



Improvement of antibacterial and mechanical properties of chitosan matrix biodegradable polymer coating materials with green synthesized nanosilver obtained from aromatic arugula (*Eruca vesicaria*)

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Abstract

This study explores the development of antibacterial and mechanically enhanced nanocomposite films based on biodegradable chitosan (CS) and alginate (ALG) matrices. Initially, four polymer film formulations (CS1, CS2, CS-ALG, and ALG) were prepared and characterized to assess their baseline physical, mechanical, and antimicrobial properties. Among them, CS2 exhibited the highest tensile strength (31.68 MPa), while CS-ALG displayed the greatest elongation (19.68%), although their inherent antibacterial performance was limited. To enhance their functionality, silver nanoparticles (AgNPs) were synthesized through a green method using *Eruca vesicaria* (arugula) extract and incorporated into the CS2 matrix at different concentrations (1%, 2.5%, and 5%). The resulting CSNP films were comprehensively characterized by UV–Vis spectroscopy, SEM, FT-IR, EDX, and XRD, which confirmed the formation of well-dispersed, crystalline AgNPs with an average size of 16 nm. The CSNP3 sample (5% AgNPs) demonstrated significant improvement in both antibacterial activity (inhibition zones: 11–15 mm) and mechanical strength (54.6 MPa), while CSNP2 offered an optimal balance between flexibility and antibacterial efficacy. Overall, the results confirm that green synthesized AgNPs significantly improve the structural, mechanical, and biological performance of CS-based films. These eco-friendly nanocomposites have strong potential for biomedical applications, including antimicrobial wound dressings and sustainable surface coatings.

Keywords Biodegradable film · Chitosan · Silver nanoparticles · Green synthesis · Mechanical properties · Antibacterial activity

Introduction

Today, there is an increased need for new materials that are environmentally and human-friendly, sustainable, and high performance. Composite materials meet these criteria and are considered important due to properties such as lightness and strength. In particular, polymer matrix composites play a crucial role in this context [1]. Due to the increasing carbon footprint, rising oil prices, and challenges associated with recycling and disposing of plastics, there is growing interest in polymeric materials derived from renewable raw materials instead of petroleum-based polymers [2].

The use of biopolymers, especially biodegradable biopolymers, is a key factor in addressing the aforementioned issues [2, 3]. Biopolymers derived from natural macromolecules offer high biocompatibility and sufficient biodegradability and refer to materials that are either bio-based, biodegradable, or both [4, 5]. "Bio-based" indicates that the polymer is made from renewable resources, but this does not mean it is biodegradable—some petroleum-based plastics can also be biodegradable. The biodegradation of a biopolymer occurs through its decomposition into low molecular mass species, bio-assimilation of fragments by microorganisms, and mineralization through the action of enzymes and/or chemical degradation associated with living organisms [2].

Biopolymers, including proteins, nucleic acids, and polysaccharides, are natural macromolecules formed by the polymerization of monomers. They offer benefits such as reduced CO₂ emissions, biodegradability, and lower environmental pollution compared to petroleum-based polymers [6, 7]. Their renewable origin, low density, affordability, and ease of production make them increasingly attractive. Drop-in bioplastics, a subset of biopolymers, can be made from biomass rather than petrochemicals, and agricultural or industrial waste conversion has gained interest [8, 9].

Polysaccharides are biopolymers naturally plentiful in nature, non-toxic and biodegradable, derived from plants, animals or microbes. Cellulose, the most abundant biopolymer, is strong, thermally resistant, and easily degradable [7, 8, 10]. Some polysaccharides like chitin and chitosan can be derived from both plant and animal sources. Protein-based biopolymers consist of amino acid chains and can be plant-derived or animal-derived [6]. Biopolyesters, such as PLA, are biodegradable polymers from microbial or plant sources [11]. Chitosan and alginate are the most widely available biodegradable biopolymers in the world [12].

Alginate (ALG), derived from non-refined brown algae, is used in various pharmaceutical applications and tissue engineering due to its biocompatibility. It is a heteropolysaccharide consisting of β -(1-4)-D-mannuronic acid and α -(1-4)-L-guluronic acid units. Sodium alginate, the most commonly used alginate salt, serves as a stabilizer, encapsulating agent, and suspension agent in various industries. It is also employed in the production of films, membranes, and gels in the food, textile, and pharmaceutical sectors due to its low toxicity and cost-effectiveness [13, 14].

Chitosan (CS), a linear polysaccharide formed by the β -(1-4) linkage of *N*-acetylglucosamine and glucosamine units, has natural antibacterial, antiviral, and antifungal properties. Due to its multifunctionality, particularly in film formation, chitosan and its derivatives are widely used in pharmaceutical and biotechnological applications [15, 16]. Chitosan exhibits bactericidal, bacteriostatic, and antifungal properties, while sodium alginate is entirely biocompatible and provides a strong film structure for food packaging applications [4, 12].

The presence and proliferation of harmful microorganisms increase infection rates, posing significant risks to human health [17, 18]. Some biopolymers, such as chitosan, have antibacterial properties and also function as backbone polymers. Antimicrobial materials can inhibit and kill microbes on or around their surfaces [19]. However, brittleness, high water sensitivity, low thermal stability, and poor gas barrier properties are challenges in the commercial use of both pure and blended biopolymer films [20, 21]. To overcome these disadvantages, composites with biopolymer matrices have been developed. The development of polymer-based biocomposite scaffolds, such as PLA–hydroxyapatite systems, has shown promise in orthopedic applications due to their enhanced mechanical integrity and compatibility with additive manufacturing technologies [22]. Furthermore, the incorporation of elements such as fluorine and titania into hydroxyapatite-based systems has been shown to significantly improve chemical stability and biological performance, making them suitable candidates for biomedical coatings [23]. Recent studies have highlighted the importance of nanocomposite coatings with improved mechanical and biological performance, such as diopside-reinforced hydroxyapatite systems which demonstrate excellent bioactivity and wettability on titanium substrates [24]. In addition, bio-nanoceramic coatings composed of natural hydroxyapatite and diopside have been reported to enhance osseointegration and mechanical stability of implant surfaces [25].

Nowadays, significant efforts have been made to enhance the properties of biopolymers by incorporating nanoparticles [12, 26, 27]. The combination of biopolymers and nanoparticles leads to substantial improvements in material properties, including optics, electronics, strength, and antimicrobial and antibacterial characteristics [28, 29]. To enhance the antibacterial properties of the matrix material, AgNPs were incorporated into the matrix. Silver is the most commonly used metal for antibacterial materials due to its excellent bactericidal properties, low toxicity to human health, and ease of acquisition [30]. In this study, alginate, chitosan, and AgNPs were selected for their natural, sustainable, inexpensive, and antimicrobial characteristics, enabling the creation of coatings.

Previous studies have indicated that metal nanoparticles, particularly silver nanoparticles (AgNPs) and ions, exhibit promising antimicrobial properties in various applications, especially in manufacturing of the coating materials. When added to coatings for high-touch surfaces, textile fabrics, polymers, dental materials, medical devices, and burn dressings, AgNPs have demonstrated effectiveness against approximately 650 types of pathogens, including SARS-CoV-2, *Staphylococcus aureus* (MRSA), *Escherichia coli*, Influenza A, and Norovirus [31–35].

AgNPs can be produced by two main methods: chemical and physical [36]. However, these methods can be associated with large energy consumption, the release of toxic and harmful chemicals, the use of complex equipment and synthesis conditions, the need for high temperatures and pressures, and absorption of toxic substances onto silver. Green synthesis approaches can overcome this limitation [36, 37]. Considering NP production processes, green synthesis methods, also known as "Green Chemistry", play a vital role in preventing the production of harmful compounds and waste. It is easier to prevent waste than to treat or clean it after it has been created [38].

Green synthesis of AgNPs has emerged as an environmentally friendly and cost-effective approach, relying on natural reducing agents derived from plant extracts. In this method, silver nitrate (AgNO_3) solution is mixed with plant extracts prepared through standard procedures involving boiling, filtering, or soaking plant parts in solvents like water. The formation of AgNPs is typically indicated by a color change to brown [36, 38, 39]. The synthesis process and properties of AgNPs are influenced by factors such as plant species, pH, temperature, and reaction time. While most green synthesized Ag NPs are spherical, other shapes like triangular and hexagonal forms have also been reported, with particle sizes ranging from nanometers to a few micrometers depending on the extract used [36, 40]. In terms of applications, AgNPs demonstrate strong antibacterial activity against both Gram-positive and Gram-negative bacteria, outperforming gold nanoparticles in antimicrobial performance [41].

Various plant materials have successfully been used for this purpose like grape seed [40], *Euphrasia officinalis* [42], *Citrus sinensis* [43]. Moreover, agricultural waste such as hay has also been utilized, offering a sustainable alternative while reducing environmental pollution caused by biomass burning [44]. *Eruca vesicaria* (arugula/rocket) has been known since ancient times and is commonly consumed in different Mediterranean countries, such as Italy, Portugal, Egypt and Türkiye. Besides being a diuretic and antiscorbutic, it contains glucosides, mineral salts and vitamin C [45]. Arugula has been reported to possess several beneficial medicinal properties including antimicrobial, antioxidant, anticancer, analgesic and anti-inflammatory properties [46]. Andrade et al. [47] investigated edible coatings obtained from flours derived from various fruit and vegetable residues, including arugula, and obtained homogeneous, flexible films without the addition of plasticizers. Brito et al. [48] obtained flours from various fruit and vegetable residues, including arugula, and combined them with 0.25% high methoxylated pectin to contribute to the improvement of film-forming properties. They concluded that flour containing arugula, especially when combined with a small amount of pectin, is a promising raw material for the development of biodegradable packaging films.

In addition, the antibacterial activity of AgNPs biologically synthesized from arugula leaf extracts was evaluated against *S. pneumonia* and *S. aeruginosa* and showed more effective bactericidal activity against gram-negative bacteria than gram-positive bacteria. Arugula leaf extract has been found to change the silver ion oxidation state from Ag^+ to Ag^0 , which allows AgNPs to act as synthesizing agents without the inclusion of a reducing agent in the reaction mixture [49].

Arugula leaves are known to be rich in polyphenols and flavonoids, which have been shown to significantly contribute to the reduction and stabilization of silver ions during green nanoparticle synthesis. In addition, AgNPs synthesized from arugula leaves were found to exhibit anticancer activity [46]. García-Gurrola et al. [50] incorporated polyphenolic-rich extract from arugula leaves into succinylated starch nanocapsules. They found that starch nanoparticles were effective for encapsulating the polyphenolic-rich ethanolic extract and that succinylation affected nanoparticle-extract molecular interactions and arrangements.

Arugula is cultivated on more than 10 thousand hectares with a total of 13 thousand tons of arugula produced in Türkiye. It can be found on the market every month of the year. If there is sufficient temperature and humidity for the plants to develop, arugula reaches harvest maturity 30–40 days after sowing [51]. In this context, it can be considered as a sustainable green synthesis material. Although the mechanical and antibacterial properties obtained by adding AgNPs to chitosan and alginate matrices were investigated [37, 52–54], In the literature search, no studies involving chitosan and/or alginate films doped with AgNPs derived from arugula were found.

This study aimed to develop a sustainable, safe, non-toxic, and antimicrobial coating. Antibacterial AgNPs were synthesized using the green synthesis method with the aromatic arugula plant. Films were prepared by the solvent casting method, incorporating 1%, 2.5%, and 5% AgNPs to biodegradable polymer matrices, prepared in four different combinations using chitosan and alginate using biodegradable and edible sources. After characterizing and determining the mechanical properties of the prepared biodegradable composite films, chitosan prepared at 45 °C was chosen as the matrix. The antibacterial properties of silver were evaluated against two gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and two gram-negative bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*) using the disc diffusion technique.

Materials and methods

In this study, biopolymer matrices were first produced and investigated to develop biopolymer nanocomposites with enhanced mechanical and antibacterial properties suitable for use as coating materials.

The generated biopolymer matrices underwent initial tensile tests to assess their mechanical properties. Subsequently, the matrix material exhibiting the highest strength value was reinforced with AgNPs. The experimental phase of the study was conducted following this work plan:

- a. Production of biopolymer matrices, and determination of their properties
- b. Production and characterization of AgNPs
- c. Production of biopolymer nanocomposites and determination of their properties

Materials

Production of biodegradable polymer matrix

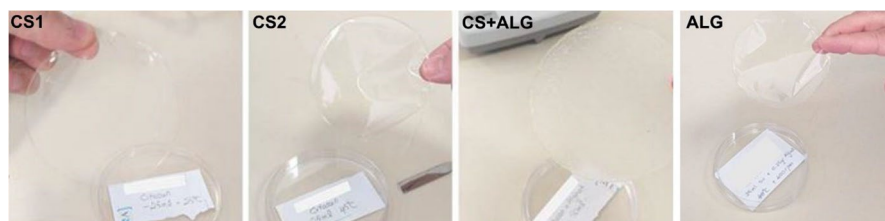
Biodegradable composite matrices were prepared as solutions using various combinations of chitosan (Sigma Aldrich), acetic acid (purity $\geq 99.8\%$) (Sigma Aldrich), alginate as alginic acid sodium salt (RPI Research Products International), and distilled water. These formulations resulted in four distinct thin films, designated as CS1, CS2, CS-ALG, and ALG. All parameters including mixing speeds and temperatures were selected based on prior optimization studies and align with literature protocols. The prepared solutions were cast into petri dishes using the solvent casting method, a conventional technique widely used for producing polymer films. In this method, the polymer is first dissolved in a suitable solvent to achieve a homogeneous solution, which is then poured onto a flat surface and left to dry under controlled conditions. After drying, the resulting thin films are carefully removed from the mold. This method is preferred for its ability to produce optically clear, isotropic, and dimensionally stable films, which are essential for high-quality film applications [55].

The chitosan (CS) solution was prepared following protocols similar to those reported in the literature, where chitosan was dissolved in acetic acid and stirred at controlled speeds and temperatures using a magnetic stirrer before casting onto petri dishes for film formation [53, 56]. A CS solution was prepared by combining 100 mL of distilled water, 1 mL of acetic acid, and 1000 mg of chitosan. The solution was stirred on a magnetic stirrer for 1 h and 50 min at 250 min^{-1} and 25°C . The stirring speed was then increased to 300 min^{-1} and stirring continued for an additional 10 min. The 100 mL chitosan solution was then divided into two equal 50 mL portions. One portion was stirred at 475 min^{-1} . After 3 h, this pure chitosan solution was further divided into two equal 25 mL portions. One portion, designated CS2, was maintained at a temperature of 45°C with stirring at 475 min^{-1} , while the other portion, CS1, was kept at 25°C with stirring at 300 min^{-1} . Both solutions were allowed to be dissolved for a total of 24 h until a homogeneous mixture was achieved. After 24 h, the solutions were poured into petri dishes using the solvent casting method. After 6 days of drying, thin films were obtained by removing them from the petri dishes.

Chitosan-alginate (CS-ALG) solution was prepared following protocols similar to described in the literature with minor modifications [12, 57]. For the CS-ALG solution, 500 mg of alginate was added to one of the 50 mL chitosan solutions, and the mixing speed was set to 315 min^{-1} . After 30 min, the temperature of the CS-ALG solution was raised to 40°C . As the dissolution progressed, the temperature was further increased to 45°C after an additional 20 min. The CS-ALG solution was allowed to dissolve for 24 h until a homogeneous mixture was achieved. After 24 h, the homogeneity of the solution was enhanced by adjusting the temperature to 55°C and increasing the mixing speed to 900 min^{-1} for an additional 4 h. The homogeneous solution was then slowly poured into a petri dish using the solvent casting method and left to dry for 6 days to form thin films.

Table 1 Components and production temperatures of biodegradable matrix material combinations

Material	Chitosan	Alginate	Acetic acid	Distilled water	Temperature (°C)
CS1	+		+	+	25
CS2	+		+	+	45
CS-ALG	+	+	+	+	55
ALG		+		+	40

**Fig. 1** Biodegradable polymer matrix materials obtained by solvent casting method

Alginate (ALG) solution was prepared using only distilled water following protocols similar to those reported in the literature [57]. For the ALG solution, 0.25 g of ALG was mixed with 25 mL of distilled water, creating a homogeneous solution through 24 h of stirring at 40 °C and 400 min⁻¹. The resulting ALG solution was then cast into a petri dish using the solvent casting method and allowed to dry. The thin film was removed from the petri dish and left to dry for 1 day. Table 1 summarizes the ingredients and production temperatures of biodegradable matrices. The resulting thin biodegradable films can be seen in Fig. 1.

Production of silver nanoparticles

The AgNPs utilized in this study were synthesized via a green synthesis method. Sigma Aldrich brand silver nitrate (AgNO₃) and sodium hydroxide (NaOH), and ethanol (Merck) were used to synthesize AgNP. All the reagents and solvents used were of analytical purity.

Nanoparticles (NPs) can be produced by physical, chemical, and biological means. The most significant disadvantage of the synthesis of NPs by chemical methods is the use of harsh reducing agents and organic solvents such as sodium borohydride and sodium citrate [36, 37]. To overcome these drawbacks, a green synthesis method was used in this study.

Arugula contains high amounts of phenolic compounds and flavonoids. These compounds are natural antioxidants with a high capacity to reduce Ag⁺ ions [58]. The main components found in arugula are acids including caffeic, ferulic, gallic, and quercetin and kaempferol. These compounds are known for their capacity to capture free radicals and reduce metal ions. For example, quercetin reduces silver ions with high efficiency, while also contributing to the stabilization of nanoparticles

[59]. Arugula contains high amounts of glucosinolates since it is in the cruciferous family. These compounds can play a role in oxidative and reduction reactions by turning into isothiocyanates by hydrolysis [58]. This makes arugula not only capable of reducing phenolic compounds but also sulfur-containing compounds—an advantage over many other plants. This indicates that it has both a high reducing capacity and stabilizing secondary metabolites.

Arugula, sourced from a local market in Denizli, Türkiye, underwent a series of preparatory steps. The steps for obtaining the extract from arugula were based on the similar works [39, 60]. The plant was washed with distilled water, dried, grinded, and pulverized into a powder. Next, 10 g of the extracted powder was suspended in 250 mL of water using ultrasonic treatment for 1 h to prepare the plant extract. The suspension underwent stirring for 1 h to obtain a cold extract, boiled for 10 min for a hot extract, and then the resulting mixture was filtered using Whatman (Grade GF/B: 1.0 μm) filter paper [61].

For the green synthesis of AgNPs, 10 mg of arugula was added into 10 ml water. This extract was added dropwise to a 0.01 M AgNO_3 solution. After 10 min of stirring, the pH of the solution was adjusted to 10 using 1 M NaOH, followed by continued stirring until a color change indicated nanoparticle formation. The resulting AgNPs were separated by centrifugation at 10,000 rpm for 20 min and subsequently washed twice with distilled water and ethanol to remove impurities.

These parameters were determined by considering both literature support and experimental preliminary tests in order to increase the homogeneity and stability of nanoparticles. Similar parameters have been successfully applied in other plant-mediated AgNP syntheses [49, 62]. The mixing time and the ratio between arugula extract and AgNO_3 solution used in this study are based on similar green synthesis approaches in the literature and findings from preliminary studies. In the literature, it has been shown that the ratio between the plant extract and metal salts generally varies between 1:1 and 1:10 and that these ratios have significant effects on nanoparticle size, morphology, and stability [63, 64]. In addition, 10 min was preferred as the mixing time because, in preliminary trials, it was observed that full-color change did not occur in shorter times (e.g. 1–9 min), i.e., the reduction of silver ions was not completed. On the other hand, it was determined that particle growth increased, and the risk of agglomeration increased in longer mixing times. Therefore, 10 min was found to be suitable for optimum results.

Production of biodegradable polymer matrix composites

In the production of biodegradable composites, the CS2 material which was prepared at 45 °C and exhibited superior mechanical properties compared to other materials underwent further investigation with the incorporation of AgNPs. In the production of AgNPs reinforced CS matrix composite, protocols similar to those reported in the literature were modified and prepared [12, 56]. For the CS2 matrix, the temperature increased to 45 °C and mixed at 315 min^{-1} for 12 h, the mixing speed increased to 475 min^{-1} for 24 h to ensure that AgNPs were evenly distributed.

Table 2 Components and production temperatures of biodegradable composites

Composite	Matrix	AgNPs (in wt%)
CSNP1	CS2	1
CSNP2	CS2	2.5
CSNP3	CS2	5

At the end of 24 h, 100 mL of the solution was transferred into three separate 25 mL beakers and different concentrations of AgNPs (in wt%), namely 1% (2.5 mg), 2.5% (6.25 mg), 5% (12.5 mg) were added into them and continued to be stirred at 45 °C at 1000 min⁻¹ for another 24 h. CSNP1, CSNP2, CSNP3 samples were obtained respectively by solvent casting method. The samples were left to dry in the natural environment for a week and then dried in an oven at a temperature between 50 and 80 °C. The dried samples were removed from the petri dishes as thin films and prepared for the tests Table 2.

Characterization and tests

Optical microscopy and scanning electron microscopy (SEM)

Optical microscopy and Scanning Electron Microscopy (SEM) were employed to illustrate the biocomposite matrix samples and nanosilver particles. Optical microscopy images were obtained using a Nikon Eclipse LV150NL device in the Materials Engineering Laboratory at Pamukkale University's Faculty of Technology. SEM analysis of the prepared samples was performed using a Zeiss Supra V40 FESEM device, operating at 20 kV, located at Pamukkale University's Advanced Research Center (ILTAM).

Because the biocomposite matrices were non-conductive, they underwent a coating process to enhance conductivity. This was done using a Quorum Q150R ES device, which applied a coating of 80% gold and 20% palladium. The samples were mounted on conductive carbon tapes with the surfaces to be imaged facing upwards. Three-dimensional images were acquired using the Secondary Electron (SE) detector for SEM image analysis.

For SEM analysis, the nanoparticles were dispersed in ethanol using an ultrasonic bath for ten minutes to prevent agglomeration. Quantitative analyses were performed using Energy Dispersive X-ray Spectroscopy (EDX) in conjunction with SEM.

Fourier transform infrared spectroscopy analysis (FT-IR)

FT-IR provides information about the chemical structure of a material by using infrared light, making it essential for identifying organic, inorganic, and polymeric materials. This method is particularly useful for examining the structure of bioactive plant extracts [65]. In this study, the FT-IR analysis was conducted using the Nicolet

IS50 FT-IR model, located at Pamukkale University's Advanced Research Center (ILTAM).

UV–Vis Spectroscopy is a simple and useful method used to measure the absorbed and scattered light by a sample, facilitating mass concentration determination according to Beer's law [66]. UV–Vis spectroscopy is particularly assessing the optical properties of nanoparticles due to its sensitivity to light scattering near the surface, which is influenced by factors such as size, shape, aggregation state, and surface properties [67]. The stability of AgNPs is typically assessed via UV–Vis spectroscopy over time, with most stable nanoparticles showing a single absorbance peak around 420 nm [53]. In this study, the optical properties of AgNP were examined using UV/Visible spectroscopy (UV–Vis 1601, Shimadzu, Kanagawa, Japan) at Pamukkale University Advanced Research Center (ILTAM). To prepare the sample, 20 mg of AgNPs were mixed with ethanol/water solution in an ultrasonic bath for half an hour and then filtered through a membrane filter. UV scanning was performed between 200 and 800 nm using a quartz cell, with ethanol as the reference.

X-ray diffraction analysis

X-ray Diffraction (XRD) is a technique that provides detailed information about material's structure, grain size, strain, crystallinity, and crystal defects, making it essential for the characterization of crystalline materials. XRD was utilized in this study to examine the structural details of the metallic nanoparticles. XRD patterns of AgNPs were carried out at Pamukkale University Advanced Research Center (ILTAM) using a GNR APD PRO 2000 brand instrument. The X-ray source was Cu-K α ($\lambda = 1.5406 \text{ \AA}$) radiation, and the measurements were performed in the scan width range $2\theta = 5\text{--}90^\circ$.

Thickness and density measurements

The dried samples were cut into $1 \times 1 \text{ cm}^2$ sizes and weighed using a precision balance, with an average of 10 measurements taken for accuracy. The thickness of each sample was measured at 10 different places using a Louis Schopper Automatic Micrometer, and the average thickness values were recorded in mm. The density of the prepared film samples was calculated using the method applied by Lozano-Navarro et al. [68], as expressed in Eq. (1):

$$\rho = m/(A * \delta) \quad (1)$$

Here, A represents the film area (cm^2), δ is the film thickness (cm), m is the dry weight of the film (g), ρ is the density of the film (g/cm^3).

Swelling test

The biodegradable composite samples were immersed in distilled water ($\text{pH} \approx 7$) at room temperature for 2, 4, 8, 12, and 24 h to determine their swelling rates.

To calculate the degree of swelling, the dry weight (m_{dry}) and the swollen weight (m_{swell}) of the samples were measured individually using a precision balance through five measurements. The swelling ratio was then calculated using Eq. (2) [68]:

$$\text{Swelling Ratio (\%)} = (m_{\text{swell}} - m_{\text{dry}} / m_{\text{dry}}) \times 100 \quad (2)$$

Moisture test

The moisture content in biocomposites films, crucial for controlling microbiological growth in food and maintaining freshness [69], was calculated following the parameters from Giyatmi et al. [70]. The samples were kept at 105 °C for 2 h to determine moisture content through five measurements. The percentage of moisture loss in the film samples was calculated by Eq. (3):

$$\% \text{ Moisture loss} = [(M_1 - M_2) / M_1] \times 100 \quad (3)$$

Here, M_1 (g) is the mass value measured before the film pieces were placed in the oven, and M_2 (g) is the mass value measured after the film pieces were dried in the oven.

Tensile test

The tensile test is utilized to determine the mechanical properties of materials including elongation at break (A , %) and tensile strength (Rm , MPa). Thin film biocomposites samples, measuring 10×80 mm [48], were cut and the tests were conducted using a Tinius Olsen H10KT device at a tensile speed of 5 mm/min in the Textile Engineering Laboratory of Pamukkale University Faculty of Engineering using 3 samples of each parameter. The Rm of the films, defined as the maximum force sustained by the sample until it ruptures (F_{max}), was calculated by dividing this force by the cross-sectional area (A) of the film, expressed in megapascals (MPa). Based on the results obtained, σ – ε graphs of the samples were plotted.

Antimicrobial activity tests

The antibacterial properties of biodegradable polymers reinforced with AgNP were investigated in the Department of Biomedical Engineering at Pamukkale University, Faculty of Technology. The research aimed to comprehensively assess the efficacy

Table 3 Characteristics of bacterial cultures used in antimicrobial testing

Bacteria strain	Gram type	ATCC NO
<i>Bacillus subtilis</i>	Gram-positive	6633
<i>Staphylococcus aureus</i>	Gram-positive	25,923
<i>Escherichia coli</i>	Gram-negative	25,922
<i>Pseudomonas aeruginosa</i>	Gram-negative	700,603

of nanoparticles for antibacterial applications, following the rigorous standards set by the Clinical Laboratory Institute [71]. The experimental design involved the evaluation of four bacterial strains, encompassing two Gram-positive strains (*Bacillus subtilis*, *Staphylococcus aureus*) and two Gram-negative strains (*Escherichia coli*, *Pseudomonas aeruginosa*). The bacterial cultures used in the antibacterial tests are given in Table 3. Antimicrobial tests were performed on both biodegradable polymer matrices (CS1, CS2, CS-ALG, and ALG) and AgNPs reinforced films (CSNP1, CSNP2, and CSNP3).

The method of choice for assessing antibacterial sensitivity, the disk diffusion test originally developed by Kirby Bauer, was adopted in this research. To mitigate the possibility of interference and overlapping inhibition zones during the experimental process, a standardized spacing of 22 mm between disks and 14 mm from the edge of the petri dish was diligently maintained. Within the scope of antimicrobial tests, 8 log cfu/mL stock of bacteria prepared on Mueller Hinton Agar was prepared, transferred to 100 μ L agar surface and spread evenly on the surface with a drigalski spatula [72]. The films were sterilized under UV light for 10 min before the tests. The inhibition zone diameters were determined by placing 5 mm diameter film samples, cut in disk shapes, onto agar plates with bacterial cultures and incubating them at 37 °C for 24 h, followed by the measurement and assessment of the resulting zones. After incubating, the inhibition zones were measured in millimeters. The experiment was conducted three times.

Results

Surface morphology analysis by SEM images and optical microscope

Biodegradable polymer matrix

The optical microscope and SEM surface images of the matrix materials prepared for composite production are shown in Fig. 2. Among the samples, CS1, CS2, and ALG exhibited a smooth and homogeneously mixed structure, with the exception of CS-ALG.

The surfaces of the pure CS2 displayed a very smooth, uniform, and crack-free appearance, consistent with findings reported by the other authors [56, 73, 74]. Although CS1 film also has a relatively smooth, uniform, and flat surface, air bubbles are observed on the surface. It can be seen from the surface condition of CS2 that this can be eliminated by increasing the processing temperature. ALG film had a flat surface appearance without cracks, but pores of different sizes were observed in this film. In contrast, CS-ALG film showed a microstructure that was smooth yet highly porous. Notably, a significant number of air bubbles and particles were observed on the surface of the CS-ALG, indicating less homogeneity of the components in the film, aligning with the characteristics reported in the work of Venkatesan et al. [75].

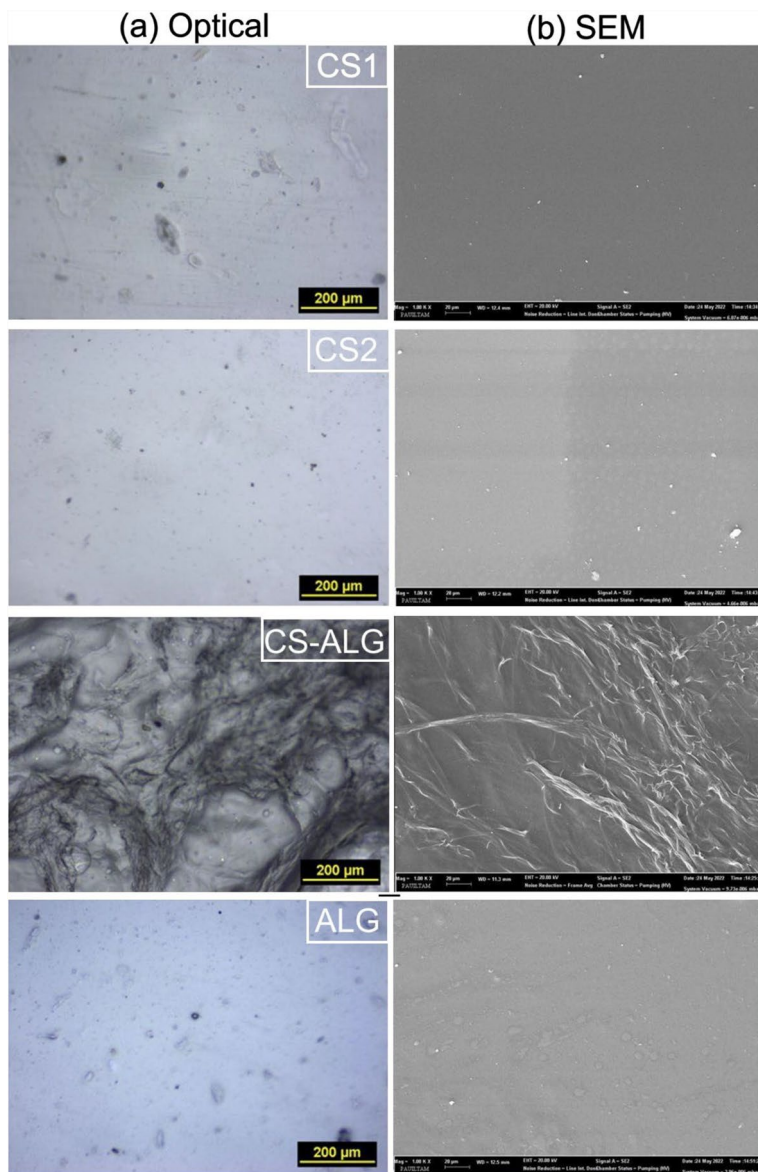
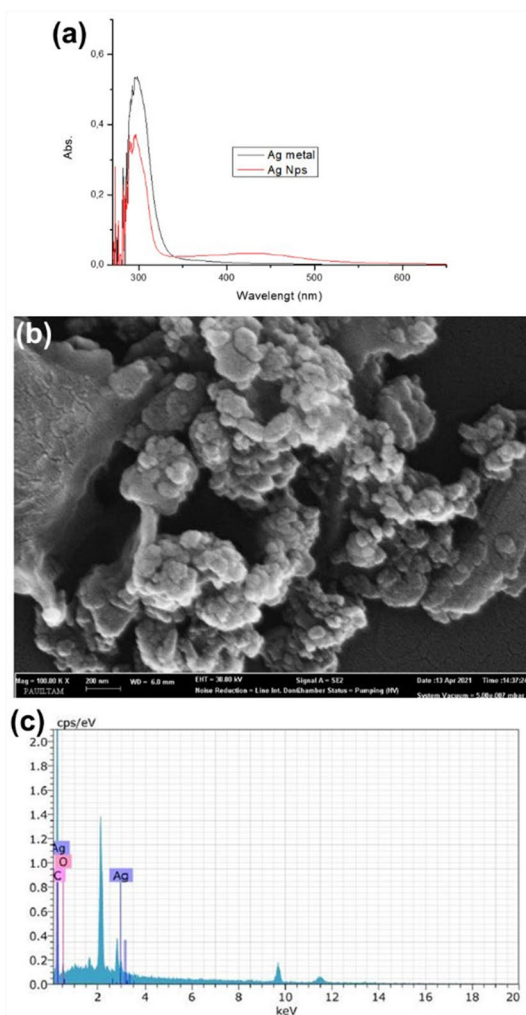


Fig. 2 Micrograph images of the matrix materials: **a** Optical and **b** SEM

Silver nanoparticles

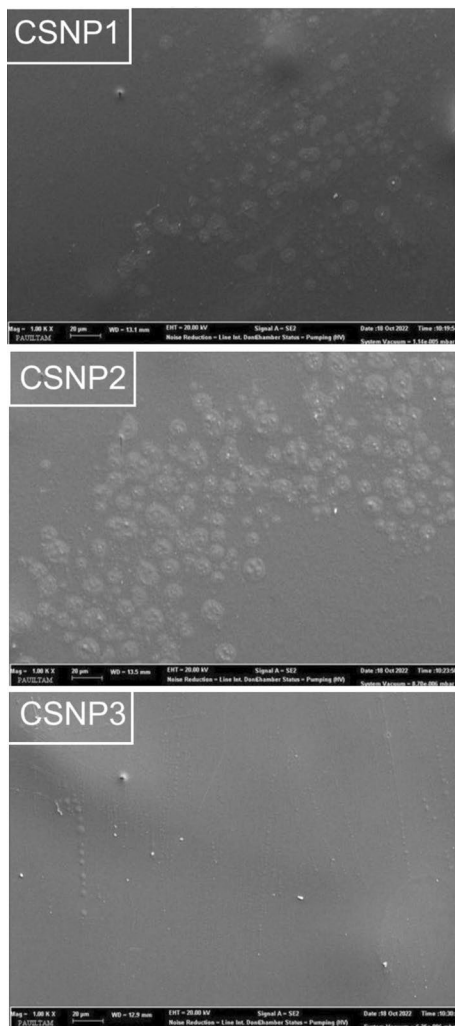
The synthesis of AgNPs was carried out as an environmentally friendly biosynthesis that does not harm the nature thanks to the arugula extract (Fig. 3). The dark brown color of the arugula extract, which turns black as a result of the reaction with water, was the first evidence of the formation of AgNPs. UV–visible absorption spectra

Fig. 3 **a** UV–visible spectrophotometry of arugula extract and silver nanoparticles maximum absorbances, **b** SEM images of the silver nanoparticles, **c** EDX analysis of the silver nanoparticles



between 200 and 800 nm were taken to determine the optical properties of AgNPs obtained from arugula extract. Figure 3a, it is seen that AgNPs have maximum absorption at 300 nm. There are number of intense peaks observed in the ultraviolet region in the range of 250 to 300 nm. The absorbance decreases abruptly from 300 to 400 nm. In the visible region, absorption further decreases with small peaks at 430 nm. The absorbance spectra of the UV–Vis study revealed that AgNPs showed less absorbance in the entire visible region. In this respect, the study is in agreement with the study in which AgNPs were obtained from *Artocarpus heterophyllus* leaves extract [76]. A clearly absorption spectrum with a peak maximum at 424 nm was also obtained in AgNPs from arugula leaves in the study by Awadelkareem et al. [46]. It was stated that the free electrons in AgNPs contribute to the absorption band's appearance resulting from the mutual vibration of light wave events, resulting

Fig. 4 SEM images of the composite materials



in a Surface Plasmon Resonance (SPR) absorption band. In the same study, spectroscopic measurements were performed two weeks after synthesis and it was stated that the synthesized AgNPs did not show any visible change in the spectroscopic results, indicating that arugula leaf extract may act as a capping agent or a binding agent to stabilize AgNPs.

SEM images of the green synthesized AgNPs are shown in Fig. 3b. The analysis revealed that the AgNPs were randomly distributed, with an approximate size of 16 nm. The nanoparticles did not exhibit a fully spherical structure, likely due to the aggregation of two or more AgNPs during the synthesis process. This observation aligns with findings from other studies in the literature [42]. The chemical compositions of nanoparticles were further analyzed by the Energy Dispersive

X-ray Spectroscopy (EDX). EDX analysis of the powdered sample, shown in Fig. 3c, indicates the presence of silver as the primary element, with trace amounts of carbon and oxygen. The EDX spectrum of AgNPs showed the highest peak at 3 keV for AgNPs [42, 49]. The sharply marked peak of Ag strongly demonstrated the reduction of AgNO_3 to AgNPs. The appearance of carbon and oxygen signals is potentially attributable to biomolecules surrounding the AgNPs or may have occurred during sample preparation [39, 49].

Biodegradable polymer matrix composites

Previous analysis revealed that the surface of the CS2 sample was smooth and free of air bubbles. The biocomposite films, which were prepared by incorporating nano-clusters, maintained smooth surfaces with preserved structural integrity. No cracks were observed in all three films (Fig. 4). However, the CSNP1 and CSNP2 samples showed the presence of air bubbles on their surfaces. Despite the films appearing macroscopically homogeneous, SEM analysis revealed that particle agglomerates formed as the silver content in the films increased from 1 to 2.5%. This irregular morphology might be due to the increase in the viscosity of the solution observed in some parts of the surface matrix and the macroscopic inhomogeneity of the distribution of AgNPs in some parts of the matrix [56]. Moreover, this was observed to be reduced in the CSNP3 sample which reinforced with 5% of AgNPs, compared to the other two samples. Importantly, no signs of burning were observed in the films as evidenced by the SEM images obtained through field scanning.

Similar observations regarding the microstructure of CS films with various additives have been reported by other researchers. For instance, Zhang et al. [77] studied the mechanical and thermal properties of CS reinforced with lignin nanoparticles (LNPs). They found out that increasing the LNP content in the film, relatively rough morphology was obtained with cracks forming in the cross-sections and a rough surface with visible agglomeration. In another study, Vimala et al. [53] used SEM to examine the porosity in CS-AgNP composites. Their findings revealed that, while pure CS films exhibited a dense and uniform plain microstructure, the CS-AgNP composite films showed the presence of numerous pores throughout the film.

Chemical characterization by FT-IR

The FT-IR spectra of CS2 and CSNP3 samples were analyzed to investigate the structural interactions between chitosan and silver nanoparticles synthesized via green chemistry. These results are shown in Fig. 5. In both spectra, characteristic bands associated with $-\text{OH}$, $-\text{NH}_2$, $-\text{CH}$, amide, and $\text{C}-\text{O}-\text{C}$ stretching vibrations were observed, and notable spectral differences were identified following the incorporation of AgNPs [78]. In CS2, a broad peak at 3288 cm^{-1} corresponds to $-\text{OH}$ and $-\text{NH}_2$ stretching vibrations. In the CSNP3 spectrum, this peak became broader and less intense, indicating the formation of hydrogen bonds between the chitosan chains

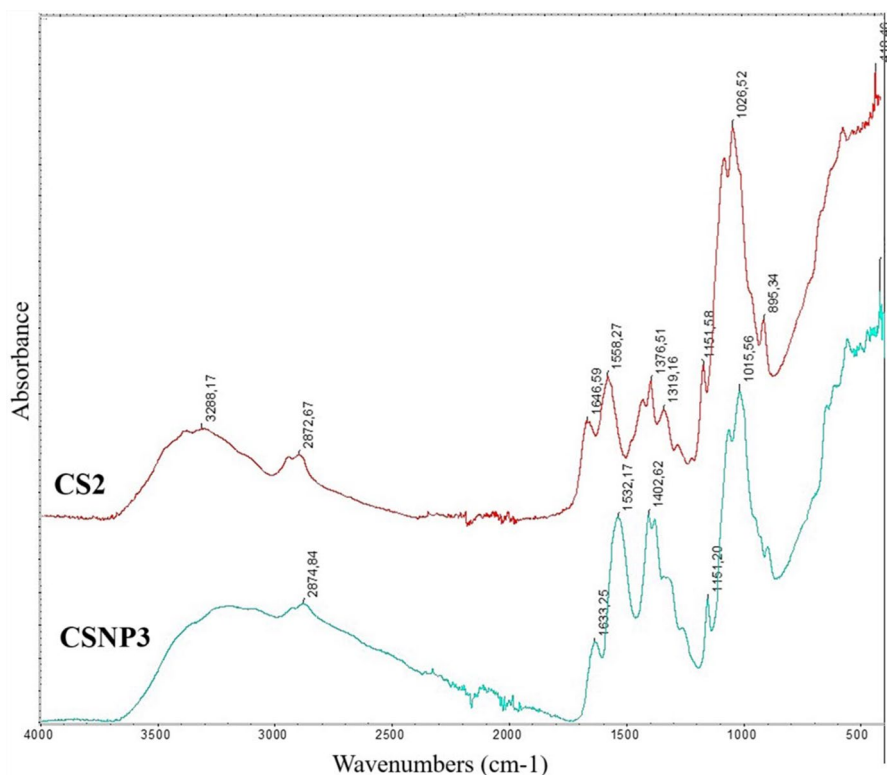


Fig. 5 FT-IR spectrum of CS2 and CSNP3

and AgNPs. The -CH stretching band at around 2872 cm^{-1} in CS2 shifted slightly to 2874 cm^{-1} in CSNP3, which may suggest minor conformational changes in the polymer backbone. A more prominent shift was detected in the amide I region, where the peak at 1532 cm^{-1} in CS2 shifted to 1633 cm^{-1} in CSNP3. This shift is typically attributed to interactions between AgNPs and the carbonyl groups of the polymer, likely resulting in coordination or electrostatic interactions. While the amide II band around 1402 cm^{-1} remained nearly unchanged, additional differences were observed in the fingerprint region: peaks related to C-N and C-O-C stretching shifted and altered in intensity, indicating changes in the molecular environment and possibly improved dispersion or bonding of AgNPs within the matrix [79], FT-IR spectra of chitosan showed prominent peaks at 3352 cm^{-1} , 2921 cm^{-1} , 2877 cm^{-1} , 1550 cm^{-1} , and 1640 cm^{-1} . These peaks were attributed to various functional group vibrations, such as the -NH_2 and -OH stretching at 3352 cm^{-1} , the -CH stretching at 2921 cm^{-1} and 2877 cm^{-1} , the bending vibration of -NH in primary amides at 1550 cm^{-1} , and the -C=O stretching at 1640 cm^{-1} . The peaks at 1423 and 1377 cm^{-1} were associated with the stretching in the -NH amide bonds and the NH stretching of the amide(III) band. Additionally, the peaks at 1028 and 1067 cm^{-1} were attributed to IR bands at infinite frequency. In the same study, a shift in the peaks from 1640

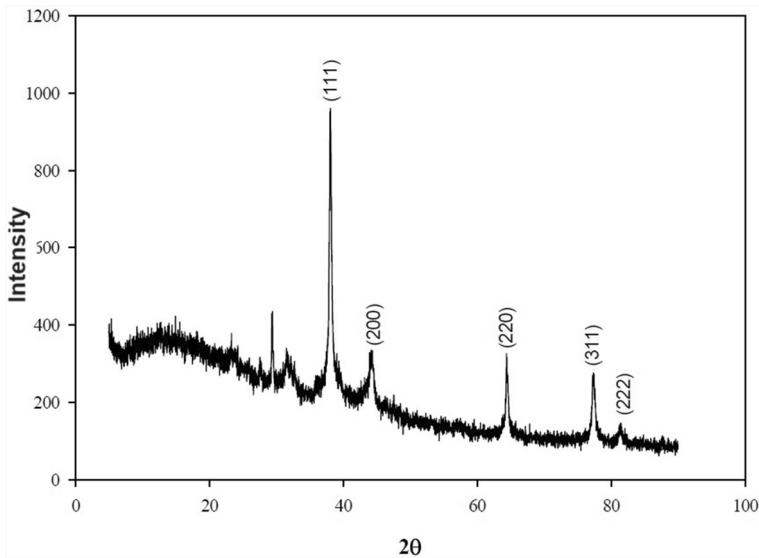


Fig. 6 X-ray diffraction pattern of the green synthesized silver nanoparticles

and 1550 cm^{-1} to 1648 and 1553 cm^{-1} in the nano-enhanced chitosan was reported, which was ascribed to interactions between the cationic groups of chitosan and the additive material. The sharpness and increased peak intensity at 3352 cm^{-1} were attributed to enhanced hydrogen bonding due to the formation of nanocomposites [79, 80].

Overall, these spectral variations confirm that AgNPs are effectively integrated into the chitosan matrix and influence its chemical structure through hydrogen bonding and secondary interactions. This integration is consistent with the enhancements observed in the mechanical and antibacterial performance of the composite films.

X-ray diffraction analysis

The crystalline nature of the green synthesized AgNPs using arugula was confirmed by XRD pattern analysis. The XRD pattern in Fig. 6 indicates the face-centered cubic structure of the AgNPs. There are five distinct reflections in the diffractogram at 37° (111), 44° (200), 63° (220), 77° (311), and 82° (222). Particle sizes were calculated using the Debye–Scherrer equation. The intensities and positions of the obtained peaks indicated that the Ag nanoparticles were face-centered cubic with an average grain size of about 16.37 nm. These results are in agreement with the results of AgNPs obtained using both arugula and other plants in the literature and confirmed that XRD method is an important tool to demonstrate the formation of AgNPs and crystal formation of the prepared AgNPs [37, 42, 49, 81].

Physical and mechanical tests results

Mass, thickness, and density results

The masses of CS1 and CS2 samples were measured at 3.0 mg and 2.8 mg, respectively. The increased production temperature (45 °C) of the CS2 sample resulted in a decreased mass compared to CS1. The CS-ALG sample weighed 8.5 mg, with the ALG sample exhibiting the smallest mass at 1.9 mg. The reduced mass of the ALG sample is attributed to the formation of a solution with water and subsequent water evaporation during drying. The thickness values for CS1 and CS2 were measured at 0.02 mm, while the CS-ALG and ALG samples had thicknesses of 0.08 mm and 0.01 mm, respectively. The density values of CS1 and CS2 samples were calculated as 1.5 g/cm³ and 1.4 g/cm³, respectively. The density of the CS2 sample prepared at 45 °C decreased slightly compared to the CS1 sample prepared at 25 °C. The CS-ALG sample exhibited a calculated density of 1.0625 g/cm³, despite having a larger volume than other materials. This is directly related to the fact that the CS-ALG film shows a smooth macrostructure, but highly porous microstructure and the thickness obtained compared to other films was considerably thicker (Fig. 2). Notably, the ALG sample, with the smallest volume, showed the highest density at 1.9 g/cm³. The ALG sample was prepared with 25 mL of water. It has the thinnest thickness measurement due to evaporation of water during drying and its value was measured as 0.01 mm. Table 4 provides the obtained values and their standard deviations.

Compared to CS, CS/Ag films' thickness was close to each other in CSNP2 and CSNP3 (approx. 0.11 ± 0.034), it was considerably lower (0.02 ± 0.005) in the matrix material CS2 film, indicating that the film thickness is critically affected by the presence of silver nanoparticles [56]. When the nanoparticle ratio was increased from 1 to 5%, the density of the composites decreased from 0.81 to 0.49 g/cm³. CS's density was reported as to be 0.0993, 1.22, and 2.00 g/cm³ [57, 74, 82]. It is worth noting that the mass and density of thin films of the same size may vary based on the monomer ratio forming the polymer and any additional materials used. By adding cellulose nanocrystals (CNC), nano-fibrils (CNF), AgNPs, and bacterial nanocellulose (BNC) to chitosan and alginate, their densities were decreased [20, 83].

Swelling and moisture test values of thin films

Table 5 presents all obtained swelling ratios (%) that were calculated from Eq. (2), and Fig. 7 shows the mass increase (%) as a function of soaking time.

The time-dependent mass increases and swelling values of CS1 and CS2 samples closely align. After 24 h of immersion in water, the mass of CS1 increased by 33.3%, and the mass of CS2 increased by 28.5%. Liu et al. [74] determined the water swelling value as 660.8% for chitosan film at 25 °C for 24 h in their study. In this study, alginate (ALG) dissolved in water before completing the 2-h retention time. ALG, belonging to the ionic polymer group, exhibits limitations in aqueous applications due to its high swelling and dissolution properties in water [84, 85].

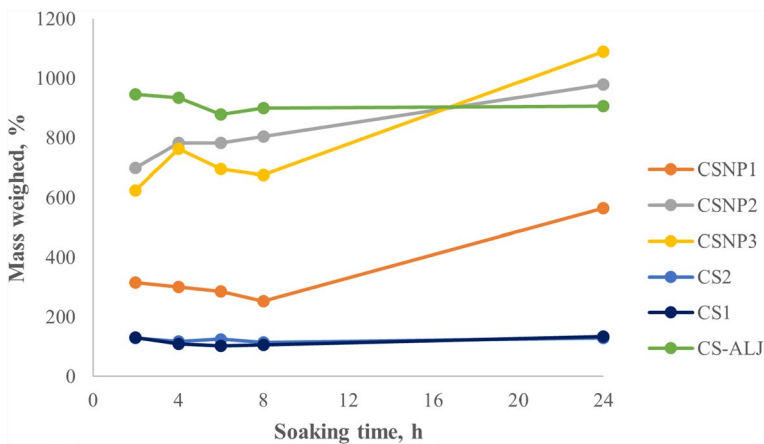
Table 4 Mass, thickness, and density values of the thin films

Material	Mass* (mg)	Standard deviation	Thickness (mm)	Standard deviation	Volume (cm ³)	Density (g/cm ³)	Standard deviation
CS1	3.0	0.242	0.02	0.005676	0.002	1.5	0.391578
CS2	2.8	0.116	0.02	0.005676	0.002	1.4	0.51875
CS-ALG	8.5	0.106	0.08	0.013166	0.008	1.0625	0.179736
ALG	1.9	0.019	0.01	0.003213	0.001	1.9	0.329908
CSNP1	5.7	0.156	0.070	0.007379	0.0070	0.81	0.009808
CSNP2	5.8	0.597	0.11	0.034383	0.011	0.52	0.081359
CSNP3	4.9	0.275	0.10	0.018135	0.010	0.49	0.079242

*Mass of dried sample with 1 cm² surface area (mg)

Table 5 Water swelling rates of thin films (%)

Material	2 h	4 h	6 h	8 h	24 h
CS1	130	110	103.3	106.66	133.3
CS2	128.5	117.8	125	114.2	128.5
CS-ALG	945.8	935.2	877.6	900	905.8
ALG	Dissolved	Dissolved	Dissolved	Dissolved	Dissolved
CSNP1	315.78	300	285.96	253.50	564.91
CSNP2	698.27	782.75	782.75	803.44	979.31
CSNP3	624.48	762.24	695.910	675.51	1089.79

**Fig. 7** The mass increase of the materials as a function of soaking time

Remarkably, the mass of CS-ALG increased by 900% after 24 h of soaking, yet it did not dissolve.

Compared to CS2, swelling values appear to increase in composites. As the AgNP ratio also increased, the swelling ratio increased. In CSNP2 and CSNP3 samples, swelling values depending on time were obtained close to each other in composites containing 2.5% and 5% AgNP. Compared to CSNP1, the swelling ratio increased approximately 2 times in these two samples. Compared to CS2 under the same conditions, swelling ratio increased by 439%, 762%, and 848% for CSNP1, CSNP2, and CSNP3, respectively.

Literature reports indicate that the swelling rates of CS composites reinforced with NPs are typically reduced compared to CS films [86, 87]. In the study by De Matteis et al. [88], swelling properties of composites obtained with CS/AgNPs were examined. The pure chitosan showed a high swelling degree as to be 260% after 3 h. For nanocomposite samples, it was determined that the swelling ratio of films was influenced by NPs concentrations and size, and it was determined that after 3 h swelling values decreased to 235–195%. In this study, the swelling values of the AgNPs reinforced CS composite films were found to be much higher than those of

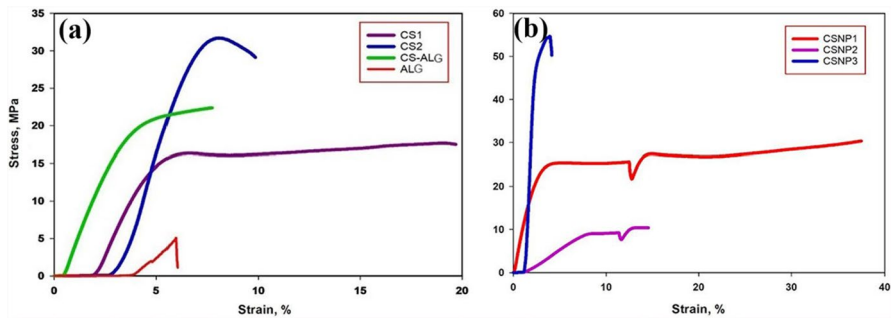


Fig. 8 The tensile test result of biodegradable composite matrices (a) and biodegradable composites

unreinforced CS2. It can be seen that this is in parallel with the density values of the films given in Table 4. The density of the composites decreased compared to CS2. It can be concluded that with decreasing density, water soaking capacity increases.

Tensile test results

Firstly, the mechanical properties of the biodegradable composite matrices were determined by tensile testing (Fig. 8a). As a result of the tests, the highest tensile strength (R_m) value was determined as 31.68 MPa for the CS2 sample. This was followed by CS1 with 22.375 MPa, CS-ALG with 17.48 MPa, and ALG with 5.028 MPa, respectively. The highest elongation at break value was obtained in the CS-ALG sample with 19.68%. This was followed by CS2 with 9.84%, CS1 with 7.2%, and ALG with 6.032%, respectively.

The tensile strength of CS films increases with increasing molecular weight of chitosan. In the study by Nunthanid et al. [89], R_m values ranged between 45.07 and 61.76 MPa, while elongation at break ranged between 9.3 and 18.25% for CS produced from starting material with different molecular weights. The film-forming properties of ALG are significantly influenced by the concentration of sodium alginate. As the concentration of sodium alginate increases, the tensile strength gradually increases; however, this increase is not significant beyond a concentration of 2% [90]. Additionally, the film's thickness obtained also affects the mechanical strength [91]. Reported values for R_m and elongation at break for ALG in the literature vary widely. The range for the R_m value is 3.72–60 MPa, and the values for elongation at break range between 0.7 and 17% [90–92]. ALG films are generally not used alone due to their insufficient strength. Instead, the composition can be modified by adding one or more additives to improve adhesion properties and overall performance of the film [93, 94]. The R_m of the film prepared with ALG content was increased by the addition of CS in the CS-ALG sample.

After obtaining these results, the CS2 sample, exhibiting the highest R_m value, was chosen for reinforcement with AgNP at three different ratios. Figure 8b presents the results of the tensile tests conducted on the composite materials. Following the

Table 6 Tensile test results of the thin films

Material	Rm (MPa)	Elongation at break (%)
CS1	22.37	7.2
CS2	31.68	9.84
CS-ALG	17.48	19.68
ALG	5.02	6.03
CSNP1	10.43	37
CSNP2	30.40	14.56
CSNP3	54.60	4.14

tensile tests on AgNP reinforced thin films with a biodegradable polymer matrix, the highest Rm value was observed in the CSNP3 sample (with 5% nanosilver additive) at 54.6 MPa. This was followed by CSNP1 (with 1% nanosilver additive) at 30.4 MPa and CSNP2 (with 2.5% nanosilver additive) at 10.43 MPa. When analyzing the elongation at break values, the highest value was determined at 37% in the CSNP1 sample, followed by CSNP2 at 14.56% and CSNP3 at 4.14%, respectively. All mechanical values obtained in this study agree with the literature and are shown in Table 6.

The addition of AgNPs in increasing concentration exhibits an increase in the strength value of the CS polymer matrix as evident from the changes in the mechanical properties of the CS/Ag nanocomposite film. CSNP3 has the lowest density and the highest water absorption capacity. It also has the surface with the fewest air voids and defects in SEM examinations. This demonstrated also a robust molecular association between AgNPs and CS chains through the creation of H-bonds and ester linkages, resulting in stronger, stiffer films with a discernible capacity for stretching. Similarly in the study by Kadam et al. [56], the Rm value of CS was increased from 51.67 to 65.04 MPa. Elongation at break increased from 25.75 to 26.94%. The observed drop in tensile strength in CSNP2 is attributed to nanoparticle agglomeration at intermediate concentrations, as evidenced by SEM images. At this level, partial clustering may lead to poor stress transfer and weakened interfacial bonding. This interpretation is consistent with literature reports on nanoparticle dispersion behavior in polymer matrices [56, 79].

Table 7 Antibacterial test results for biodegradable materials

Sample	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>S. aureus</i>
CS1	2	3	2	2
CS2	3	4	3	3
CS-ALG	4	4	5	3
ALG	1	1	2	2
CSNP1	6	8	8	9
CSNP2	7	8	8	10
CSNP3	11	14	13	15

Antibacterial test results

Table 7 presents antibacterial test results of the biodegradable matrix samples, with zone measurements (mm) considered effective if equal to or greater than 5. The results indicate that among the samples tested, CS-ALG exhibited antibacterial activity against *B. subtilis*, while CS1, CS2, and ALG demonstrated no significant antibacterial effects. The observation that the cross-linking of CS with ALG, as represented by the CS-ALG sample, demonstrated antibacterial efficacy against *B. subtilis* is particularly noteworthy. The absence of antibacterial effects in the unmodified samples emphasizes the relevance of the cross-linking method in enhancing the antibacterial activity of biodegradable polymers. These findings not only contribute valuable insights into the antibacterial properties of biodegradable polymer samples but also highlight the importance of cross-linking techniques, particularly in the design and development of antimicrobial materials in the field of material and biomedical engineering.

According to the results obtained, it can be seen that CSNP1, CSNP2, and CSNP3 composite coating materials are antibacterial. It is seen in Table 7 that as the amount of AgNPs added to the CS2 sample increases, its antibacterial properties also increased. It can be seen that the antibacterial activity values of CSNP1 and CSNP2 materials obtained by adding 1% and 2.5% nanosilver to the CS2 material are close to each other, while the antibacterial activity value of CSNP3 material obtained with 5% nanosilver addition increases rapidly.

In the study conducted by Sharma et al. [52], it was stated that, while CS and ALG composite films did not show antibacterial properties against gram-negative and gram-positive bacteria, the antibacterial properties were increased when AgNPs with sizes ranging from 10 to 20 nm were added to CS and ALG solutions. Chicea et al. [95] reported that AgNPs below 100 nm showed strong antibacterial effects on both *E. coli* and *S. aureus*. It is clear from these studies that the antibacterial effect of AgNPs depends on the particle size and distribution as well as the proportion of AgNPs. In this study, the average size of the AgNPs produced to reinforce the CS matrix measured 16 nm. This optimal distribution resulted in both the highest tensile strength (Rm) and the strongest antimicrobial activity against four different bacteria in the CSNP3 sample. The samples CSNP1→CSNP3 show a clear, consistent increase in inhibition zones for all bacteria. This confirms the dose-dependent antimicrobial efficacy of silver nanoparticles, as widely supported in literature [96].

Comparison of physical and mechanical properties

The integration of microstructural, chemical, mechanical, and antibacterial data provides a comprehensive understanding of the structure–property–performance relationship in AgNP-doped CS composites.

SEM analyses revealed that AgNP dispersion significantly affects the morphology and thus the mechanical performance. While CSNP1 and CSNP2 exhibited air bubbles and partial agglomeration, CSNP3 displayed a more uniform surface (Fig. 4), aligning with its superior Rm (54.60 MPa) and the highest antibacterial

Table 8 A practical interpretation for antibacterial and mechanical properties

Application type	Sample	Reasoning
Flexible antibacterial film	CSNP1	High elongation, moderate antimicrobial
Balanced mechanical and bioactivity	CSNP2	Strong, reasonably flexible, high antimicrobial
Rigid antibacterial barrier	CSNP3	Maximum strength and antimicrobial effect, low flexibility

activity (inhibition zones up to 15 mm). FTIR spectra indicated strong hydrogen bonding and molecular interactions between CS and AgNPs, especially in CSNP3, supporting enhanced stiffness and structural integrity [79, 80]. XRD confirmed the formation of crystalline AgNPs with ~ 16 nm average size, which is considered optimal for both antimicrobial efficiency and reinforcement [37, 49, 52].

Density and swelling analyses supported these observations: as AgNP content increased, film density decreased (0.81 to 0.49 g/cm³) and water uptake rose (564.91 to 1089.79%), indicating increased porosity and hydrophilicity—factors that may assist antimicrobial functionality without compromising mechanical robustness when particle distribution is well controlled [56, 83, 88].

Mechanically, CSNP1 stood out with the highest flexibility (elongation: 37%) and moderate antibacterial effect. CSNP2 provided a balance between strength (30.40 MPa), elasticity (14.56%), and microbial inhibition. CSNP3 demonstrated the highest strength and antimicrobial effect but had the lowest flexibility (elongation: 4.14%). These findings validate that higher AgNP concentration improves antibacterial activity but may reduce flexibility due to matrix hardening or NPs agglomeration [52, 95, 96].

These results show that higher concentrations of AgNPs result in increased antibacterial activity but may reduce flexibility due to nanoparticle agglomeration or matrix hardening. A practical interpretation is given in Table 8.

Conclusions

This study demonstrates the successful green synthesis of antibacterial AgNPs using arugula extract and their effective integration into biodegradable CS matrices. Characterization by UV–Vis, SEM, FT-IR, EDX, and XRD confirmed that the AgNPs possess a face-centered cubic structure with an average size of ~ 16 nm, ideal for nanocomposite formation.

Incorporation of AgNPs into the CS matrix significantly improved both the mechanical and antibacterial properties. Notably, the CSNP3 sample, containing 5% AgNPs, exhibited the highest tensile strength (54.6 MPa) and the strongest antibacterial activity, with inhibition zones of 11 mm against *E. coli* and 15 mm against *S. aureus*. Comprehensive characterization confirmed the structural and chemical integration of AgNPs within the chitosan matrix. UV–Vis spectroscopy indicated successful nanoparticle synthesis, while SEM and EDX analyses demonstrated their homogeneous dispersion and elemental composition. FT-IR

results revealed molecular-level interactions between AgNPs and the polymer network, and XRD patterns validated the crystalline nature and nanoscale size of the particles. These results indicate that optimal nanoparticle loading can reinforce the structural properties of biodegradable polymers, while imparting effective antimicrobial functionality.

Overall, the findings support the potential of green synthesized AgNPs as functional additives in biodegradable polymer systems, particularly for biomedical and environmentally sensitive applications. Future research should optimize nanoparticle concentrations to balance mechanical robustness, biodegradability, and sustained antimicrobial efficacy under practical use conditions.

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Data availability The data that support the findings of this study are available on request from the corresponding authors. The data are not publicly available due to privacy or ethical restrictions.

Declarations

Conflict of interest The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript. We certify that the submission is original work and is not under review at any other publication.

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References

1. Pethrick RA (1995) Polymer surface modification and characterization. *Polym Int* 37:84–84. <https://doi.org/10.1002/pi.1995.210370115>
2. Vroman I, Tighzert L (2009) Biodegradable polymers. *Materials* 2:307–344. <https://doi.org/10.3390/ma2020307>

3. Ribba L, Lopretti M, de Oca-Vásquez GM et al (2022) Biodegradable plastics in aquatic ecosystems: latest findings, research gaps, and recommendations. *Environ Res Lett* 17:033003. <https://doi.org/10.1088/1748-9326/ac548d>
4. Petzold Pauli F, Silva Freitas C, Ribeiro Pereira P et al (2023) Exploring the antimicrobial and antitumoral activities of naphthoquinone-grafted chitosans. *Polymers* 15:1430. <https://doi.org/10.3390/polym15061430>
5. European Bioplastics (2024) What are bioplastics? In: What are bioplastics? <https://www.european-bioplastics.org/bioplastics/>. Accessed 25 Aug 2024
6. Flórez M, Cazón P, Vázquez M (2023) Selected biopolymers' processing and their applications: a review. *Polymers (Basel)* 15:641. <https://doi.org/10.3390/polym15030641>
7. Işıtan A, Pasquardini L, Bersani M et al (2025) Sustainable production of microcrystalline and nanocrystalline cellulose from textile waste using HCl and NaOH/Urea treatment. *Polymers (Basel)*. <https://doi.org/10.3390/polym17010048>
8. Balart R, García-García D, Fombuena V et al (2021) Biopolymers from natural resources. *Polymers (Basel)* 13:2532. <https://doi.org/10.3390/polym13152532>
9. Gök C, Sulak M, Işıtan A (2024) Definition of Polymers. In: Oztekin Y, Sayin U (eds) *Biodegradable plastics for a green future*, 1st ed. pp 1–3
10. Silva RRA, Marques CS, Arruda TR et al (2023) Biodegradation of polymers: stages, measurement, standards and prospects. *Macromol* 3:371–399. <https://doi.org/10.3390/macromol3020023>
11. Okolie O, Kumar A, Edwards C et al (2023) Bio-based sustainable polymers and materials: from processing to biodegradation. *J Compos Sci* 7:213. <https://doi.org/10.3390/jcs7060213>
12. Khachatryan G, Khachatryan K, Szczepankowska J et al (2023) Design of carbon nanocomposites based on sodium alginate/chitosan reinforced with graphene oxide and carbon nanotubes. *Polymers* 15:925. <https://doi.org/10.3390/POLYM15040925>
13. Hay ID, Rehman ZU, Moradali MF et al (2013) Microbial alginate production, modification and its applications. *Microb Biotechnol* 6:637–650. <https://doi.org/10.1111/1751-7915.12076>
14. Yoo SM, Ghosh R (2014) Fabrication of alginate fibers using a microporous membrane based molding technique. *Biochem Eng J* 91:58–65. <https://doi.org/10.1016/J.BEJ.2014.07.006>
15. Ghosh S, Ranebennur TK, Vasan HN (2011) Study of antibacterial efficacy of hybrid chitosan-silver nanoparticles for prevention of specific biofilm and water purification. *Int J Carbohydr Chem* 2011:693759. <https://doi.org/10.1155/2011/693759>
16. Kong M, Chen XG, Xing K, Park HJ (2010) Antimicrobial properties of chitosan and mode of action: a state of the art review. *Int J Food Microbiol* 144:51–63. <https://doi.org/10.1016/J.IJFOODMICRO.2010.09.012>
17. Lode HM (2009) Clinical impact of antibiotic-resistant Gram-positive pathogens. *Clin Microbiol Infect* 15:212–217. <https://doi.org/10.1111/J.1469-0691.2009.02738.X>
18. Mahira S, Jain A, Khan W, Domb AJ (2019) Antimicrobial materials—an overview. In: *Antimicrobial materials for biomedical applications*. The Royal Society of Chemistry, pp 1–37
19. Jain A, Duvvuri LS, Farah S et al (2014) Antimicrobial polymers. *Adv Healthc Mater* 3:1969–1985. <https://doi.org/10.1002/ADHM.201400418>
20. Rhim JW, Park HM, Ha CS (2013) Bio-nanocomposites for food packaging applications. *Prog Polym Sci* 38:1629–1652. <https://doi.org/10.1016/j.progpolymsci.2013.05.008>
21. Hou X, Xue Z, Xia Y (2018) Preparation of a novel agar/sodium alginate fire-retardancy film. *Mater Lett* 233:274–277. <https://doi.org/10.1016/j.matlet.2018.09.026>
22. Esmaeili S, Akbari Aghdam H, Motififard M et al (2020) A porous polymeric-hydroxyapatite scaffold used for femur fractures treatment: fabrication, analysis, and simulation. *Eur J Orthop Surg Traumatol* 30:123–131. <https://doi.org/10.1007/s00590-019-02530-3>
23. Karamian E, Abdellahi M, Khandan A, Abdellah S (2016) Introducing the fluorine doped natural hydroxyapatite-titania nanobiocomposite ceramic. *J Alloys Compd* 679:375–383. <https://doi.org/10.1016/j.jallcom.2016.04.068>
24. Khandan A, Abdellahi M, Ozada N, Ghayour H (2016) Study of the bioactivity, wettability and hardness behaviour of the bovine hydroxyapatite-diopside bio-nanocomposite coating. *J Taiwan Inst Chem Eng* 60:538–546. <https://doi.org/10.1016/j.jtice.2015.10.004>
25. Khandan A, Abdellahi M, Barenji RV et al (2015) Introducing natural hydroxyapatite-diopside (NHA-Di) nano-bioceramic coating. *Ceram Int* 41:12355–12363. <https://doi.org/10.1016/j.ceramint.2015.06.065>

26. Arfat YA, Benjakul S, Prodpran T et al (2016) Physico-mechanical characterization and antimicrobial properties of fish protein isolate/fish skin gelatin-zinc oxide (ZnO) nanocomposite films. *Food Bioproc Tech* 9:101–112. <https://doi.org/10.1007/S11947-015-1602-0/METRICS>
27. Shipway AN, Willner I (2001) Nanoparticles as structural and functional units in surface-confined architectures. *Chem Commun* 20:2035–2045
28. Beecroft LL, Ober CK (1997) Nanocomposite materials for optical applications. *Chem Mater* 9:1302–1317. <https://doi.org/10.1021/CM960441A/ASSET/IMAGES/MEDIUM/CM960441AN00001.GIF>
29. Wetzel B, Hauptert F, Friedrich K et al (2002) Impact and wear resistance of polymer nanocomposites at low filler content. *Polym Eng Sci* 42:1919–1927. <https://doi.org/10.1002/PEN.11084>
30. Zhao G, Stevens SE (1998) Multiple parameters for the comprehensive evaluation of the susceptibility of *Escherichia coli* to the silver ion. *Biomaterials* 11:27–32. <https://doi.org/10.1023/A:1009253223055/METRICS>
31. Atiyeh BS, Costagliola M, Hayek SN, Dibo SA (2007) Effect of silver on burn wound infection control and healing: review of the literature. *Burns* 33:139–148. <https://doi.org/10.1016/J.BURNS.2006.06.010>
32. Raffi M, Hussain F, Bhatti TM et al (2008) Antibacterial characterization of silver nanoparticles against *E. coli* ATCC-15224. *J Mater Sci Technol* 24:192–196
33. Ahmad N, Sharma S (2012) Biosynthesis of silver nanoparticles from biowaste Pomegranate peels. *Int J Nanopart* 5:185–195. <https://doi.org/10.1504/IJNP.2012.048013>
34. Michels C, Perazzoli S, Soares MH (2017) Inhibition of an enriched culture of ammonia oxidizing bacteria by two different nanoparticles: Silver and magnetite. *Sci Total Environ* 586:995–1002. <https://doi.org/10.1016/J.SCITOTENV.2017.02.080>
35. Birkett M, Zia AW, Devarajan DK et al (2023) Multi-functional bioactive silver- and copper-doped diamond-like carbon coatings for medical implants. *Acta Biomater* 167:54–68. <https://doi.org/10.1016/J.ACTBIO.2023.06.037>
36. Abbasi E, Milani M, Aval SF et al (2016) Silver nanoparticles: synthesis methods, bio-applications and properties. *Crit Rev Microbiol* 42:173–180. <https://doi.org/10.3109/1040841X.2014.912200>
37. Reiad NA, Abdel Salam OE, Abadir EF, Harraz FA (2013) Green synthesis of antibacterial chitosan films loaded with silver nanoparticles. *Chin J Polym Sci* 31:984–993. <https://doi.org/10.1007/S10118-013-1263-2>
38. Ying S, Guan Z, Ofoegbu PC et al (2022) Green synthesis of nanoparticles: current developments and limitations. *Environ Technol Innov* 26:102336. <https://doi.org/10.1016/J.ETI.2022.102336>
39. Kajani AA, Bordbar A-K, Hamid S et al (2014) Green synthesis of anisotropic silver nanoparticles with potent anticancer activity using *Taxus baccata* extract. *RSC Adv* 4:61394–61403. <https://doi.org/10.1039/C4RA08758E>
40. Ping Y, Zhang J, Xing T et al (2018) Green synthesis of silver nanoparticles using grape seed extract and their application for reductive catalysis of Direct Orange 26. *J Ind Eng Chem* 58:74–79. <https://doi.org/10.1016/j.jiec.2017.09.009>
41. Abu Nayem SM, Sultana N, Haque MA et al (2020) Green synthesis of gold and silver nanoparticles by using *amorphophallus paeoniifolius* tuber extract and evaluation of their antibacterial activity. *Molecules*. <https://doi.org/10.3390/molecules25204773>
42. Singh H, Du J, Singh P, Yi T (2018) Ecofriendly synthesis of silver and gold nanoparticles by *Euphrasia officinalis* leaf extract and its biomedical applications. *Artif Cells Nanomed bBiotechnol* 46:1163–1170. <https://doi.org/10.1080/21691401.2017.1362417>
43. Annu SA, Nirala RK et al (2021) Green synthesis of chitosan/nanosilver hybrid bionanocomposites with promising antimicrobial, antioxidant and anticervical cancer activity. *Polym Polym Compos* 29:199–210. <https://doi.org/10.1177/0967391121993977>
44. Khatami M, Sharifi I, Nobre MAL et al (2018) Waste-grass-mediated green synthesis of silver nanoparticles and evaluation of their anticancer, antifungal and antibacterial activity. *Green Chem Lett Rev* 11:125–134. <https://doi.org/10.1080/17518253.2018.1444797>
45. Jilani MI, Ali A, Rehman R, et al (2015) Health benefits of Arugula: a review
46. Awadelkareem AM, Al-Shammari E, Elkhaila AO et al (2022) Biosynthesized silver nanoparticles from *Eruca sativa* miller leaf extract exhibits antibacterial, antioxidant, anti-quorum-sensing, antibiofilm, and anti-metastatic activities. *Antibiotics*. <https://doi.org/10.3390/antibiotics11070853>
47. Andrade RMS, Ferreira MSL, Gonçalves ECBA (2016) Development and characterization of edible films based on fruit and vegetable residues. *J Food Sci* 81:E412–E418. <https://doi.org/10.1111/1750-3841.13192>

48. Brito TB, Carrajola JF, Gonçalves ECBA et al (2019) Fruit and vegetable residues flours with different granulometry range as raw material for pectin-enriched biodegradable film preparation. *Food Res Int* 121:412–421. <https://doi.org/10.1016/j.foodres.2019.03.058>
49. Alaraidh IA, Ibrahim MM, El-Gaaly GA (2014) Evaluation of green synthesis of Ag nanoparticles using *Eruca sativa* and *Spinacia oleracea* leaf extracts and their antimicrobial activity. *Iran J Biotechnol*. <https://doi.org/10.5812/ijb.12392>
50. García-Gurrola A, Escobar-Puentes AA, Rincón S et al (2021) Succinylated starch nanocapsules loaded with the polyphenolic extract from arugula (*Eruca sativa*) Leaves: colloidal, chemical, and structural properties. *Starch/Staerke*. <https://doi.org/10.1002/STAR.202100059>
51. Gözükar G, Altunbaş S, Sönmez NK et al (2019) The effect of solid and liquid organic fertilizer applications on spectral reflection in rocket (*Lactuca sativa* L.) cultivation. *Yuzuncu Yil Univ J Agric Sci* 29:641–651. <https://doi.org/10.29133/yyutbd.595836>
52. Sharma S, Sanpui P, Chattopadhyay A, Ghosh SS (2012) Fabrication of antibacterial silver nanoparticle—sodium alginate—chitosan composite films. *RSC Adv* 2:5837–5843. <https://doi.org/10.1039/C2RA00006G>
53. Vimala K, Mohan Y, Sivudu K et al (2010) Fabrication of porous chitosan films impregnated with silver nanoparticles: a facile approach for superior antibacterial application. *Colloids Surf B Biointerfaces* 76:248–258. <https://doi.org/10.1016/j.colsurfb.2009.10.044>
54. Pinto R, Fernandes S, Pinto RJ et al (2012) Antibacterial activity of optically transparent nanocomposite films based on chitosan or its derivatives and silver nanoparticles. *Carbohydr Res* 348:77–83. <https://doi.org/10.1016/j.carres.2011.11.009>
55. Siemann U (2005) Solvent cast technology—A versatile tool for thin film production. *Prog Colloid Polym Sci* 130:1–14. <https://doi.org/10.1007/B107336>
56. Kadam D, Momin B, Palamthodi S, Lele SS (2019) Physicochemical and functional properties of chitosan-based nano-composite films incorporated with biogenic silver nanoparticles. *Carbohydr Polym* 211:124–132. <https://doi.org/10.1016/j.carbpol.2019.02.005>
57. Lavrič G, Oberlintner A, Filipova I et al (2021) Functional nanocellulose, alginate and chitosan nanocomposites designed as active film packaging materials. *Polymers (Basel)* 13:2523. <https://doi.org/10.3390/polym13152523>
58. Testai L, Pagnotta E, Piragine E et al (2022) Cardiovascular benefits of *Eruca sativa* mill. Defatted seed meal extract: potential role of hydrogen sulfide. *Phytother Res* 36:2616–2627. <https://doi.org/10.1002/PTR.7479>
59. Tasca F, Antiochia R (2020) Biocide activity of green quercetin-mediated synthesized silver nanoparticles. *Nanomaterials* 10:909. <https://doi.org/10.3390/NANO10050909>
60. Baláž M, Balážová L, Daneu N et al (2017) Plant-mediated synthesis of silver nanoparticles and their stabilization by wet stirred media milling. *Nanoscale Res Lett* 12:1–9. <https://doi.org/10.1186/S11671-017-1860-Z/FIGURES/8>
61. Sulak M (2021) Preparation of G-CuO NPs and G-ZnO NPs with mallow leaves, investigation of their antibacterial behavior and synthesis of bis (indolyl) methane compounds under. *Turk J Chem* 45:1517–1532. <https://doi.org/10.3906/kim-2105-31>
62. Al-Fakeh MS, Gassoumi M, Rabhi M et al (2021) Biosynthesis and characterization of Saudi propolis-mediated silver nanoparticles and their biological properties. *Open Phys* 19:753–757. <https://doi.org/10.1515/PHYS-2021-0091/HTML>
63. Garibo D, Borbón-Núñez HA, de León JND et al (2020) Green synthesis of silver nanoparticles using *Lysiloma acapulcensis* exhibit high-antimicrobial activity. *Sci Rep* 10:1–11. <https://doi.org/10.1038/s41598-020-69606-7>
64. Chinni SV, Gopinath SCB, Anbu P et al (2021) Characterization and antibacterial response of silver nanoparticles biosynthesized using an ethanolic extract of *Coccinia indica* Leaves. *Crystals* 11:97. <https://doi.org/10.3390/CRYST111020097>
65. Rakhee M, Mishra J, Sharma P, Misra K (2018) Chromatographic determination of bioactive compounds in Hippophae leaf extracts: a comparative study of three varieties. *Def Life Sci J* 3:255. <https://doi.org/10.14429/dlsj.3.12913>
66. Tejamaya M, Römer I, Merrifield RC, Lead JR (2012) Stability of citrate, PVP, and PEG coated silver nanoparticles in ecotoxicology media. *Environ Sci Technol* 46:7011–7017. <https://doi.org/10.1021/ES2038596>
67. Dağlioğlu Y (2018) Nanopartikül Karakterizasyon Yöntemleri ve Ekotoksiste Deneylerindeki Önemi. *Marmara Fen Bilimleri Dergisi* 30:1–17. <https://doi.org/10.7240/MARUFBD.346547>

68. Lozano-Navarro JI, Díaz-Zavala NP, Melo-Banda JA et al (2019) Chitosan–starch films modified with natural extracts to remove heavy oil from water. *Materials* 11:120. <https://doi.org/10.3390/w12010017>
69. Masamba K, Li Y, Hategekimana J et al (2016) Effect of Gallic acid on mechanical and water barrier properties of zein-oleic acid composite films. *J Food Sci Technol* 53:2227–2235. <https://doi.org/10.1007/S13197-015-2167-7>
70. Giyatmi G, Irianto H, Anggoro B, Fransiska D (2021) Use of basil leaf ethanol extract in alginate base edible film. *J Phys Conf Ser*. <https://doi.org/10.1088/1742-6596/1933/1/012001>
71. The Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing. <https://clsi.org/about/press-releases/clsi-publishes-m100-performance-standards-for-antimicrobial-susceptibility-testing-31st-edition/>. Accessed 31 Aug 2024
72. Öztaş E, Ünver Koluman B, Koluman A (2024) Kültür Mantarından (*Agaricus bisporus*) İzole Edilen Kitosanın Hemostatik ve Antimikrobiyal Yara Örtüsünde Kullanımın Araştırılması
73. Jakubowska E, Gierszewska M, Nowaczyk J, Olewnik-Kruszkowska E (2020) Physicochemical and storage properties of chitosan-based films plasticized with deep eutectic solvent. *Food Hydrocoll* 108:106007. <https://doi.org/10.1016/j.foodhyd.2020.106007>
74. Liu T, Wang J, Chi F et al (2020) Development and characterization of novel active chitosan films containing fennel and peppermint essential oils. *Coatings* 10:936. <https://doi.org/10.3390/coatings10100936>
75. Venkatesan J, Lee J, Kang D et al (2017) Antimicrobial and anticancer activities of porous chitosan-alginate biosynthesized silver nanoparticles. *Int J Biol Macromol* 98:515–525. <https://doi.org/10.1016/j.ijbiomac.2017.01.120>
76. Albert HM, Mendam K, Bansod PG et al (2023) Biosynthesis, spectroscopic, and antibacterial investigations of silver nanoparticles. *J Fluoresc*. <https://doi.org/10.1007/s10895-023-03398-7>
77. Zhang W, Gao P, Jiang Q, Xia W (2023) Green fabrication of lignin nanoparticles/chitosan films for refrigerated fish preservation application. *Food Hydrocoll* 139:108548. <https://doi.org/10.1016/j.foodhyd.2023.108548>
78. Krishnaveni B, Ragunathan R (2015) Extraction and characterization of Chitin and Chitosan from *Aspergillus terreus* sps, synthesis of their bionanocomposites and study of their productive applications. *J Chem Pharm Res* 7:115–132
79. Ghosh T, Katiyar V (2022) Nanochitosan functionalized hydrophobic starch/guar gum biocomposite for edible coating application with improved optical, thermal, mechanical, and surface. *Int J Biol Macromol* 211:116–127. <https://doi.org/10.1016/j.ijbiomac.2022.05.079>
80. Mondéjar-López M, Rubio-Moraga A, José López-Jimenez A et al (2022) Chitosan nanoparticles loaded with garlic essential oil: a new alternative to tebuconazole as seed dressing agent. *Carbohydr Polym* 277:118815. <https://doi.org/10.1016/j.carbpol.2021.118815>
81. Khan Z, Al-Thabaiti SA, Obaid AY, Al-Youbi AO (2011) Preparation and characterization of silver nanoparticles by chemical reduction method. *Colloids Surf B Biointerfaces* 82:513–517. <https://doi.org/10.1016/j.colsurfb.2010.10.008>
82. Mushi NE, Utsel S, Berglund LA (2014) Nanostructured biocomposite films of high toughness based on native chitin nanofibers and chitosan. *Front Chem* 2:99. <https://doi.org/10.3389/FCHEM.2014.00099/FULL>
83. Russo R, Malinconico M, Santagata G (2007) Effect of cross-linking with calcium ions on the physical properties of alginate films. *Biomacromol* 8:3193–3197. <https://doi.org/10.1021/BM700565H>
84. Krumova M, Lopez D, Benavente R, Mijangos C (2000) Effect of crosslinking on the mechanical and thermal properties of poly (vinyl alcohol). *Polymer* 41:9265–9272. [https://doi.org/10.1016/S0032-3861\(00\)00287-1](https://doi.org/10.1016/S0032-3861(00)00287-1)
85. Kim Y, Yoon K, Ko S (2000) Preparation and properties of alginate superabsorbent filament fibers crosslinked with glutaraldehyde. *J Appl Polym Sci* 78:1797–1804.
86. Kahya N, Kestir S, Öztürk S et al (2022) Antioxidant and antimicrobial chitosan films enriched with aqueous sage and rosemary extracts as food coating materials: characterization of the films and detection. *Int J Biol Macromol* 217:470–480. <https://doi.org/10.1016/j.ijbiomac.2022.07.073>
87. Ji S, Sun R, Wang W, Xia Q (2023) Preparation, characterization, and evaluation of tamarind seed polysaccharide-carboxymethylcellulose buccal films loaded with soybean peptides-chitosan. *Food Hydrocoll* 141:108684. <https://doi.org/10.1016/j.foodhyd.2023.108684>
88. De MV, Cascione M, Costa D et al (2023) *Aloe vera* silver nanoparticles addition in chitosan films: improvement of physicochemical properties for eco-friendly food packaging material. *J Mater Res Technol* 24:1015–1033. <https://doi.org/10.1016/j.jmrt.2023.03.025>

89. Nunthanid J, Puttipipatkachorn S, Yamamoto K, Peck GE (2001) Physical properties and molecular behavior of chitosan films. *Drug Dev Ind Pharm* 27:143–157. <https://doi.org/10.1081/DDC-100000481>
90. Zhang G, Guan T, Zhang J, Zhang T (2021) Theoretical and experimental investigation of sodium alginate composite films containing star anise ethanol extract/hydroxypropyl- β -cyclodextrin inclusion complex. *J Food Sci* 86:434–442. <https://doi.org/10.1111/1750-3841.15564>
91. Hadi A, Nawab A, Alam F, Zehra K (2022) Alginate/*Aloe vera* films reinforced with tragacanth gum. *Food Chem Mol Sci* 4:100105. <https://doi.org/10.1016/j.fochms.2022.100105>
92. Rahman N, Dafader N, Banu P (2017) Preparation and property analysis of biodegradable packaging film from alginate, starch and citric acid. *J Polym Sci Technol* 2:20–35
93. Al-Remawi M (2012) Sucrose as a crosslinking modifier for the preparation of calcium alginate films via external gelation. *J Appl Sci* 12:727–735. <https://doi.org/10.3923/jas.2012.727.735>
94. Tavassoli-Kafrani E, Shekarchizadeh H, Masoudpour-Behabadi M (2016) Development of edible films and coatings from alginates and carrageenans. *Carbohydr Polym* 137:360–374. <https://doi.org/10.1016/j.carbpol.2015.10.074>
95. Chicea D, Nicolae-Maranciuc A, Chicea LM (2024) Silver nanoparticles-chitosan nanocomposites: A comparative study regarding different chemical syntheses procedures and their antibacterial effect. *Materials*. <https://doi.org/10.3390/ma17051113>
96. Shehabeldine AM, Salem SS, Ali OM et al (2022) Multifunctional silver nanoparticles based on chitosan: antibacterial, antibiofilm, antifungal, antioxidant, and wound-healing activities. *J Fungi* 8:612. <https://doi.org/10.3390/JOF8060612>
97. Erkan HE (2023) Production of nanosilver reinforced biodegradable polymer coating material obtained by green synthesis method. Pamukkale University, Kınıklı
98. Future Bio. <https://www.futurebioproject.eu/en#ev>. Accessed 1 Sep 2024

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