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Green Synthesis and Antibiofilm Activity of Silver Nanoparticles by *Camellia sinensis* L. (White Tea Leaf)

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ABSTRACT

Biosynthesis of nanoparticles refers to the production or synthesis of nanoparticles using organisms, such as bacteria, fungi, plants or their byproducts. This approach offers several advantages over conventional chemical methods, including eco-friendliness, cost-effectiveness, and potential for large-scale production. The silver nanoparticles (AgNPs) synthesized using aqueous *Camellia sinensis* L. (white tea leaf) extracts as reducing and stabilizing agents were reported and evaluated for antibiofilm activity against test microorganisms (*Acinetobacter baumanii* ATCC 19606 NRRLB 3704, *Pseudomonas aeruginosa* ATCC 27853 (Gram –), and *Bacillus subtilis* ATCC 6633, *Staphylococcus haemolyticus* ATCC 43252 (Gram +), and *Candida albicans* ATCC 10231) in the study. The synthesized AgNPs were observed and characterized using Uv-Vis spectroscopic analysis, scanning electron and transmission electron microscopy energy-dispersive spectra and Fourier transform infrared spectroscopy. The synthesized AgNP was also screened for antibiofilm activity against test microorganisms. Our results show that the synthesized AgNPs have the potential to be used for antibiofilm materials and different biomedical applications.

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Keywords
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Camellia sinensis L. (Beyaz Çay Yaprağı) ile Gümüş Nanopartiküllerin Yeşil Sentezi ve Antibiyofilm Aktivitesi

ÖZET

Nanopartiküllerin biyosentezi bakteriler, mantarlar, bitkiler veya yan ürünleri gibi organizmaları kullanarak nanopartiküllerin üretimini veya sentezini ifade eder. Bu yaklaşım, çevre dostu, maliyet etkinliği ve büyük ölçekli üretim potansiyeli dahil olmak üzere geleneksel kimyasal yöntemlere göre çeşitli avantajlar sunar. İndirgeyici ve stabilize edici ajan olarak *Camellia sinensis* L. (beyaz çay yaprağı) ekstraktları kullanılarak sentezlenen gümüş nanopartiküller (AgNP'ler) rapor edilmiş ve çalışmada test mikroorganizmalarına (*Acinetobacter baumanii* ATCC 19606 NRRLB 3704, *Pseudomonas aeruginosa* ATCC 27853 (Gram –), *Bacillus subtilis* ATCC 6633, *Staphylococcus haemolyticus* ATCC 43252 (Gram +), ve *Candida albicans* ATCC 10231) karşı antibiyofilm aktivitesi açısından değerlendirilmiştir. Sentezlenen AgNP'ler Uv-Vis spektroskopik analizi, taramalı elektron mikroskobu, enerji dağıtıcı spektrumları ve Fourier dönüşümü kızılötesi spektroskopisi kullanılarak gözlemlendi ve karakterize edildi. Sentezlenen AgNP'nin test mikroorganizmalarına karşı antibiyofilm aktivitesi de taranmıştır. Sonuçlarımız, sentezlenen AgNP'lerin antibiyofilm materyalleri ve farklı biyomedikal uygulamalar için kullanılma potansiyeline sahip olduğunu göstermektedir.

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INTRODUCTION

The term nanoparticle refers to particles with dimensions of 100 nm and below. Unlike large-structured materials, nanoparticles have unique physico-chemical, industrial and biological properties with their nano-sized structures. Thanks to their unique structure, metal nanoparticles have a high function for use in the electronics and materials industry and allow the development of new methods in medical applications such as the production of antimicrobial substances, drug delivery, disease diagnosis and treatment (Składanowsk et al. 2017). Due to this feature of nanoparticles, synthesizing them has gained a very important dimension in recent years. Chemical methods are expensive and unstable methods that require high energy, reducing/stabilizing agents and toxic chemicals. In addition, the produced nanoparticles may have biological side effects (Składanowski et al. 2017). Therefore, biological, non-toxic, environmentally friendly and economical syntheses come to the fore in nanoparticle production.

Plants, fungi, actinomycetes and bacteria can be used for non-toxic nanoparticles such as gold, silver, cadmium sulfide, selenium, zinc oxide, and copper (Qi et al. 2016; Umaz et al. 2019; Atalar et al. 2022; İpek et al. 2023). *Camellia sinensis* L., whose homeland is known as China and Thailand, is a drink as important as water in the world. It is obtained from the leaves and buds of the plant *C. sinensis*, and different varieties such as black, green, oolong, white, and pu-erh tea are obtained according to different processing methods, and they are becoming important commercial products that are widely used in the world (Ilgaz et al. 2006). It has been investigated in type of research that different tea varieties produced as a result of differences in business have different biological activities in addition to having unique tastes and aromas. Tea polyphenols, especially catechin derivatives, are powerful antioxidant agents with positive effects on human health. Antioxidant compounds have aroused great interest due to their ability to scavenge free radicals and thus inhibit oxidative stress. Many studies have focused on the natural antioxidants found in tea extracts, especially because of the chemical compounds they contain. In addition, the polyphenols contained in teas are thought to be responsible for anticarcinogenic, antimutagenic and protection. Among commercial tea varieties, white tea undergoes very little processing and is reported to have strong hypoactivity such as antioxidant, anti-inflammatory, anti-mutagenic, and anti-cancer activities (El-Taher, 2011). Especially silver nanoparticles (AgNPs) are being used in various fields such as medicine and healthcare, electronics, textiles, water treatment, cosmetics - personal care products and food packaging, due to their unique properties and potential applications. The AgNPs with small particle size and without bulking between particles are also favorable to exploring new pharmaceutical, antimicrobial, anticancer and antibiofilm agents (Ghaffari-Moghaddam & Hadi-Dabanlou, 2014).

Biofilm is a complex community of microorganisms that form a protective matrix or slime layer, adhering to surfaces such as rocks, pipes, medical devices, and even living tissues. It is composed of various species of bacteria, fungi, algae, and other microorganisms embedded within a self-produced extracellular polymeric substance (EPS) matrix. This matrix provides structural support and protection to the microorganisms within the biofilm. The high antibiotic resistance of microbial biofilms has necessitated the investigation of new antimicrobial agents, such as the use of green synthesis nanoparticles Biofilms provide pathogens with a protected niche where they can thrive, resist treatment, and cause chronic infections, making them an important focus of research and clinical management. Understanding how biofilms function and their role in infection is crucial for developing effective strategies to prevent and treat biofilm-associated diseases (Göse & Hacıoğlu Doğru, 2021).

The current study was to explore the use of *C. sinensis* L. for the synthesis and characterization of AgNPs. The AgNPs were also investigated for antibiofilm activity against some human pathogens.

MATERIALS and METHODS

Synthesis of AgNPs by Green Method

Air-dried *C. sinensis* (CS) leaves (5 g) obtained from herbalists were ground and extracted with 100 mL of sterile distilled water at 65-70°C for 60 minutes. For AgNP synthesis, an aqueous solution of $AgNO_3$ (1 mM - 500 mL) and CS extract (125 mL) prepared beforehand were left to react in a flask at room temperature. The dark solution was centrifuged at 10,000 rpm for 5 minutes. The obtained solid part (AgNP) was left to dry in an oven at 65°C for 48 hours (Bayğu, 2020). The synthesized AgNPs were dried and collected in eppendorfs and used for other analyses. The silver nitrate solution was obtained by dissolving the appropriate amount of silver nitrate salt and double distilled water, with a final concentration of 1 mM.

Characterization of Synthesized AgNPs

UV-Visible (Uv-Vis) Spectroscopy Analysis

UV-Vis provides knowledge about the structure, size, stability, concentration and aggregation of nanoparticles (Chandran et al. 2006). Synthesized AgNPs were characterized by measuring the UV-Vis spectra at wavelengths between 200 and 875 nm.

Scanning Electron Microscopy (SEM) Analysis

Synthesized powder crystal AgNPs were performed using a Quanta FEG 250 (FEI, USA) SEM as service procurement by DAYTAM, (Atatürk University Eastern Anatolia High Technology Application and Research Center).

Transmittance Electron Microscopy (TEM) Analysis

The size and shape of powder AgNPs were determined using TEM (Hitachi HT-7700) by service procurement by DAYTAM.

Energy Distribution Spectroscopy (EDS) Analysis

EDS is an analytical test system applied for chemical analysis of nanoparticles (Hodoroaba, 2020). A powder crystal structure of AgNPs was carried out as service procurement by DAYTAM.

Fourier Transforms Infrared Spectroscopy (FT-IR) Analysis

The FT-IR (KBr disk) spectra of the extracts and AgNPs were recorded in the Perkin Elmer BX II FT spectrometer with 16 scan numbers, in the range of 4000-400 cm^{-1}, at 1 cm^{-1} resolution.

Antibiofilm Activity of AgNPs

Acinetobacter baumanii ATCC 19606 NRRLB 3704, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Staphylococcus haemolyticus* ATCC 43252, and *Candida albicans* ATCC 10231 test cultures were used for antibiofilm activity of AgNPs extract. For antibiofilm detection, the minimum inhibitory concentrations (MIC) (Barry, 2007) of CS and AgNP were used. The medium, 0.1% (w/v) Streptomycin (ST), NYS100 and 10% DMSO were used as the non-treated, positive and negative controls, respectively. The antibiofilm capacity of CS and AgNPs was determined using the crystal violet (CV) staining method (Merritt et al. 2005). According to Göse & Hacıoğlu Doğru (2021), a measurement of the anti-biofilm effect of the extracts was made.

% Inhibition = $(A_{control} - A_{sample} / A_{control}) x100$; $A_{control}$: Absorbance of the control reaction; A_{sample}: Absorbance of test compounds

Statistical analyzes of the obtained data were carried out using the SPSS program. Differences between data were compared with ANOVA using the minimum significant difference test (LSD, P ≤0.05).

RESULTS and DISCUSSION

Characterization Results of AgNPs

The toxicity and structural properties of metallic nanoparticles can be affected by many properties such as their size, load, dimensional structure, and chemical activities (Mohanta et al. 2020). For this reason, good characterization of nanoparticles is very important in order to obtain accurate results in studies (Bayğu, 2020). The reduction of Ag^{+} into AgNPs during exposure to the leaves of CS extract could be seen by the color change. During formation; we just noted the colors as the first color, the intermediate color, and the last color. Afterward, we continued our experiment according to these colors.

The Uv-Vis Spectroscopy

The first obvious observation of nanoparticles is color change. Ag ions reduced by white tea leaves are observed as the extract changes from yellowish to burgundy-brown (Figure 1). The resulting color change is thought to be an indicator that plant components are successful in the synthesis of AgNPs (Mohanta et al. 2020).

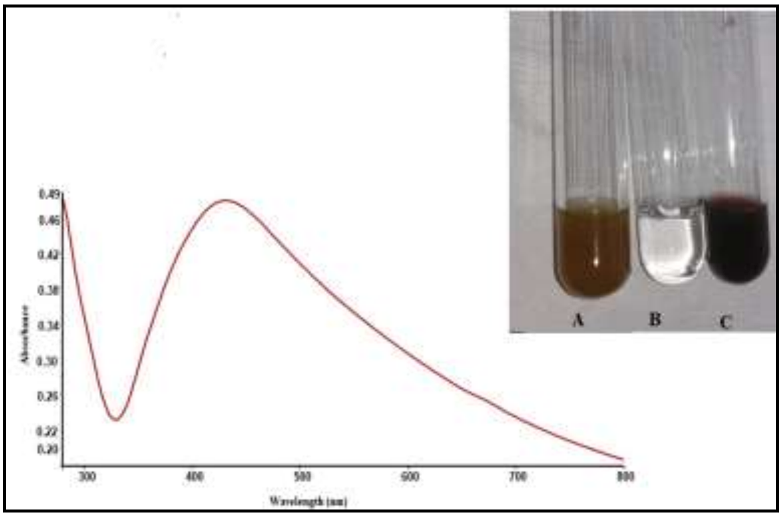

Figure 1. UV-Vis spectra of AgNP using CS extract; Inset figure: A: CS extract, B: $AgNO_3$ solution, CS – AgNPs
Şekil 1. CS ekstraktı kullanılarak AgNP'nin UV-Vis spektrumları; Ek şekil: A: CS ekstraktı, B: $AgNO_3$ çözeltisi, CS – AgNP'ler

Synthesized AgNPs were measured in the wavelength range of 200-800 nm. The peak at 432 nm in the UV-Vis spectrum is due to the plasmon vibration of the metallic Ag particles, which is also the peak range characteristic for AgNPs (Figure 1). Findings showed that the UV-Vis analyzes of the synthesized AgNPs were compatible with the literature (Gan & Li, 2012; Baran, 2019). In the study conducted in 2021, AgNP synthesis was performed using olive leaves and the maximum absorbance value was determined at 433.5 nm (Atalar et al. 2022).

SEM and TEM Analysis of AgNPs

SEM determines size, shape and surface morphology of nanoparticles (Joshi & Viswanathan, 2006). SEM is an important technique used to investigate the surface morphology of nanostructures. It is seen that AgNPs are randomly dispersed on the plant platform and have sizes ranging from 49 to 89 nm (Figure 2). Normally spherical AgNPs have an expanded structure due to the aggregation of two or more Ag nanoparticles during the synthesis in our study. This finding have also similar to the literatures (Tamuly et al. 2013; Baran et al. 2019; Atalar et al. 2022).

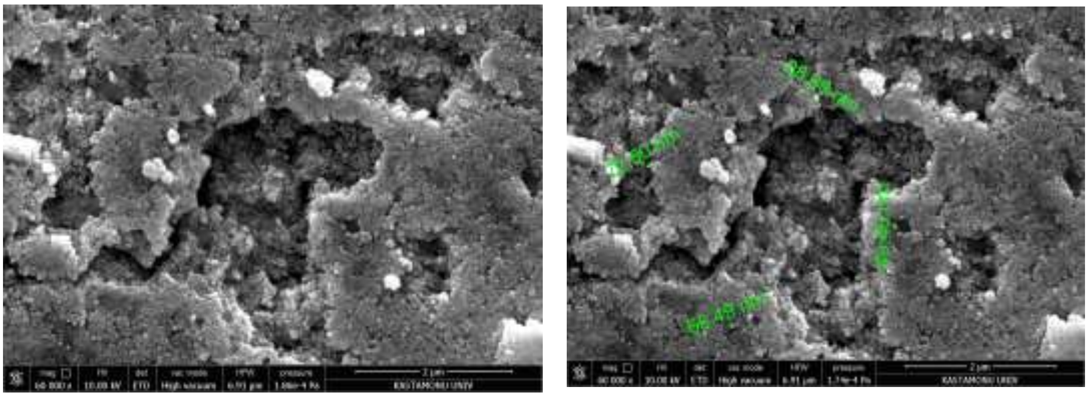

Figure 2. SEM images of synthesized AgNPs
Şekil 2. Sentezlenen AgNP'lerin SEM görüntüleri

TEM images of AgNPs, which is almost spherical in particle size and shape, ranges from 51 to 56 nm (Figure 3). TEM images revealed that the small particle aggregates were covered with a thin organic layer that acts as a sealing organic agent. This can also explain the fact that nanoparticles show very good dispersion in bio reduced aqueous solution, even on a macroscopic scale (Kouvaris et al. 2012).

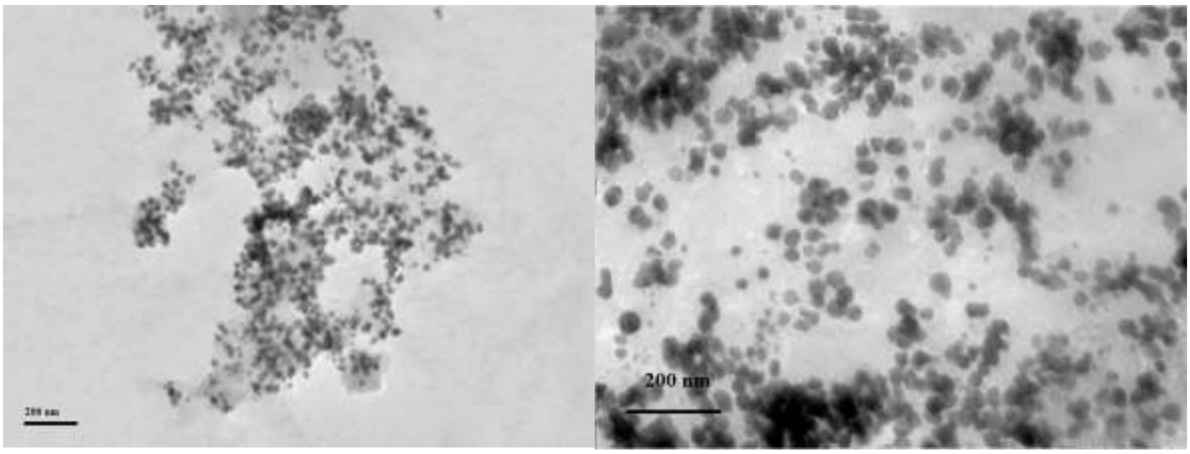

Figure 3. TEM images of synthesized AgNPs
Şekil 3. Sentezlenen AgNP'lerin TEM görüntüleri

EDS Analysis of AgNPs

One of the methods used to determine the elemental composition of solids is EDS analysis (Hodoroaba, 2020) which is also evidence of the formation of AgNPs, as silver ions produce strong signals at about 3.15 KeV (Kumar et al. 2011). AgNPs typically showed an absorption peak at 3 KeV. Enzymes or proteins found in CS extract can be cited as the possible cause of other weak element signals (such as oxygen) (Baran, 2019). The presence of elemental silver by EDS analysis is given in Figure 4. In a similar study, it was said that

AgNPs were in spherical appearance (Pallela et al. 2018). In another study, the peaks of the EDX silver were evaluated (Veisi et al. 2018).

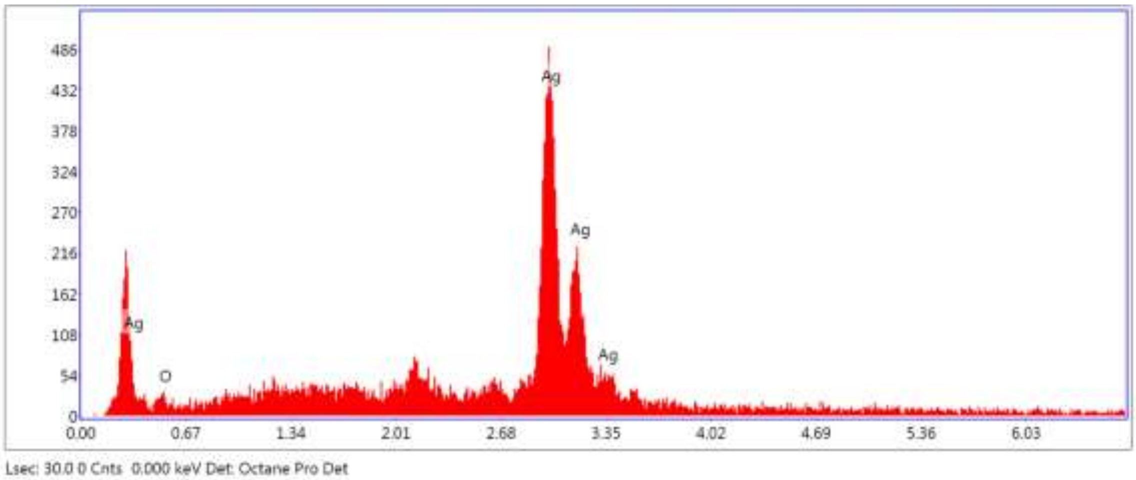

Figure 4. EDS spectra of AgNPs
Şekil 4. AgNP'lerin EDS spektrumları

FTIR Spectroscopy Analysis of AgNPs

FT-IR analysis is accepted as a method used to make sense of the relationship of functional groups between biomolecules and metal nanoparticles (Chandran et al. 2006). Measurements taken with FT-IR analyzes are performed to identify the biomolecules responsible for the coating, effective stabilization and reduction of silver nanoparticles. The peaks at 3272 cm^{-1} and 1615 cm^{-1} in the IR spectrum are the vibrational bands of the OH and C=O groups of flavonoids/phenolic groups, respectively. The band at 1404 cm^{-1} belongs to the OH bending of the polyphenol group and confirms the presence of the aromatic group. The peak at 1026 cm^{-1} belongs to the C-N vibration of the amine groups (Figure 5 and 6).

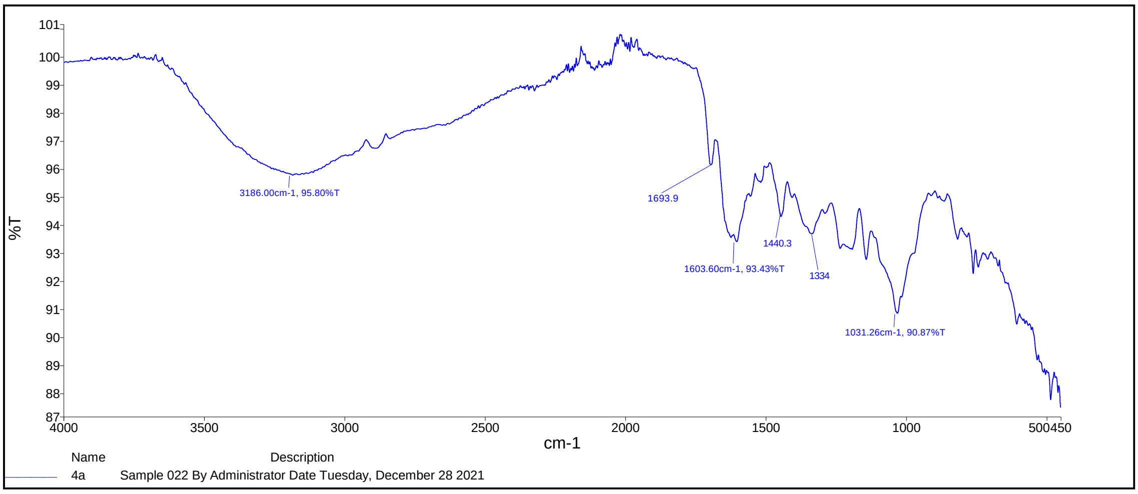

Figure 5. FTIR analysis of CS extract
Şekil 5. CS ekstraktının FTIR analizi

Antibiofilm Activity Results of AgNPs

In order to determine the antibiofilm activities of CS and AgNPs, firstly, the MIC values of both extracts were detected (Table 1). MIC values of CS and AgNPs were found in the range of 0.625- 10 µg/mL against all tested cultures. MIC of AgNPs was higher than both CS extracts and comparison antibiotic against *A. baumanii, P. aeruginosa* and *B. subtilis* test cultures. No significant antagonistic effects of CS and AgNP were detected against *C. albicans* test culture.

The results indicated that CS and AgNPs extracts reduced the metabolic activity of cells in biofilm of all test microorganisms, showing an inhibition percentage range of 15.03- 82.01% and 5.03- 92.03% respectively (Table 1). By comparison, CS and AgNPs extracts were the most effective in inhibiting the formation and growth of *S. haemolyticus* and *P. aeruginosa* biofilm by 82.01% and 92.03%, respectively.

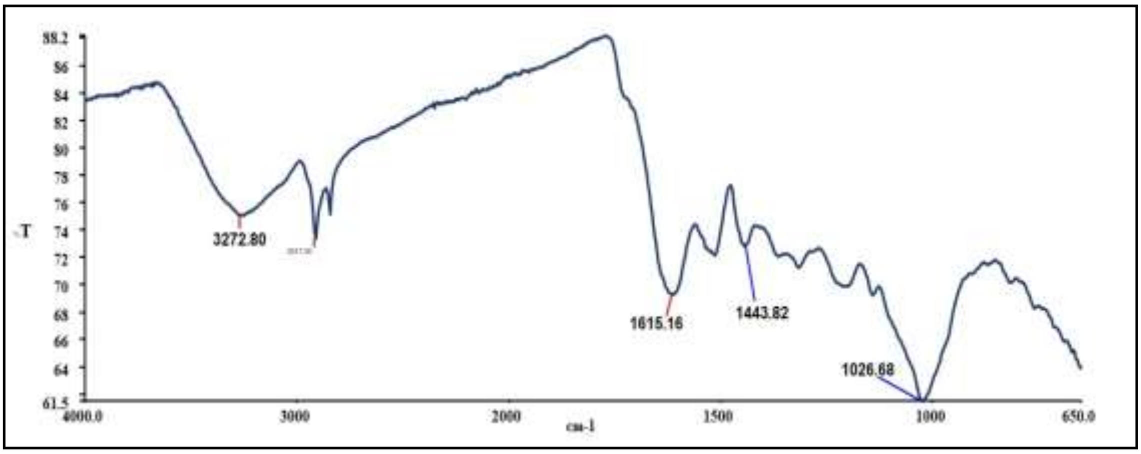

Figure 6. FT-IR analysis of synthesized AgNPs
Şekil 6. Sentezlenen AgNP'lerin FT-IR analizi

Table 1. MIC and antibiofilm activity of CS and AgNPs
Çizelge 1. CS ve AgNP'lerin MIC ve antibiyofilm aktivitesi

Test Cultures	MIC (µg mL^{-1})				Antibiofilm (% inhibition rates)			
	Extracts		**Standard antibiotics**		**Extracts**			
	CS	**AgNP**	**ST**	**NY100**	**CS**		**AgNP**	
					MIC	**MIC/2**	**MIC**	**MIC/2**
A. baumanii	**2.5**	**0.625**	4.0	NT	**74.19±6.41**	43.03±3.13	**82.03±0.22**	55.03±0.13
P. aeruginosa	5.0	**0.625**	1.0	NT	55.21±0.11	50.01±1.11	**92.03±0.21**	56.03±0.13
B. subtilis	10.0	**2.5**	4.0	NT	31.02±0.01	15.03±0.8	**70.09±0.41**	63.03±0.13
S. haemolyticus	**1.25**	10.0	5.0	NT	**82.01±0.15**	65.01±0.3	21.01±0.02	12.01±0.1
C. albicans	10.0	10.0	NT	5.0	22.02±0.01	15.03±0.8	12.02±0.01	5.03±0.8

ST: Streptomycin (10 ug/disc); NYS100: Nystatin (100 ug/disc) NT: Not detected

Although there are similar studies in the examples of black (Göl et al. 2020; Lake et al. 2020) and green tea (Elbossaty, 2017), white tea (palladium synthesis) (Azizi et al. 2017), this study has been shown for the first time that the use of *C. sinensis* (white tea) leaf extract as a reducing agent for the controlled preparation of NPs in size and shape may be a valuable source in the biological synthesis of NPs in an environmentally friendly way. In this study, *C. sinensis* was preferred for NP synthesis due to its rich content of naturally derived polyphenolic compounds and flavonoid group (Salah et al. 1995).

Before investigating any biological activity of AgNPs, it is important to reveal particle size, surface charge, morphology, and particle composition, which provides information on in vitro toxicity of AgNPs (Bhanumathi et al. 2017). Particle sizes <100 nm have greater potential in biomedical applications, as the type of interaction that occurs between nanoparticles and cells is highly dependent on the size of the nanoparticle. This shows that the particle synthesized in terms of size can be used for biomedical purposes.

CONCLUSIONS

It has been demonstrated by this study that white tea leaves can be used as both reducing and stabilizing agents. This procedure offers a cost-effective and "green" alternative to traditional protocols and demonstrates an easily scalable synthesis process for the industry given the low synthesis temperatures and time requirement. In addition, the high antibiofilm activity obtained against some bacteria indicates that AgNPs synthesized by *C. sinensis* may be an important pharmaceutical agent.

It seems to be much more common and intense towards applications of NPs. However, NP will come with further scrutiny and ethics of the biosynthesis exam without the underlying research at hand and the purchase of new forms of synthesis. Evaluating the endemic plant potential of the land, different plants are among those exhibited in the products.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest Statement

The authors of the article declare that there is no conflict of interest between them.

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