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Karabiber özütü kullanılarak sentezlenen biyojenik gümüş nanopartiküller ile grafen yapıların kombine antibakteriyel ve antioksidan etkisi

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ÖZET

Nanoteknoloji, malzemelerin nanometre ölçeğinde, özellikle 1 ila 100 nanometre aralığında tasarım ve manipülasyonuna odaklanan öncü bir araştırma alanıdır. Gümüş nanoparçacıkları, antiseptik özellikleri ile bilinir ve genellikle malzemelerin antibakteriyel etkinliğini artırmak amacı ile diş hekimliği gibi alanlarda kullanılır. Son zamanlarda, grafen bazlı malzemeler de antibakteriyel özellikleri nedeniyle önem kazanmıştır. Bu çalışma ile karabiber ekstraktı kullanılarak mikrodalga yöntemi ile sentezlenmiş gümüş nanoparçacıkları ile grafen yapılarının kombinasyonunun antibakteriyel ve antioksidan aktiviteleri incelenmiştir. Sentezlenen malzemeler, UV-Vis spektroskopisi, taramalı elektron mikroskobu (SEM) ve enerji dağıtıcı X-ışını spektroskopisi (EDS) gibi çeşitli analitik yöntemlerle karakterize edilmiştir. Grafen ve gümüş nanoparçacık kompozitlerinin antibakteriyel etkinliği, *Staphylococcus aureus* (*S. aureus*) üzerine agar well difüzyon yöntemi kullanılarak değerlendirilmiştir. Ayrıca, kompozitlerin serbest radikal süpürme aktiviteleri DPPH, FRAP ve ABTS testleri ile test edilmiştir. Sonuçlar, grafen oksit ve gümüş nanoparçacıklarının kombinasyonunun 14.40 mm ile en büyük inhibisyon alanını oluşturduğunu, antioksidan aktivitenin ise genel olarak gümüş nanoparçacıklar için grafen ve kompozit yapılara kıyasla daha üstün olduğunu göstermiştir. Bu sonuçlar, sentezlenen grafenin, biyosentezlenmiş gümüş nanoparçacıkları ile entegrasyonunun antibakteriyel aktiviteyi geliştirebileceği ve biyomedikal alanlarda kullanılabileceğini göstermektedir.

Combined Antibacterial and Antioxidant Effect of Graphene Structures with Biogenic Silver Nanoparticles Synthesized by Using Black Pepper Extract

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ABSTRACT

Nanotechnology is a pioneering research field that focuses on the design and manipulation of materials at the nanoscale, particularly within the range of 1 to 100 nanometers. Silver nanoparticles are known for their antiseptic properties and are commonly used in fields such as dentistry to enhance the antibacterial efficacy of materials Recently, graphene-based materials have also gained importance due to their antibacterial properties. In this study, antibacterial and antioxidant activities of the combination of silver nanoparticles and graphene structures synthesized by microwave method using black pepper extract were investigated. The synthesized materials were characterized by various analytical methods such as UV-Vis spectroscopy, scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). The antibacterial activity of graphene and silver nanoparticle composites was evaluated using agar well diffusion method on *Staphylococcus aureus* (*S. aureus*). In addition, the free radical scavenging activities of the composites were tested by DPPH, FRAP and ABTS assays. The results showed that the combination of graphene oxide and silver nanoparticles produced the largest inhibition area of 14.40 mm, while the antioxidant activity was generally superior for silver nanoparticles compared to graphene and composite structures. These results suggest that the integration of synthesized graphene with biosynthesized silver nanoparticles can improve antibacterial activity and can be used in biomedical fields.

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INTRODUCTION

Nanotechnology is an important and developing field that is utilized in many scientific disciplines and having an impact on human life. It entails creating and working with materials that range in size from 1 to 100 nanometers and have special properties like a high surface area-to-volume ratio, which contribute to their diverse applications and effects [1]. The applications for nanoparticle-based technologies are numerous and include semiconductors, batteries, pharmaceuticals, and industrial catalysis. Fullerenes, liposomes, metal nanoparticles, nanodroplets, and dendrimers are some types of nanomaterials [2]. The synthesis of metallic nanoparticles is primarily conducted through two main methodologies. The first is the "bottom-up" approach, which involves constructing materials from atomic or molecular components. The second is the "top-down" approach, which focuses on breaking down larger bulk materials into nanoscale particles. Nowadays, silver nanoparticles (AgNPs) are one of the most popular materials because they have important antibacterial properties. In addition to their enhanced antibacterial activity, AgNPs have other beneficial properties that make them useful for a variety of applications [3]. The biological synthesis of AgNPs has become increasingly popular in recent years. The use of microorganisms and plant extracts for this synthesis represents a strong alternative to traditional chemical and physical synthesis methods as they are simpler to use, more economical, more environmentally friendly, and easily scalable for high-yield production [4].

AgNPs can effectively inhibit the growth of a variety of bacteria, making them a useful addition to materials such as dental composites and other restorative materials [5,6]. AgNPs have an antibacterial effect because they release silver ions, which are toxic to bacteria and can damage bacterial cell membranes, impairing bacterial cell functions and causing cell lysis [7]. This property is particularly important in some areas such as prosthetic dentistry, where the risk of infection is increased due to the presence of foreign materials in the oral cavity. Without substantially altering their mechanical qualities, AgNPs can be added to a variety of dental materials, including acrylic and composite resins, to increase their antimicrobial efficacy [8,9]. In addition, AgNPs exhibit antioxidant activities due to their ability to scavenge free radicals. This effect is particularly significant in dental applications, as oxidative stress can accelerate the progression of oral diseases and lead to the degradation of dental materials [10,11]. The antioxidant capacity of AgNPs has been shown to be comparable to that of ascorbic acid, indicating their potential role in protecting dental materials from oxidative damage. Additionally, adding AgNPs to dental materials enhances their antibacterial properties while halting oxidative deterioration, which increases the materials' stability and durability [12,13]. Investigating environmentally friendly methods of synthesizing AgNPs, like plant extracts, further increases their appeal for dental applications. Because they still have antimicrobial and antioxidant qualities but are less damaging to human cells, the green synthesized AgNPs are safe for use in dental procedures [14,15]. Nanomaterials have shown significant potential to control bacterial colonization in prosthetic dentistry by highlighting the antibacterial properties of silver nanoparticles (AgNPs), which can effectively inhibit bacterial growth and biofilm formation on prosthetic materials. This capability is crucial for enhancing the longevity and success of dental implants, as bacterial biofilms are a primary cause of implant failure [16].

The antibacterial and antioxidant properties of GO have made it a significant material in various fields. The unique physical and chemical characteristics of GO primarily contribute to its antibacterial effects [17] . Studies have demonstrated that GO can damage bacteria membranes, resulting in cell lysis and death. The bacterial cells suffer physical harm and membrane stress due to the sharp edges of the graphene sheets, which facilitate this mechanism [18,19]. Furthermore, electron transfer interactions between graphene and microbial membranes, which depend on direct physical contact and membrane perturbation rather than just reactive oxygen species (ROS), strengthen the antibacterial effects [20]. GO is a versatile treatment for dental infections because research shows that it has broad-spectrum antimicrobial activity against a variety of bacterial strains, including both Gram-positive and Gram-

negative bacteria [21,22]. In addition, GO has significant antioxidant properties. GO has been shown to be an effective free radical scavenger, outperforming conventional antioxidants such as ascorbic acid and mannitol under certain conditions [23]. This antioxidant activity is critical in prosthodontics, as oxidative stress can degrade dental materials and compromise the integrity of prosthetic devices. Incorporating GO into dental materials can increase their resistance to oxidative degradation, hence extending their useful lifespan [24]. GO has also been shown to increase the activity of antioxidant enzymes in biological systems, including catalase (CAT) and superoxide dismutase (SOD), which are essential for cellular defense against oxidative stress [25].

In this study, we aimed to investigate the synergistic antibacterial and antioxidant properties of green synthesized AgNPs coupled with graphene materials. To achieve this, we characterized the synthesized nanoparticles using various analytical methods. The antimicrobial efficacy of the nanoparticles and graphene structures, both individually and in combination, was evaluated against *Staphylococcus aureus* through the agar well diffusion method. Additionally, we conducted DPPH, FRAP, and $ABTS^{+}$ analyses to assess their antioxidant capacity.

MATERIALS AND METHODS

Chemicals used in the study

All materials used in this study were rinsed with deionized water. The chemicals employed were sourced from various suppliers, including sulfuric acid (H_2SO_4), iron (III) chloride ($FeCl_3$), and ABTS (2,2'-Azino-Bis (3-Ethylbenzothiazoline-6-Sulfonic Acid) from Merck. Additionally, sodium nitrate ($NaNO_3$), potassium permanganate ($KMnO_4$), Müller Hinton agar, hydrogen peroxide (H_2O_2), silver nitrate ($AgNO_3$), DPPH (2,2-Diphenyl-1-picrylhydrazyl), potassium ferricyanide ($K_3Fe(CN)_6$), trichloroacetic acid (TCA), and ammonium acetate (CH_3COONH_4) were obtained from Sigma-Aldrich. The graphene structures were purchased from MediSen (Türkiye) company.

Graphene oxide synthesis

A modified version of the Hummers method was used to synthesize GO from natural graphite. In brief, 1 g of $NaNO_3$ and 46 mL of H_2SO_4 were mixed with 1 g of natural graphite powder, and the mixture was continuously stirred in an ice bath to keep the temperature below 20 °C. Gradually, 6 g of $KMnO_4$ was added while stirring. After 60 min., the mixture was taken out of the ice bath, and the temperature was raised to 35 °C, where it was maintained for an additional 30 min. After that, 70 mL of water was gently added to the mixture, which was agitated for another 15 min. Then, 80 mL of hot water (at 60 °C) and a 30% H_2O_2 solution were added to reduce any remaining $KMnO_4$ until effervescence ceased. The resulting product was washed multiple times to remove any residual salt impurities. After undergoing a thermal reduction process at 200 °C for 3 h, a black powder of GO was obtained.

Synthesis of silver nanoparticles and characterization

The black pepper was subjected to a washing process that included three rinses with distilled water, followed by removal of impurities and subsequent drying. The dried plant materials were then ground into a fine powder. 10 g of this powder was boiled in 100 mL of distilled water to extract plant material. The resulting extracts were centrifuged at 5000 rpm (at 4 °C for 12 min). The obtained supernatant was collected and then filtered with Whatman filter paper (Grade No. 1). 100 mL of distilled water was used to dissolve 0–017 g of AgNO3 to create a 1 mM AgNO3 solution. The extract was then mixed with 80 milliliters of AgNO3 solution to synthesize AgNPs. After that, the mixture was exposed to microwave radiation for 25 min. in a laboratory-grade microwave with a 700-watt power setting. AgNP colloidal suspensions were sonicated for 10 min. at 55% power after the synthesis process. The

resulting pellets were discarded after the suspensions were centrifuged at 4 °C (5000 rpm) for 15 min. The centrifugation process was carried out again for the supernatants. The residual colloidal nanoparticles underwent additional purification through centrifugation using ultra-pure water at 10.000 rpm and 4°C for 15 min. to eliminate any residual plant extract. This washing step was performed twice. Finally, the purified AgNPs were lyophilized and stored at 4°C in a dark container for future applications. The resulting nanoparticles were then characterized by UV-Vis, SEM, DLS and EDS.

Antibacterial activity test using agar well diffusion assay

The *Staphylococcus aureus* strain (ATCC 6538P) was provided in lyophilized form. To evaluate the antibacterial activity, Mueller Hinton agar was prepared. The turbidity of the bacterial suspension intended for testing was calibrated to match the McFarland 0.5 standard. Following the homogenization of the liquid culture through shaking, approximately 100 µL was dispensed onto a Petri dish to evenly moisten the surface, which was then spread using a Drigalski spatula. To assess the antibacterial activity, wells with a diameter of approximately 7 mm were created on the agar surface, into which composite suspensions with nanoparticles and graphene structures were introduced in a final volume of 100 µL. Gentamicin at a concentration of 10 µg/mL served as the positive control. After a pre-incubation period of 1 h at 25 °C, the bacterial isolate was incubated at 37 °C for 24 h. The zones of inhibition surrounding each sample were subsequently measured. Each experimental trial was conducted in triplicate for both the samples and the bacterial isolate.

Free radical (DPPH) scavenging activity

The evaluation of free radical scavenging activity using DPPH (2,2-diphenyl-1-picryl hydrazyl) was conducted following the protocol established by Blois et al. [26]. A 0.26 mM solution of DPPH was prepared in ethanol. A specified quantity of stock solutions containing GO and AgNPs, ranging from 10 to 200 mg/mL, was measured out. To achieve a total volume of 4 mL in the reaction vessel, ethanol was added after adding 1 mL of the DPPH solution (0.26 mM). After that, the mixture was vortexed, and it was left to incubate for 30 min. Following incubation, a spectrophotometer was used to measure each sample's absorbance at a wavelength of 517 nm.

Ferric reducing power (FRAP) activity

The analysis was conducted utilizing the Oyaizu method [27], with modifications as described by Elmastas et al. [27,28]. A stock solution containing 100 µL of GO and AgNPs was prepared by diluting with phosphate buffer (0.2 M, pH 6.6) to a final volume of 1.25 mL. Subsequently, 1.25 mL of 1% $K_3Fe(CN)_6$ was added to the mixture. This combination was incubated at 50 °C for 20 min. After incubation, 1.25 mL of trichloroacetic acid 10% TCA and 0.25 mL of 0.1% FeCl3 were added to the mixture. The absorbance of the final solution was measured at 700 nm.

ABTS radical scavenging activity

This study was conducted following the methodology established by Re et al. [29]. A 0.1 M phosphate buffer solution (pH 7.4) was prepared. Subsequently, a 2 mM ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) solution was prepared using the phosphate buffer, and this was mixed with a 2.45 mM K2S2O8 (potassium persulfate) solution in a 1:2 ratio. The mixture was allowed to stand in the dark for 6 h. After this period, an appropriate volume of stock solutions was taken and diluted with phosphate buffer to a final volume of 3 mL. Then, 1 mL of the ABTS solution was added, and the mixture was vortexed to ensure thorough mixing. The resulting solution was incubated at room temperature for 1 h, after which the absorbance was measured at 734 nm using a spectrophotometer.

RESULTS AND DISCUSSION

The aqueous extract of black pepper was utilized for the synthesis of AgNPs from silver nitrate ($AgNO_3$). The first signs of AgNPs formation were observed almost immediately after 25 min. constant stirring and microwave irradiation. A rapid change in color from the initially colorless reaction mixture to a brownish yellow was the first sign of this formation. UV-Vis spectroscopy was then used to confirm the presence of AgNPs. This technique is effective because the free electrons within the nanoparticles generate surface plasmon resonance [30]. In line with earlier findings in the literature, the samples showed a peak at a wavelength of about 440 nm, according to the UV-Vis analysis [31]. Figure 1 illustrated the UV-Vis spectrum of the synthesized AgNPs.

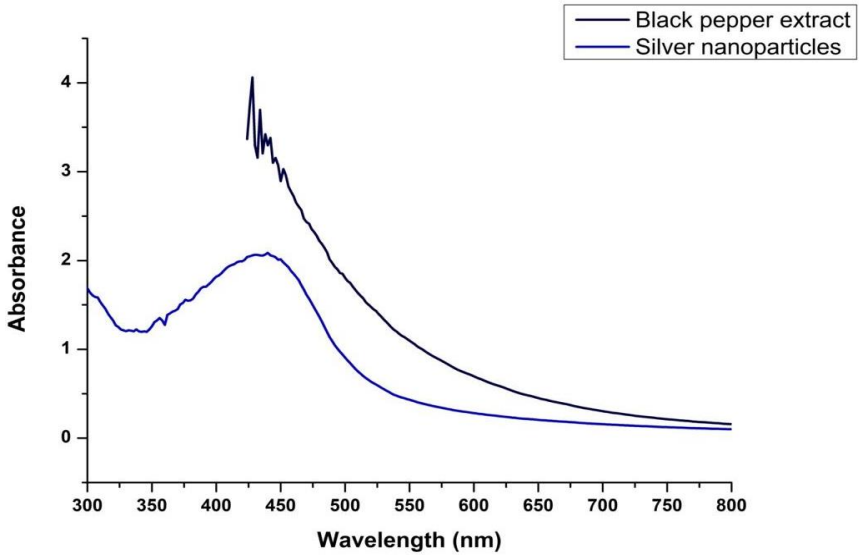

Figure 1
UV-Vis analysis of silver nanoparticles and black pepper extract synthesized by microwave method

Dynamic light scattering (DLS) was used to measure the polydispersity index (PDI), mean particle size, and particle size distribution of AgNPs in the reaction mixtures. The DLS analysis revealed that the average particle size distribution of the nanoparticles in the solution was measured at 60.95 nm, with a polydispersity index of 0.278 (Figure 2).

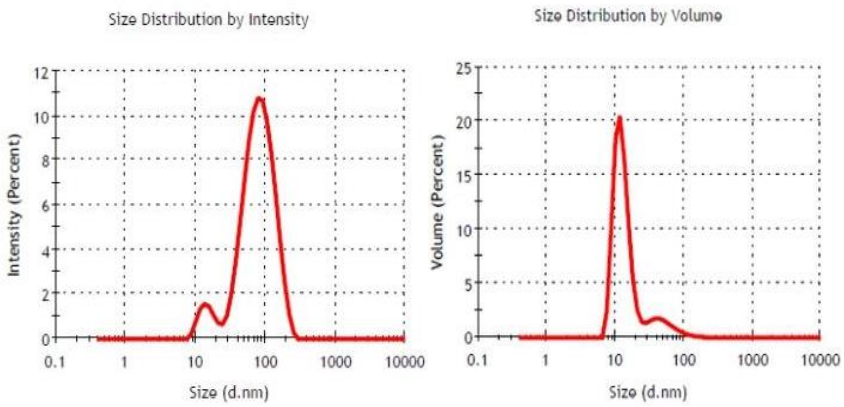

Figure 2
Particle size distribution by density and by volume

Zeta potential is an essential parameter for assessing the stability of nanofluids, which are defined

as liquid mixtures containing water and conductive solid particles with sizes typically below 100 nm [32,33]. Zeta potential is a crucial metric for evaluating the stability of metal nanoparticles in aqueous solutions because it reflects the overall charge present on the nanoparticles. The synthesized AgNPs have a negative charge, as indicated by their zeta potential value of -22.5 mV (Figure 3). This is significant because it implies that the nanoparticles are coated with biomolecules, which increases their stability through electrostatic repulsion. This helps keep the nanoparticles from aggregating and maintains their dispersion in solution [34].

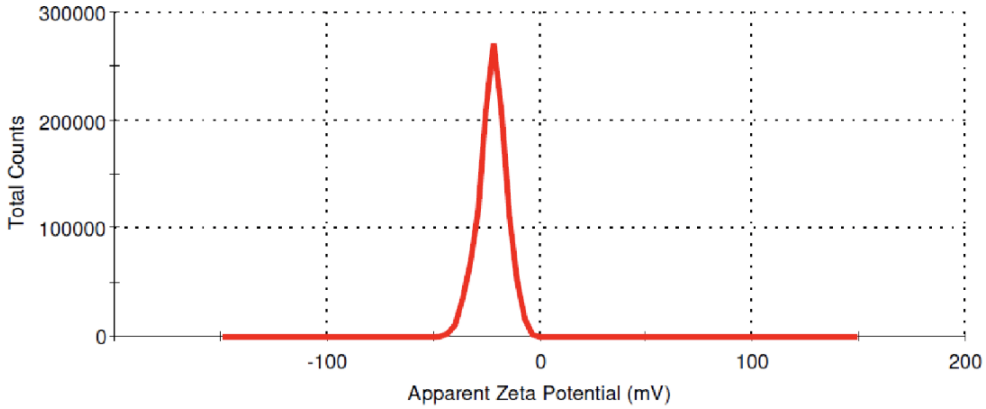

Figure 3
Zeta potential analysis of synthesized nanoparticles.

To investigate the morphology of the synthesized structures of graphene, GO and AgNPs, scanning electron microscopy (SEM) analyzes were carried out (Figure 4 and 6). The SEM examination provided insights into the overall structure of the materials.

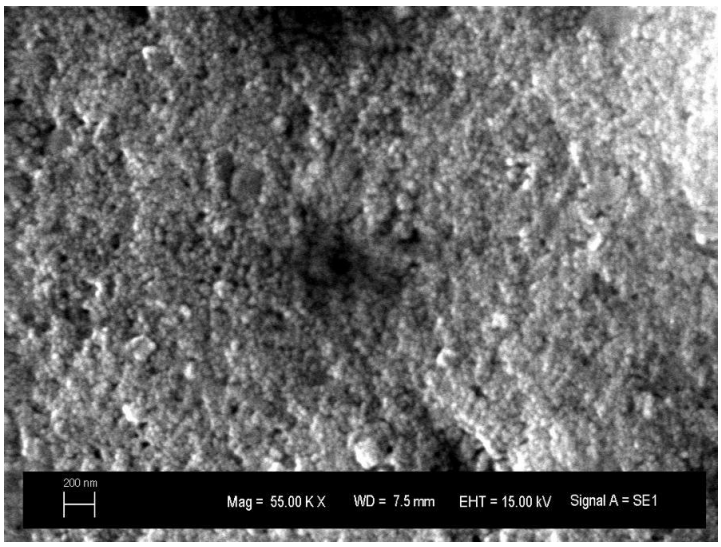

Figure 4
SEM image of silver nanoparticles.

Energy Dispersive Spectroscopy (EDS) analysis provides both qualitative and quantitative insights into the elemental composition of nanoparticles, which is crucial for understanding their formation and stability. The EDS spectrum for the synthesized AgNPs reveals a prominent signal around 3 keV, confirming the presence of silver. This peak is characteristic of metallic silver nanocrystals, which typically exhibit optical absorption peaks due to their surface plasmon resonance (SPR) [35]. Together with other elements, the presence of silver suggests that black pepper extract was used to create

the nanoparticles, where biomolecules might have helped stabilize them.

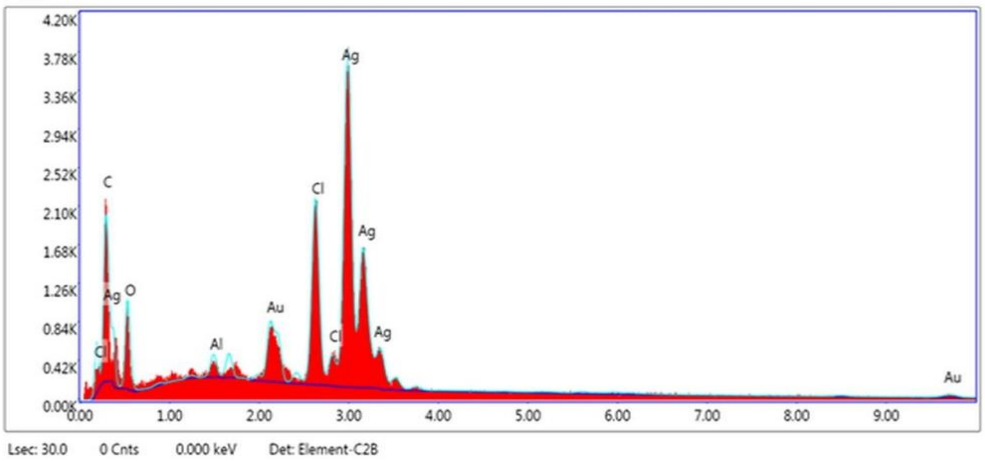

Figure 5

EDS analysis of silver nanoparticles

As shown in Figure 5, silver was the most prevalent element in the EDS analysis, making up roughly 56.2% of the total composition. Cl, O, Al, and Au were among the other elements that were observed with Ag. The coating is responsible for the Au peaks in the spectrum, while plant extract may be the source of other elements. This high silver content is noteworthy because it shows that AgNPs were successfully synthesized.

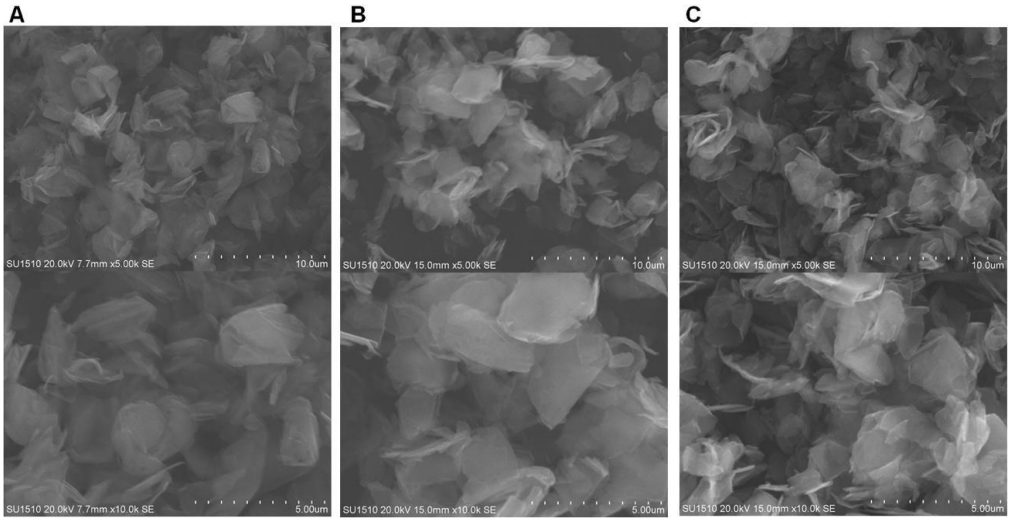

Figure 6

SEM images of A) graphene 1, b) graphene 2 and c) graphene oxide structures

In Figure 6, two distinct structures of graphene and graphene oxide are depicted through scanning electron microscopy (SEM) images. These images illustrate the unique morphological characteristics of each material. The SEM images clearly demonstrated that both graphene oxide and graphene have been effectively exfoliated to form separated thin sheets.

In this study, the antibacterial activities of several structures such as GO, G1, G2, AgNPs, GO+AgNPs, G1+AgNPs and G2+AgNPs against *Staphylococcus aureus* at a concentration of 100 µg/mLwas evaluated. Gentamicin was employed as a positive control in the experiment.

The investigation into the antibacterial properties of GO and AgNPs reveals significant findings

regarding their individual and combined effects. The inhibition zone measured for GO was recorded at 9.90 ± 0.85 mm, while AgNPs exhibited a larger inhibition zone of 12.37 ± 0.75 mm. Notably, when GO and AgNPs were combined in equal proportions and maintained at the same concentration, the resulting inhibition zone increased to 14.40 ± 1.08 mm. This increase suggests a synergistic effect between GO and AgNPs, which is not typically observed with other graphene-based materials (Figure 7 and 8).

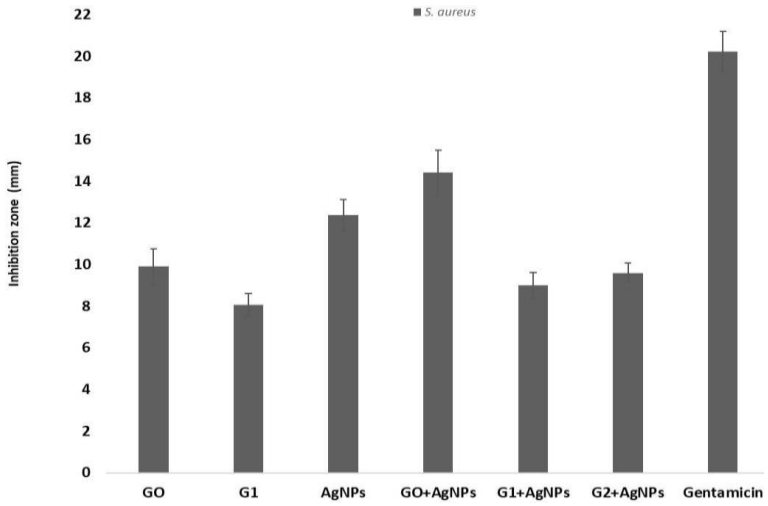

Figure 7
Inhibition zones of the materials used against S. aureus

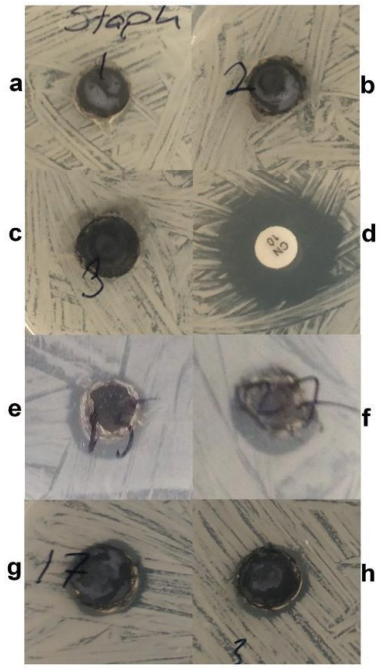

Figure 8
Antibacterial activity of synthesized silver nanoparticles and graphene structures a) GO b)G1 c)G2 d)Gentamicin e)AgNP f)GO+AgNP g) G1+AgNP h) G2+AgNP

The combination of graphite oxide and AgNPs demonstrated strong antibacterial activity, according to the results. Additionally, it was discovered that structures based on graphene have unique antibacterial properties. In recent times, there has been a concerning rise in the resistance of pathogenic bacteria and fungi to commercially available antimicrobial agents, posing a significant global threat. Drug resistance represents one of the most critical and prevalent challenges [36]. The treatment of bacterial infections is becoming increasingly complicated, as pathogens are capable of developing resistance to current antimicrobial agents and antibiotics. Furthermore, resistant pathogens have the potential to disseminate within healthcare settings and communities, leading to broader issues related to infection control [37]. To address this issue, innovative methods and strategies are essential. Promising approaches include the utilization of natural antimicrobials, combination therapies, synergistic treatments, and more recently, the application of metal nanoparticles in composite structures [38]. Numerous studies have confirmed that silver-graphene-based nanocomposites have antimicrobial properties. For example, graphene oxide-silver (GO-Ag) nanocomposites have shown antibacterial qualities against a number of pathogens [39] . Graphene oxide-silver nanoparticles (GO-AgNPs) have been shown to exhibit antibacterial activity due to a synergistic effect rather than just the additive effects of bare GO and AgNPs [40].

Antioxidant substances can neutralize radicals present in the environment. Among them, DPPH radical (2,2-diphenyl-1-picrylhydrazyl) is widely used due to its easy measurement, stability, simplicity and reproducibility in assessing radical scavenging activity by observing discoloration [41]. The DPPH radical provides a tool to measure the antioxidant efficacy of different compounds by assessing their potential to act as hydrogen donors or free radical scavengers. In this study, to determine the radical scavenging activity of the materials, we used the DPPH radical, which has a maximum absorption at 517 nm.

Table 1

Antioxidant activity (DPPH free radical scavenging activity)

	IC50 (µg/mL)
Trolox	5.77±0.11
BHA	4.89±0.15
BHT	7.65±0.16
GO	ND
G1	ND
G2	ND
AgNP	83.51±1.37
GO+ AgNP	161.,33±1.99
G1+ AgNP	183.23±1.23
G2+ AgNP	350.84±3.89

A higher level of antioxidant activity was indicated by a lower IC50 value. AgNPs produced by biosynthesis notably exhibited the highest scavenging activity (83.51 µg/mL) (Table 1). Compared to positive controls, this value was much lower. According to the DPPH scavenging analysis, AgNPs showed significant inhibitory activity compared to common antioxidants such as Trolox, BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene), although this activity decreased, especially when graphene-based materials were present. Additionally, graphene structures were found to have no inherent radical scavenging activity.

As indicated in Table 2, the antioxidant effect of graphene materials, measured by their Ferric Reducing Antioxidant Power (FRAP) reduction capacity, was significantly lower compared to the standard antioxidants BHT and BHA.

Table 2
Ferric reducing power (FRAP) activity

	µmol TE/mg sample
BHA	5.36±0.16
BHT	3.96±0.12
GO	0.26±0.03
G1	0.57±0.06
G2	0.62±0.08
AgNP	3.46±0.18
GO+AgNP	2.26±0,21
G1+AgNP	2.38±0.15
G2+AgNP	2.25±0.08

Table 3
ABTS+ Radical Removal Activity

	IC50 (µg/mL)
Trolox	6.54±0.15
BHA	5,87±0.18
BHT	6.12±0.23
GO	139.82±1.23
G1	162.09±2.45
G2	184.74±2.57
AgNP	5.74±0.14
GO+AgNP	23.64±0.15
G1+AgNP	16.12±0.12
G2+AgNP	18.64±0.13

The ABTS assay is a commonly used technique to assess the antioxidant properties of natural substances, particularly by measuring their ability to eliminate the stable radical action ABTS [42]. AgNPs were observed to have a notably high level of activity (Table 3). However, this activity was noticeably reduced when graphene structures were coupled with AgNPs.

Several studies have demonstrated that AgNPs contribute the reduction of ferric ions and stabilizes free radicals, so they increase the overall antioxidant capacity of composite materials [43,44], as correspondence to our findings. Moreover, it was also reported that graphene and AgNPs show a synergistic effect, where graphene matrix aids in the dispersion and stabilization of AgNPs, leading to increased catalytic and antioxidant activities [44,45]. In addition to their antioxidant capabilities, the antibacterial properties of GO-AgNPs further complement their potential applications in medical and environmental fields. The antibacterial action is primarily due to the oxidative stress induced by silver nanoparticles, which can damage bacterial cell walls, while graphene oxide contributes to this effect through its ability to generate reactive oxygen species [46,47]. This dual functionality of GO-AgNPs not only enhances their utility as antioxidant agents but also positions them as effective antimicrobial agents, making them suitable for various biomedical applications, including wound dressings and drug delivery systems [47,48].

CONCLUSION

In this study, composite structures obtained by combining AgNPs biologically synthesized from black pepper extract and GO structures have great potential, especially in terms of antibacterial properties. The study showed that the synergistic combination of AgNPs and GO significantly increased

antibacterial activity against *Staphylococcus aureus*. In this study, the antioxidant capacity of biologically synthesized AgNPs was found to be higher than that of GO structures. However, the combination of AgNPs with GO structures had a negative impact on the antioxidant capacity and a decrease in this capacity was observed. While the combination of AgNPs and GO nanocomposites increases antibacterial effectiveness, reducing antioxidant properties may affect long-term material performance. It is believed that such nanocomposites can contribute to the development of more biocompatible, durable and infection-resistant materials such as dental materials. Combining GO nanocomposites with AgNPs may make it possible to create dental materials that are both mechanically robust and biologically compatible.

Ethical Statement

This study was derived from a part of Master's thesis “Synthesis of grafphene oxide-silver nanoparticle composite structures, investigation of antibacterial and antioxidant properties”, presented by Selim Işıldak under the supervision of Prof. Dr. Mahfuz Elmastaş.

Author Contributions

Research Design (CRediT 1) S.I. (%50) - M.E. (%50)
Data Collection (CRediT 2) S.I. (%50) - M.E. (%30) - B.S.E. (%10) - M.E. (%10)
Research - Data Analysis - Validation (CRediT 3-4-6-11) S.I. (%50) - M.E. (%30) - B.S.E. (%10) - M.E. (%10)
Writing the Article (CRediT 12-13) S.I. (%60) - M.E. (%20) - B.S.E. (%10) - M.E. (%10)
Revision and Improvement of the Text (CRediT 14) S.I. (%40) - M.E. (%40) - B.S.E. (%10) - M.E. (%10)

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Conflict of Interest

The authors declare no conflict of interest.

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