 43.98^\circ, 64.42^\circ, 77.02^\circ, 81.65^\circ$ that can be indexed at 111, 200, 220, and 311 of the cubic silver suggesting that the AkAgNPs were crystalline (Fig. 3).

SEM micrographs of AkAgNPs at room temperature showed the presence of very well dispersed AkAgNPs. The analysis of particle size distribution revealed that the majority of the particles are between 0.4 to 0.9 μm at 1000 magnification (Fig. 4).

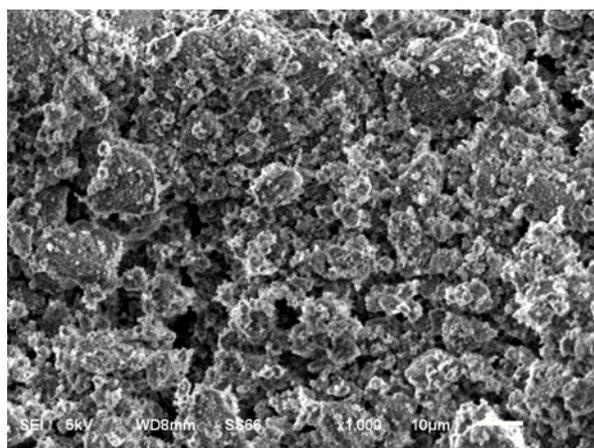


Figure 4. SEM micrograph of synthesised AkAgNPs

3.2. Antimicrobial Activity of AkAgNPs

The antimicrobial activity of AkAgNPs were tested against *E. aerogenes ccm 2531*, *B. subtilis IM 622*, *S. aureus 6538 p.*, *S. aureus ATCC 29213* and *L. monocytogenes NCTL 5348* using the disc diffusion method and the results were presented in table 1. The synthesised nanoparticles inhibited the growth of gram-negative bacterium *E. aerogenes ccm 2531* and gram positive bacteria such as *B. subtilis IM 622*, *S. aureus 6538 p.*, *S. aureus ATCC 29213* and *L. monocytogenes NCTL 5348* (2 and 4 mg/mL). 4 mg/ml AgNO_3 and Ak extract did not inhibit the growth of the microorganisms.

Table 1. Antibacterial activity of AkAgNPs against gram negative and gram positive bacteria. The inhibition zones are given as cm.

Bacteria	AkAgNPs (4 mg/ml)	AkAgNPs (2 mg/ml)	Ak extract (4mg/ml)	Antibiotic	AgNO_3 (4mg/ml)	Water
<i>E. aerogenes ccm 2531</i> (Gr -)	0.840	0.775	0.696	1.940	0.631	0
<i>B. subtilis IM 622</i> (Gr +)	0.786	0.760	0.605	1.783	0.713	0
<i>S. aureus 6538 p.</i> (Gr +)	0.812	0.793	0.700	2.316	0.806	0
<i>S. aureus ATCC 29213</i> (Gr +)	0.660	0.700	0.512	2.037	0.643	0
<i>L. monocytogenes NCTL 5348</i> (Gr +)	0	0.89	0	2.260	0.655	0

3.3. Effects of AkAgNPs on SHSY5Y cell proliferation

The antiproliferative effect of the AkAgNPs and their extracts were assessed on NB cells. The cells were treated with different concentrations of AkAgNPs (4 and 8 mg/ml) over 24 hours, and the cell proliferations are given in Fig. 5. AkAgNPs significantly decreased cell viability at 4 and 8 mg/ml while the extract did not show anticancer activity on NB cells.

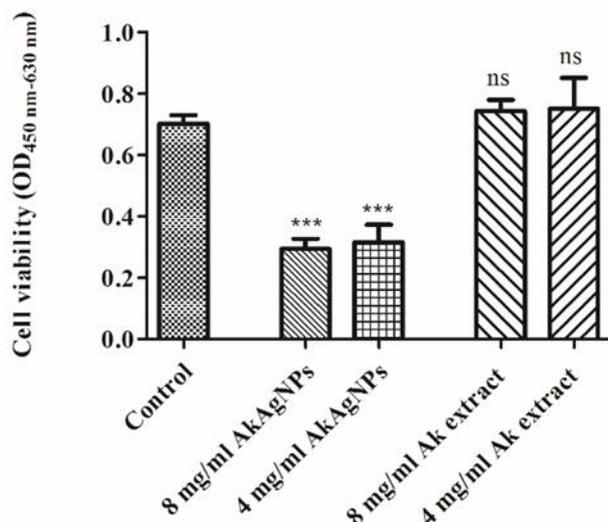


Figure 5. Anticancer activity of AkAgNPs in NB cell line. (***) $p < 0.001$; Control vs other groups 8 mg/ml, 4 mg/ml; ns: not significant)

4. DISCUSSION AND CONCLUSION

Many studies regarding the physicochemical characteristics of Ak are reported in the literature Haciseferoğulları et al., Alpaslan et al. [15,16], but the in vitro anticancer and antimicrobial activities of Ak and their NPs have not been studied in detail and the lack of the research related to the antimicrobial and anticancer activity of the apricot seeds along with their nanoparticles have stimulated the present research. In the present study, single step synthesis of the AgNPs through the reduction of aqueous AgNO₃ at room temperature by Ak methanol extracts are presented.

Ak extracts contain high levels of amygdalin, protein, fat, and various antioxidant and anticancer molecules. However, these molecules are unable to cross lipid membranes, have large molecular sizes, are poorly absorbed, and are unstable, resulting in reduced bioavailability and efficacy. Therefore, using nanoparticles is essential to enhance their absorption and increase their stability by preventing oxidation. For this reason, AgNPs were synthesized from Ak extracts, and their biological activity was investigated. The different concentrations of Ak methanol extract (4 and 8 mg/mL) did not show an antiproliferative effect on NB cells, whereas AkAgNPs significantly decreased cell viability at both concentrations. Similarly, Grunathan et al. also demonstrated that AgNPs reduced cell viability in SH-SY5Y cells in a dose-dependent manner [17]. This

suggests that nanoparticle-based kernel extracts have the potential to increase the efficiency and safety of kernel biomolecules by enhancing their capacity, improving solubility, and controlling their release.

AgNPs are reported to show their antibacterial effect through interaction with the outer membranes of the bacteria thanks to their chemical stability and tiny size [18-21]. The silver ions leads toxicity by reacting thiol group of the enzymes located outer membranes of the microorganisms [22,23]. In addition to these antimicrobial activity of the NPs are reported to be arisen from ionic (electrostatic) interaction between negatively charged cell membrane and positively charged silver [24-26].

The antimicrobial activity of AkAgNPs was tested against *E. aerogenes ccm 2531*, *B. subtilis IM 622*, *S. aureus 6538 p.*, *S. aureus ATCC 29213* and *L. monocytogenes NCTL 5348*. Based on the results, it can be concluded that AkAgNPs inhibit the growth of both Gram-negative and Gram-positive bacteria. In recent years, some studies have suggested that Gram-positive bacteria may be resistant to the effects of nanoparticles due to differences in their cell wall structures [27-29]. Vadakkan et al. also reported that biogenic AgNPs exhibit strong antibacterial properties against a wide range of both Gram-positive and Gram-negative bacteria [30]. Additionally, Saied et al. demonstrated that Ak extract possesses antimicrobial properties [31]. The data obtained in this study are consistent with the literature, showing that AkAgNPs inhibit the growth of both Gram-negative and Gram-positive bacteria.

The anticancer and antimicrobial effects of AgNPs synthesized by reducing AgNO₃ with Ak methanol extracts at room temperature are presented. The environmentally friendly synthesized AkAgNPs demonstrated significant activity against both Gram-negative and Gram-positive bacteria. Additionally, their notable effectiveness against NB cancer cells suggests potential efficacy against other cancer types. In future studies, the effects of AkAgNPs on other cancer cell lines will be further explored.

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