RESEARCH PAPER

Structural and antibacterial properties of Ag/GO wound dressing

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ABSTRACT

Objective(s): Bioactive wound dressings are essential for preventing infection and accelerating tissue regeneration. Green synthesis offers a sustainable route for producing functional nanomaterials with reduced environmental impact.
Materials and Methods: Silver nanoparticles (Ag NPs) were synthesized using aqueous extract of *Mentha pulegium* as a natural reducing and stabilizing agent. The Ag NPs were combined with graphene oxide (GO) to form Ag/GO composite nanoparticles (CNPs), which were coated onto medical gauze via a simple, cost-effective, and scalable immersion method. The products were characterized by X-ray diffraction (XRD) and elemental mapping, and their antibacterial activities were evaluated against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*.
Results: The synthesized Ag NPs had an average diameter of ~108 nm. XRD patterns revealed the (111) plane of face-centered cubic Ag and the (002) plane of GO. Elemental analysis confirmed uniform Ag, C, and O distribution on the gauze surface. The Ag/GO-coated gauze achieved > 99% reduction in bacterial colony counts compared to untreated controls.
Conclusion: Ag/GO-coated gauze demonstrates excellent antibacterial performance and strong potential as a bioactive wound dressing. This environmentally friendly, scalable approach could aid future developments in infection control and wound management.

Keywords: *Ag nanoparticles; Wound dressing; Antibacterial activity; Green synthesize; Ag/GO composite*.

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INTRODUCTION

Nanotechnology has revolutionized scientific, industrial, and medical fields with its micro-dimensions and wide range of applications. This technology enables manipulating materials at the nanometer scale, infusing them with new properties [1]. Nanotechnology allows the production of materials with unique characteristics, such as lightness, strength, and high electrical conductivity [1, 2]. In industry, nanomaterials enhance product performance and reduce energy consumption [1]. Nanotechnology facilitates drug-targeted and efficient delivery in medicine and provides more precise imaging [2]. Nanotechnology has improved various technologies, from wearable devices and mobile phones to smart cars and advanced medical equipment, and it continues to do so [3]. However, new viruses and microbes emerge daily, with increasing resistance [4], posing a significant challenge to human health. For example, Escherichia coli (E. coli), a Gram-negative bacterium, thrives in aquatic environments. This bacterium is commonly found in salt, fresh water, soil, and food. Particular species of E. coli can be pathogenic, causing serious infections, including respiratory and gastrointestinal tract infections [5]. The incidence of complications caused by bacteria varies depending on factors such as health conditions, water and food contamination levels, and national health policies. In developed countries with robust healthcare systems, mortality rates from this bacterium are lower; however, in developing countries, the issue may be more severe [6]. ecent scientific advances have brought the concept of self-cleaning fabrics with antibacterial properties to the forefront as a promising solution for improving public health. These fabrics, which incorporate nanotechnologies and antibacterial substances into their structure, can eliminate bacteria [7]. Such properties make self-cleaning

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fabrics highly effective in combating infections and diseases, particularly in environments where public hygiene is critical, such as hospitals, healthcare centers, and everyday clothing. Biological, chemical, and herbal methods can produce materials with antibacterial properties [8]. Biological methods involve extracting antibacterial substances from algae biomass [9]. While biological methods offer environmental benefits by reducing pollution and maintaining ecological balance, they may be less efficient for specific fabrics and require longer washing times [10]. Chemical methods, on the other hand, use antibacterial agents to eliminate bacteria and viruses but can pose environmental and health risks [11]. Herbal methods, however, often rely on plant-based materials, medicinal plants, and other natural substances, which reduce the risks associated with chemical agents. Additionally, some plant materials can enhance the fabric recycling due to their sustainable and recyclable properties. Therefore, using plant-based methods to produce self-cleaning fabrics supports environmental sustainability and offers safer and more effective solutions for hygiene [12, 13].

Researchers have reported that various fields, including using plant leaf extracts to synthesize metal nanoparticles and their potential applications, advance scientific understanding [14]. Historically, medicinal plants have been used as traditional medicines or pesticides to treat various disorders. Extraction methods vary in simplicity, cost, efficiency, and the degree of damage they cause to the extracted or isolated molecules. Moreover, to optimize the efficiency of each extract, specific extraction methods are required [15]. Wild oregano, a medicinal plant known for its significant antibacterial and antimicrobial properties, belongs to the Lamiaceae flowering family and is considered one of the most important medicinal plants [16]. Due to the antibacterial activity of its active compounds, wild oregano enhances the body's immune system and combats bacterial infections [17]. Silver, a material with strong antibacterial properties, is widely used across industries such as medicine, healthcare, and food production [18]. In the field of nanotechnology, researchers have conducted numerous studies to achieve antibacterial properties through the use of plant extracts and the green synthesis of metal nanoparticles. For example, in their study [5], Alamdari et al. utilized zinc oxide nanoparticles (ZnO NPs) and *Sambucus ebulus* leaf extract as natural surfactants to synthesize materials in an environmentally friendly manner. They prepared ZnO nanoparticles using *S. ebulus* leaf extract and investigated their physical and chemical properties. X-ray diffraction (XRD) analysis confirmed that the prepared ZnO nanoparticles exhibited high crystallinity and a wurtzite crystal structure, with an average crystal size of approximately 17 nm. The green-synthesized nanoparticles showed excellent absorption in the ultraviolet region and emitted strong yellow-orange fluorescence at room temperature. These active nanoparticles demonstrated significant antibacterial activity against various bacterial strains and were also effective at degrading colored pollutants, such as methylene blue, when exposed to light. In another study [19], Alamdari et al. employed a casting method to fabricate a biodegradable hybrid film by combining green-synthesized ZnO nanoparticles with a chitosan (CS) matrix. Researchers have produced ZnO nanoparticles using *Mentha pulegium* wild plant extract and investigated the prepared samples' structural, morphological, mechanical, disinfection, optical properties, and hydrophilicity. Gas chromatography-mass spectrometry (GC-MS) analysis revealed the presence of phenolic compounds in *M. pulegium* extract. Furthermore, a strong coordinated binding between Zn^{2+} and the chitosan matrix was confirmed, leading to a well-distributed dispersion of ZnO within the chitosan film. The surface of the composite films was transparent, smooth, and uniform, and the bio-based hybrid films exhibited significant antiseptic and antioxidant properties. At 23 °C, the ZnO/chitosan (ZnO/CS) films extended the shelf life of fruits by up to eight days. Valinejad et al. [20] prepared a gel from *Ferula gumosa*, chitosan, and essential oil, studying its physical, chemical, and biological properties. The results demonstrated that this nanocomposite possessed favorable characteristics, including lower crystallinity than chitosan and stronger antibacterial effects than pure chitosan. In studies [21-25], hydrothermal techniques successfully produced a nanocomposite of activated carbon, silver, and titanium dioxide using jasmine flower extract. The biomolecules in jasmine flower extract underwent reduction and stabilization reactions. The results revealed that the activated carbon/silver/titanium dioxide nanocomposite exhibited a noctis structure and significant optical activity as a catalyst. Additionally, the nanocomposite demonstrated a 96% dye degradation efficiency after 120 minutes of visible light exposure. A study on silicon oxide/dioxide (ZnO/SiO_2) nanocomposite aimed to produce antimicrobial cotton [22]. Two distinct methods were used to synthesize zinc nanoparticles in situ on cotton fabric. The first technique involved synthesizing zinc oxide nanoparticles and applying them to the fabric. The second method produced zinc oxide nanoparticles directly on cotton fabric coated with silicon dioxide. The ZnO/SiO_2 nanocomposite-coated cotton exhibited excellent antibacterial efficacy against *Staphylococcus aureus* and *Escherichia coli* bacteria. A 2022 study [23] fabricated activated carbon spheres alloyed with silver nanoparticles by carbonizing and activating silver-exchanged resins. Silver-exchanged resins were obtained by exchanging hydrogen ions in polystyrene sulfonate resin with silver ions derived from silver nitrate. This research aimed to develop a bioactive wound dressing gauze with improved antibacterial properties using green-synthesized silver/graphene oxide composite nanoparticles (Ag/GO CNPs). The study emphasizes sustainability by utilizing *Mentha pulegium* extract as a natural reductant and stabilizer, providing an environmentally friendly and cost-effective synthesis approach. This work is significant as it addresses the growing issue of bacterial resistance, particularly against pathogens such as *E. coli* and *S. aureus*. Conventional antibacterial agents often present environmental and health risks, whereas natural plant extracts offer a safer, more sustainable alternative. Incorporating graphene oxide and silver nanoparticles into wound dressings enhances antibacterial efficacy while utilizing graphene oxide's unique mechanical and structural properties for robust and successful medical applications.

MATERIALS AND METHODS

Green Synthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) were synthesized using *Mentha pulegium* extract as the reducing agent. A 0.02 M silver nitrate ($AgNO_3$, 99.99%) solution was prepared in 50 mL of deionized water. Gradual addition of 3 mL of the prepared extract at 50°C resulted in a color change from white to brown to black, indicating the formation of AgNPs. The solution was then centrifuged at 4000 rpm for 10 minutes, and the collected sediment was dried at 80°C for 24 hours using an electric heater to obtain a powdered AgNP sample.

Synthesis of Ag/GO composite

Graphene oxide (GO) was synthesized using the Hummers method. To prepare the Ag/GO composite, 1.5 g of biosynthesized AgNPs and 0.5 g of GO were dispersed in 100 mL of deionized water. The mixture was magnetically stirred for 2 hours to ensure proper blending, forming the Ag/GO composite.

Preparation of antibacterial coated fabrics

Gauze textiles were immersed in an ultrasonic bath containing the Ag/GO composite solution under UV light for 30 minutes. The treated gauze was then air-dried at room temperature for 24 hours. The fabrics were subsequently ultrasonically stirred for 1 hour to ensure uniform coating. After being washed three times with deionized water, the samples were autoclaved at 110°C for 1 hour, then dried in an oven at 50°C for 24 hours (see Fig. 1).

Characterization techniques

X-ray Diffraction (XRD): XRD patterns were recorded using a PANalytical PW3050/60 diffractometer with Cu-Kα radiation (λ = 0.15418 nm).

Field Emission Scanning Electron Microscopy (FESEM): Morphological analysis and topography were assessed using a MIRA3 TESCAN microscope.

UV-Vis and FTIR Spectroscopy: Optical properties and functional groups were analyzed using a UV-Vis spectrometer (Avaspec-2048-TEC) and a Fourier Transform Infrared (FTIR) spectrometer (Perkin Elmer FTIR).

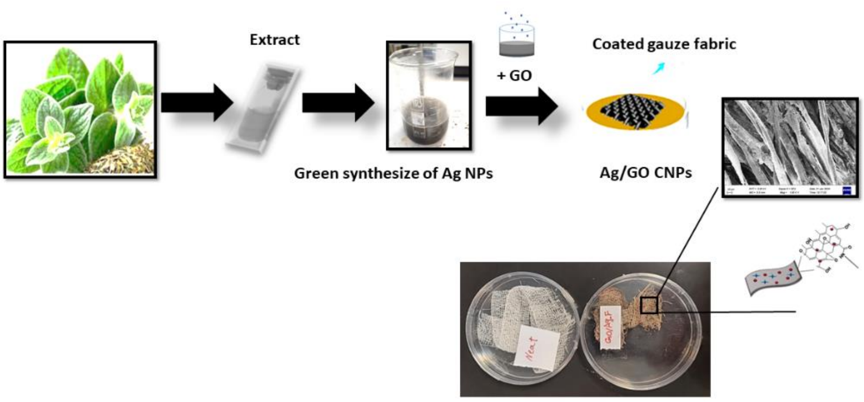


Fig. 1. Brief schematic of experimental works for preparing Ag/GO NPs using wild *Mentha pulegium* extract

Antibacterial activity evaluation

The agar diffusion method evaluated the antibacterial activity of Ag/GO-coated gauze fabrics against Staphylococcus aureus (ATCC 6538) and Escherichia coli (ATCC 25922). The viable cell count was determined at 1-, 3-, and 6-hour intervals using the direct contact method and spread plate count technique. Trypticase Soy Agar (TSA) was used as the culture medium, with incubation maintained at 30 ± 2°C for 24–48 hours. Colony-forming units (CFU) were quantified to calculate bacterial reductions. Untreated fabrics served as the control group.

RESULTS AND DISCUSSION

XRD investigation of the Ag/GO composite and treated Gauze Fabrics

XRD patterns of the Ag, GO, and Ag/GO-coated fabric are shown in Fig. 2(a-c). A prominent peak a 9-10°C indicates the presence of GO (Fig. 2(b)). Fig. 2(c) displays the X-ray diffraction (XRD) pattern of the Ag/GO-coated fabric. A peak detected at 38°C corresponds to the typical diffraction peaks of AgNPs (JCPDS file No. 04-0783). The diffraction peak observed at an angle of 2θ = 9°C corresponds to the (002) plane of graphene oxide [24]. The presence of Ag NPs and GO on the surface of the coated fabric was confirmed. The XRD patterns of the treated fabrics showed firm diffraction peaks at 2θ = ~37°C and 24°C , which correspond to the (111) crystal planes of face-centered cubic Ag NPs and the (002) plane of GO, respectively [25-30].

Fig. 3(a-e) shows the FESEM images of the synthesized Ag/GO composite nanoparticles and Ag/GO-coated fabric. Spherical silver nanoparticles, with an average diameter of 45 nm, and graphene oxide sheets comprise the composite structure. The composite nanoparticles are well-dispersed and arranged within the gauze fibers. Additionally, the EDX spectrum and elemental mapping in Fig. 4(a, b) confirm the presence of Ag nanoparticles and C and O elements on the fabric surfaces. These results indicate that Ag/GO nanoparticles were successfully distributed across all examined fabric surfaces.

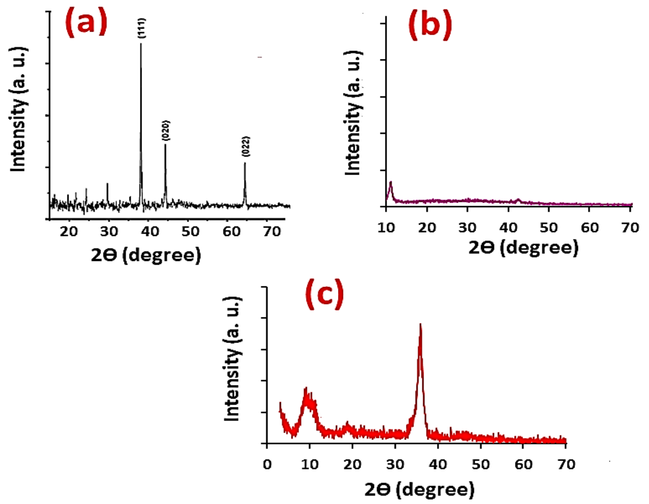
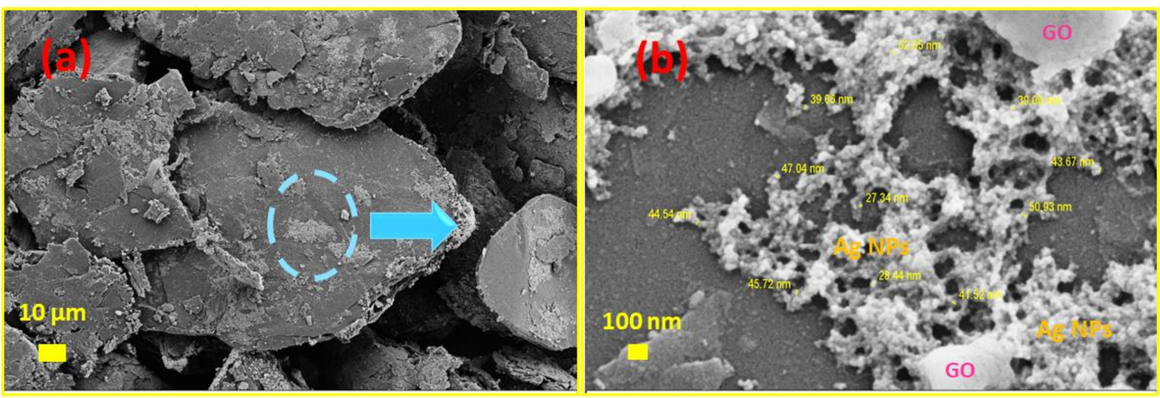


Fig.2. XRD pattern of the (a)Ag NPs, (b) GO, and (c) Ag/GO coated fabric



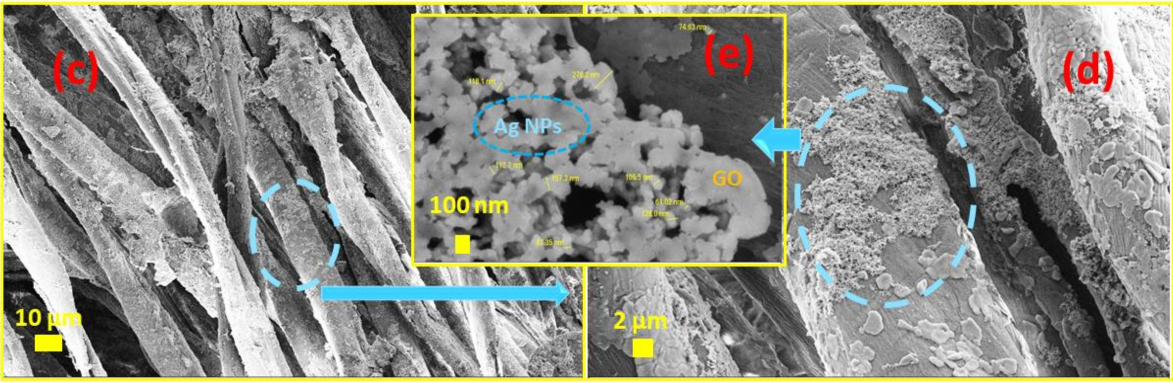
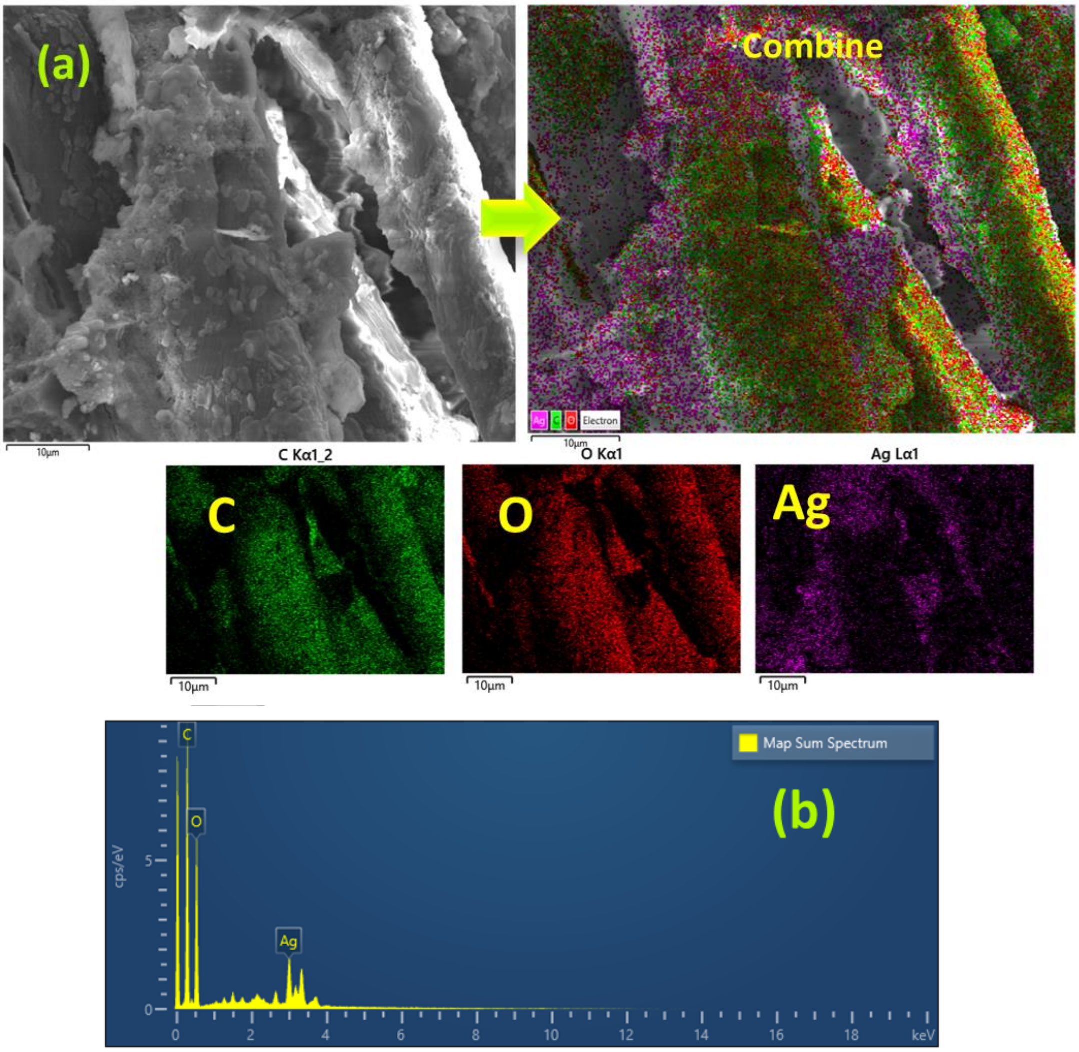


Fig.3. FESEM images of the (a, b) Ag/GO composite powders, (c, d &e) Ag/GO coated fabric



Element	Line Type	Atomic %
C	K series	54.90
O	K series	43.44
Ag	L series	1.66
Total		100.00

Fig.4. (a) Elemental mapping and (b) EDS spectrum with element concentration of Ag/GO-coated fabric

Elemental Analysis (EDS) of the Treated Gauze Fabrics

Fig. 4(a, b) shows the elemental mapping and EDX spectrum of the treated gauze fabrics. Ag, C, and O positions were identified in the coated fabric. The elemental composition obtained from the EDS analysis is summarized in Fig. 4(b). The synthesized composite is well-distributed across the fabric fibers.

Antibacterial activity of the untreated and treated Gauze Fabrics

The simple colony counting method assessed the antibacterial activity of untreated and treated gauze fabrics (Fig. 5, Table 1). A comparison of the number of colony-forming units (CFU) in the treatment group versus the control group is shown in Table 1. The sensitivity of the microorganism is indicated by the number of remaining colonies (percentage of colonies). The treated gauze fabric exhibited a decrease in viable counts of 70% for *S. aureus* and over 99% for *E. coli* after 6 hours. In contrast, untreated fabrics showed minimal antibacterial activity, with reductions of approximately 20% and 5% for *S. aureus* and *E. coli*, respectively.

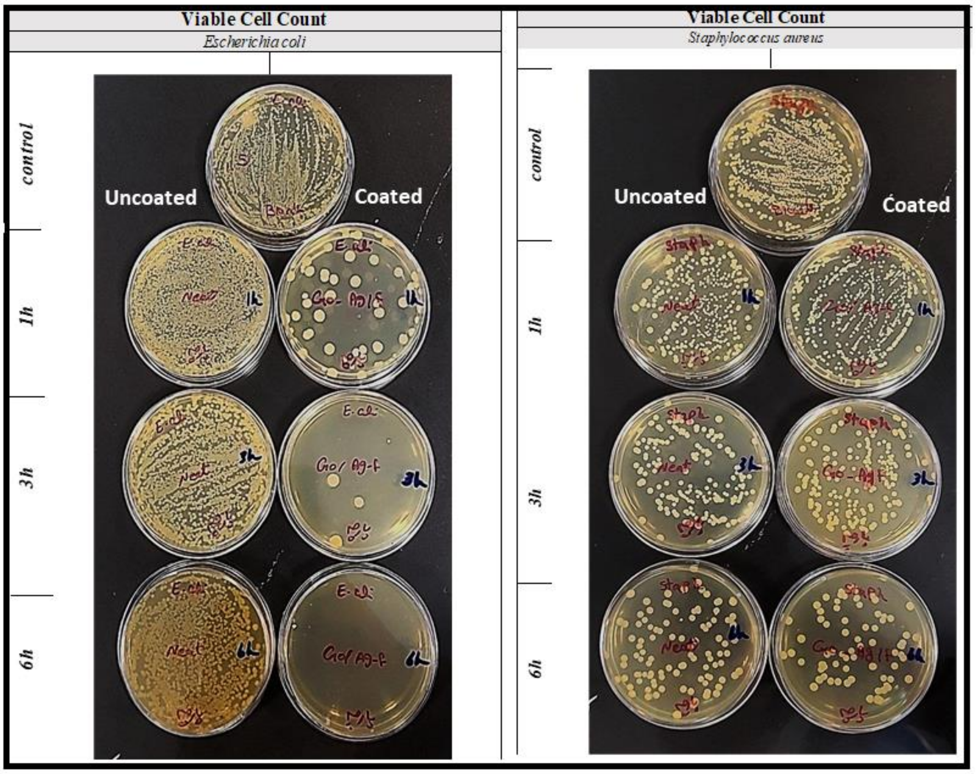


Fig.5. Antibacterial activity of the samples by the simple colony counting method.

Table 1. Reduction in colony-forming units (%) for the prepared CS and ZnO/Ag-CS films

	Strain: *S. aureus* ATCC 25923						**Gram-Positive Bacteria**		
Sample	**1h**			**3h**			**6h**		
	VC[1] (CFU/ml)	RP[2] (%)	LR[3] (Log₁₀)	VC[1] (CFU/ml)	RP[2] (%)	LR[3] (Log₁₀)	VC[1] (CFU/ml)	RP[2] (%)	LR[3] (Log₁₀)
Uncoated	9×10^5	10%	0.046	8×10^5	20%	0.097	$<8 \times 10^5$	<20%	0.097
Ag/GO Coated dressing gauze	9.5×10^5	5%	0.022	7×10^5	30%	0.155	3×10^5	70%	0.523

1- Viable Count, 2- Reduction Percentage, 3- Logarithmic Reduction

	Strain: *E. coli* ATCC 25922						**Gram-Negative Bacteria**		
Sample	**1h**			**3h**			**6h**		
	VC[1] (CFU/ml)	RP[2] (%)	LR[3] (Log₁₀)	VC[1] (CFU/ml)	RP[2] (%)	LR[3] (Log₁₀)	VC[1] (CFU/ml)	RP[2] (%)	LR[3] (Log₁₀)
Uncoated	$>9.5 \times 10^5$	<5%	<0.022	$>9.5 \times 10^5$	<5%	<0.022	$>9.5 \times 10^5$	<5%	<0.022
Ag/GO Coated dressing gauze	1×10^5	90%	1.000	$<1 \times 10^2$	>99.9%	>4.000	$<1 \times 10^2$	>99.9%	>4.000

Viable Count, 2- Reduction Percentage, 3- Logarithmic Reduction

Our study investigated the green synthesis of silver nanoparticles (Ag NPs) using Mentha pulegium leaf extract to determine its potential in bioactive wound dressings. The Ag NPs were incorporated into graphene oxide (GO) composites to create antibacterial gauze fabrics.

J. Liu et al. prepared silver nanoparticles/graphene oxide-decorated silk fabric, resulting in significant antibacterial activity with reductions of over 95% in bacterial colonies of *S. aureus* and *E. coli* [29]. J. N. Tiwari reviewed various applications of Ag/GO nanocomposites, including their use in textiles for antibacterial purposes. The Ag/GO textiles exhibited excellent antibacterial properties, with reductions in bacterial colonies often exceeding 90% [30].

Overall, using *Mentha pulegium* extract for the green synthesis of Ag NPs offers notable environmental sustainability and functionality benefits. These advantages are consistent with the results of previous research exploring the use of other plant extracts for nanoparticle synthesis. Such comparisons highlight the potential of environmentally friendly synthesis technologies in producing efficient and safe biomedical applications.

Therefore, advancements in nanostructured materials have paved the way for new developments in electrochemical energy devices and medical applications [31-35].

CONCLUSION

The current study demonstrates the successful green synthesis of silver nanoparticles (AgNPs) using *Mentha pulegium* extract, which were integrated into gauze fabrics and graphene oxide (GO) to form Ag/GO composite nanoparticles. The synthesis process was efficient, cost-effective, rapid, and scalable, yielding nanoparticles with an average size of 108 nm. XRD and FESEM analyses confirmed the presence and uniform distribution of Ag NPs and GO on the gauze fabrics. The antibacterial efficacy of the Ag/GO-treated gauze was significant, showing over 99% reduction in bacterial colonies of both *Staphylococcus aureus* and *Escherichia coli* compared to untreated fabrics. This sustainable approach offers notable benefits for biomedical applications, aligning with recent advancements in nanotechnology and antimicrobial textiles. This study presents a sustainable and effective solution for biomedical applications, reinforcing the growing potential of nanotechnology in healthcare.

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CONFLICTS OF INTEREST

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

ETHICAL APPROVAL

Not applicable.

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