Erzincan Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
2024, ÖZEL SAYI, 99-115
ISSN: 1307-9085, e-ISSN: 2149-4584
Araştırma Makalesi

Erzincan University
Journal of Science and Technology
2024, SPECIAL ISSUE, 99-115
DOI: 10.18185/erzifbed.515128
Research Article

Production of Silver Nanoparticles from Seagrass Wastes by Green Synthesis and Determination of Their Antimicrobial Activities

Aysegul INAM[1,2], Nihal OZEL[3], Zulal GUNAY[1], Murat ELIBOL[1*]

[1]Department of Bioengineering, Faculty of Engineering, Ege University, Izmir, Türkiye [2]Department of Bioengineering, Faculty of Engineering and Natural Sciences, Manisa Celal Bayar University, Manisa, Türkiye
[3]Department of Biotechnology, Graduate School of Natural and Applied Sciences, Ege University, Izmir, Türkiye

Received: 12/07/2024, **Revised:** 24/12/2024, **Accepted:** 29/12/2024, **Published:** 31/12/2024

Abstract
Green synthesis of silver nanoparticles (AgNPs) has attracted great interest in the field of nanotechnology due to their antimicrobial and antioxidant properties in different applications. Posidonia leaves, known as seagrasses, detach from their stems during their life cycle and are carried by sea currents to form deposits on the shore. These biomass wastes hold the potential to be a source for nanoparticle synthesis. In this study, we present the synthesis of AgNPs using seagrass wastes without any chemical stabilizers or reducers. The seagrass extract is used as a reducing agent for synthesizing AgNPs at room temperature. The total phenolic and carbohydrate contents of extracts were analyzed using spectrophotometric methods. Ultraviolet-visible spectroscopy (UV–Vis), dynamic light scattering (DLS), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) were used to characterize the synthesized nanoparticles. The visual color change confirmed the formation of AgNPs. The UV-visible spectrophotometer showed an absorption peak at 420 nm. DLS measurements estimated the AgNPs size at approximately 50 nm. The AgNPs exhibited antibacterial activity against Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) microorganisms and antifungal activity against Candida glabrata. Antioxidant activities of the seagrass extract and AgNPs were also evaluated. This study highlights the successful use of a waste biological material in AgNPs production via green synthesis methods, showing promise across various fields.

Keywords: Seagrass, green synthesis, silver nanoparticles, antibacterial activity, antifungal activity

Deniz Çayırı Atıklarından Yeşil Sentez ile Gümüş Nanopartikül Üretimi ve Antimikrobiyal Aktivitelerinin Belirlenmesi

Özet
Gümüş nanopartiküllerin (AgNP) yeşil sentezi, farklı uygulamalardaki antimikrobiyal ve antioksidan özellikleri nedeniyle nanoteknoloji alanında büyük ilgi görmüştür. Deniz çayırları olarak bilinen *Posidonia* yaprakları, yaşam döngüleri sırasında gövdelerinden ayrılmakta ve deniz akıntıları tarafından taşınarak kıyıda birikintiler oluşturmaktadır. Bu biyokütle atığı nanopartikül sentezi için bir kaynak olma potansiyeline sahiptir. Bu çalışmada, herhangi bir kimyasal stabilizatör veya indirgeyici olmadan deniz çayırı atığı kullanılarak AgNP'lerin sentezi hedeflenmiştir. Deniz çayırı su ekstraktı, AgNP'lerin oda sıcaklığında sentezlenmesi için indirgeyici bir ajan olarak kullanılmıştır. Ekstraktların toplam fenolik ve karbonhidrat içerikleri spektrofotometrik yöntemler kullanılarak analiz edilmiştir. Sentezlenen nanopartikülleri karakterize etmek için Ultraviyole-görünür spektroskopi (UV-Vis), dinamik ışık saçılımı (DLS), Fourier dönüşümlü kızılötesi spektroskopi (FTIR) ve X-ışını kırınımı (XRD) kullanılmıştır. Görsel renk değişimi AgNP'lerin oluşumunu doğrulamıştır. UV-görünür spektrofotometre 420 nm dalga boyunda bir absorpsiyon piki göstermiştir. DLS ölçümleri AgNP'lerin boyutunu yaklaşık 50 nm olarak tespit etmiştir. AgNP'ler Gram-pozitif (*Staphylococcus aureus*) ve Gram-negatif (*Escherichia coli*) mikroorganizmalara karşı antibakteriyel aktivite ve *Candida glabrata*'ya karşı antifungal aktivite sergilemiştir. Deniz çayırının su ekstraktı ve AgNP'lerin antioksidan aktiviteleri de değerlendirilmiştir. Bu çalışma, atık bir biyolojik materyalin yeşil sentez yöntemleriyle AgNP üretiminde başarılı bir şekilde kullanıldığını vurgulamakta ve çeşitli alanlarda umut vaat etmektedir.

Anahtar Kelimeler: Deniz çayırı, yeşil sentez, gümüş nanopartikül, antibakteriyel aktivite, antifungal aktivite

*Corresponding Author: murat.elibol@ege.edu.tr
Aysegul INAM, https://orcid.org/0000-0002-9411-1232
Nihal OZEL, https://orcid.org/0000-0002-4380-6819
Zulal GUNAY, https://orcid.org/0000-0003-3893-9876
Murat ELIBOL, https://orcid.org/0000-0002-6756-6290

1. Introduction

Nanotechnology is a new form of technology that has led to major developments in various fields. The unique properties and applications of nanoparticles make them particularly useful in the field of biotechnology [1]. There are bottom-up and top-down approaches for metal nanoparticle synthesis. In the bottom-up approach, which involves chemical and biological methods, it can be synthesized by self-assembly of atoms into new nuclei that become a particle on the nanoscale. In the top-down approach, the bulk material is broken down into fine particles through size reduction using a variety of physical lithographic techniques [2]. Green synthesis is an environmentally friendly and biocompatible nanoparticle production process, usually using a capping agent/stabilizer, yeast, bacteria or plant extract [3]. The reduction and also stabilization of metal ions are achieved by combining biomolecules already present in plant extracts such as carbohydrates, amino acids, enzymes, phenolics, alkaloids, tannins, saponins, and vitamins [2]. Biological synthesis is a good way to produce nanostructured materials with good properties, as well as to decrease the use or production of substances hazardous to the environment and human health [4].

Silver nanoparticles (AgNPs) can be synthesized using a variety of chemical, physical and biological methods. These nanoparticles have made significant contributions in several applications such as drug delivery, cell biology, nanomedicine, antioxidants, chemical sensing, agriculture, the food industry, cosmetics, textiles, photocatalytic organic dye degradation, and antimicrobial agents. Bacterial resistance caused by the overuse and even misuse of antibiotics has paved the way for the search for new antimicrobial agents. According to the studies, nanoparticles synthesized from different plant powders showed antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia*. Among the most widely used nanoparticles with a broad spectrum of antibacterial activity are AgNPs [1,3,5].

The potential for finding new, commercially valuable phytochemicals in seagrasses is enormous. Seagrass meadows are periodically shed and washed ashore by waves. They produce large quantities of leaf material and often form banks of seagrass wastes on the shore. It seems of interest to assess the potential of seagrass wastes for the production of bioactive substances [6]. Considering the studies, the carbohydrate, protein, and lipid contents, as well as the antioxidant activities, were determined by different methods even in waste biomass [7]. It aims to utilize this biomass, which is considered waste, in nanoparticle production by the green synthesis method.

In this study, a biological, non-toxic and environmentally friendly method was used to synthesize AgNPs. Seagrass wastes extract was successfully used for the reduction of $AgNO_3$ into AgNPs through its bioactive ingredients. Ultraviolet-visible spectroscopy (UV–Vis), dynamic light scattering (DLS), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) were used to characterize the synthesized AgNPs. The antimicrobial and antioxidant activities of the AgNPs were also evaluated.

2. Material and Methods

2.1. Preparation of seagrass aqueous extract

Seagrass extract was used to produce AgNPs due to its cost-effectiveness and easy availability. Seagrass leaf wastes were collected from Izmir-Karaburun. They were cleaned with running tap water to remove residues, then washed with double distilled water and dried at room temperature for 48 hours. According to preliminary experiments, 0.286 g of seagrass wastes were weighed, 10 mL of deionized water was added, and extraction was carried out at 82.5 °C in a shaking water bath (Wisd) for 1 hour. The preliminary data showed that the maximum boiling temperature was 82.5 °C, and the biomass-solvent ratio was optimized. The resulting solution was centrifuged (Nüve, NF400) at 4100 r/min for 10 min. For the synthesis of AgNPs, the supernatant was retained. The extract was cooled, filtered and stored at 4°C until needed.

2.2. Analysis of aqueous extract

2.2.1. Determination of total carbohydrates

The Dubois method was used to measure the total carbohydrate content in the extracts. Glucose (Tekkim, TK.090271) was prepared as a standard solution. Firstly, 0.5 ml of the extract was taken and transferred to a glass tube. Water was used for the blank. 0.5 ml of 5% phenol (Merck) solution was added to the prepared tubes. Then 2.5 ml of concentrated sulphuric acid (Merck) was added and vortexing was performed. After 15 minutes in a water bath, the absorbance value against the blank was recorded in a spectrophotometer (EasyPlus, Mettler Toledo) at 490 nm wavelength. Results were calculated according to the glucose standard graph [8].

2.2.2. Determination of total phenolics

Total phenolic content was determined according to the Folin-Ciocalteu method. Gallic acid (Sigma-Aldrich) was used as standard (ranging from 0.2 mg/mL to 0.005 mg/mL). 200 µL 0.2N Folin-Ciocalteu reagent (Sigma-Aldrich) was added to 100 µL sample extract and mixed. Then 2 mL of distilled water and 1 mL of 6% sodium carbonate solution (Na_2CO_3) (Merck) were added and vortexed. The mixture was kept at room temperature and in the dark for 2 hours. The absorbance was read at 765 nm in a spectrophotometer. The same procedure was applied for gallic acid standards. The gallic acid calibration curve was plotted, and the results were calculated as gallic acid equivalent (GAE) [9].

2.3. Green synthesis of silver nanoparticles

According to the results of preliminary experiments, 10 ml of extract was added to 90 ml of 1.89 mM $AgNO_3$ solution (Sigma-Aldrich) drop by drop. The solution containing AgNPs was confirmed by a visible color change following 24 hours. No external chemicals were required to stabilize the AgNPs produced by this process due to secondary metabolites in the seagrass wastes. After the synthesis of AgNPs, the solution containing the nanoparticles was centrifuged

at 9000 r/min for 15 minutes to separate the AgNPs from the rest of the solution composition. Then, AgNPs were dried at 60 °C for 48 hours and obtained in powder form.

2.4. Characterization of silver nanoparticles

2.4.1. UV-Vis Spectroscopy

The formation of AgNPs was confirmed using UV-Vis spectroscopy. The optical absorbance of nanoparticles was recorded in a UV-Vis spectrophotometer (EasyPlus, Mettler Toledo) in the wavelength range 190-900 nm. The maximum peak absorbance value of AgNP was determined.

2.4.2. Dynamic Light Scattering (DLS)

The average size of the AgNPs was measured using dynamic light scattering (Malvern Zetasizer Analyzer). It was also used to characterize the size distribution of the particles in the colloidal suspension. For the samples, the measurements were repeated three times.

2.4.3. Fourier Transform Infrared (FTIR)

Structural characteristics of the AgNPs were investigated using a Fourier Transform Infrared (FTIR) spectroscopy (Thermo Scientific, Nicolet, IS20, USA) in the wavenumber range of 4000-500 cm^{-1}. This method is used to characterize the surface chemistry and the bonds between functional groups. Organic functional groups such as carbonyl and hydroxyl are bound to the nanoparticle surface and other surface chemical residues are detected. The dried powder material was analyzed.

2.4.4. X-ray Diffractometer (XRD)

The phase compositions and quality of nanoparticles were identified by the XRD (X-ray diffractometer; PANanalytical Empyrean, U.K.) using Cu Kα radiation between 2θ = 10–80 ° with scan speed 2 °/min. Also, The Debye-Scherrer equation (1) was used to evaluate the size of the synthesized AgNPs. D is the average size of AgNPs, K is constant (K = 0.94), λ is the wavelength of X-ray (0.1546 nm), θ is diffraction angle (in degrees) and β is the width of the maximum peak at half of height [1].

$$D = \frac{K.\lambda}{\beta.cos\theta} \qquad \text{Equation (1)}$$

2.5. Antimicrobial activities of silver nanoparticles

2.5.1. Antibacterial activity

Antibacterial assays were performed on Gram-positive *Staphylococcus aureus* (ATCC-6538) and Gram-negative *Escherichia coli* (ATCC-25922) using the Kirby-Bauer disk diffusion

method [10]. The turbidity of the bacteria was diluted so that the absorbance at 600 nm wavelength corresponded to 0.6 and inoculated into Mueller Hinton Agar (Merck, 103872) by the spread plate method according to the calculations made. After inoculation, seagrass extract, $AgNO_3$, AgNP and control samples (gentamicin as positive control, distilled water as negative control) were placed on agar by impregnating sterile antibacterial assay discs (Oxoid) with a diameter of 6 mm in 25 µL volume. To examine the antibacterial effects of the samples, analyzes were also performed on cotton fabrics. In this context, fabric samples known to be 100% cotton were sterilized by cutting 1x1 cm in size, and the sterilized fabrics were immersed in the samples for 30 minutes. The prepared fabric samples were placed on agar plates and their antibacterial effects were observed as zones. The petri dishes were then incubated at 37°C for 24 hours and antibacterial inhibition zones were determined by measuring the zone diameters around the disks and fabrics [11,12].

2.5.2. Antifungal activity

Human pathogens *Candida albicans* (ATCC-10231) and *Candida glabrata* (ATCC-90018) were used to determine antifungal activity. *C. albicans* and *C. glabrata* strains were grown in Sabouraud Dextrose-Broth medium (Oxoid) and the cells were separated from the medium by centrifugation. The cells were washed with phosphate-buffered-saline (PBS Oxoid, BR0014G) to obtain pure cells. *Candida* suspensions prepared in phosphate buffer were adjusted to Mc-Farland 0.5 turbidity standard (10^8 cfu/ml) [13]. After this, dilutions were performed under aseptic conditions so that the final *Candida* concentration in Potato Dextrose Agar (PDA) media (Merck) was 10^5 cfu/ml. Wells were made in PDA plates with a 5 mm diameter hollow sterile glass rod and 100 µl of sample was added to the wells and the plates were incubated at 37°C for 24 hours. Inhibition zones formed at the end of incubation were measured [14]. The antifungal agent fluconazole was used as a positive control during the experiments.

2.6. Determination of antioxidant activity

Antioxidant activity was determined according to the DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Tokyo Chemical Industry Co. Ltd.) method. 50 µL sample extract and AgNPs were separately taken into glass test tubes and 150 µL methanol (Merck) solution was added. Then 3.8 mL of DPPH solution was added. The tubes were vortexed and kept in the dark for 30 minutes. Absorbance values were read at 515 nm in a spectrophotometer. Methanol was used as a positive control. The results obtained in this method were expressed as % inhibition (Equation 2). For comparison, the same procedure was performed for BHA (Butylated hydroxyanisole) (Sigma Aldrich) [15].

$$Inhibition\% = \frac{Absorbance_{\ positive\ control} - Absorbance_{\ sample}}{Absorbance_{\ positive\ control}} \times 100 \quad \text{Equation (2)}$$

3. Results and Discussion

3.1. Analysis of aqueous extract

Synthesis of nanoparticles is possible with compounds such as terpenoids, phenolics, flavanones, amines, amides, carbohydrates, proteins, pigments, alkaloids, etc. [16]. Phenolic compounds and carbohydrates potentially present in seagrass wastes may play a role in the reduction of silver ions. Therefore, the total carbohydrates, total amount of phenolic substances and antioxidant activity of the aqueous extract were determined by spectrophotometric methods. Total carbohydrate amounts, total phenolic amounts and antioxidant activity (DPPH) were measured as 96 ± 3.5 µg/ml, 1 ± 0.4 µg GAE/ml and 15.13 ± 2.3%, respectively. In a study, the polyphenolic content was 19.712 ± 0.496 mg GAE/g and IC_{50} value of antioxidant activity according to DPPH 0.090 µg/µL in 70% ethanolic extract of seagrass leaves. Therefore, ethanol extraction of seagrass waste may be higher in phenol compounds than extraction with water [17]. It is mainly the phenolic content that is responsible for the antioxidant activity (Table 1). Phenolics are considered powerful antioxidants which can help prevent excessive damage caused by free radicals and chronic disease. The source of the antioxidant capacity of the phenolic acids is the phenolic hydroxyl group, so the position and number of the phenolic hydroxyl groups is directly in connection with their antioxidant activity. The antioxidant capacity of phenolic acids is also significantly influenced by methoxy and carboxylic acid groups [18].

Table 1. Seagrass extract analysis

	Total carbohydrates (µg/ml)	Total phenolics (µg GAE/ml)	Antioxidant Activity (%)
Seagrass extracts	96 ± 3.5	1 ± 0.4	15.13 ± 2.3

The green synthesis of AgNPs was performed by the addition of seagrass extract to aqueous silver nitrate solutions and the reduction of Ag^{2+} ions to Ag^{0}. As a primary analysis, the changing color of the solution indicates the formation of AgNPs (Figure 1) and was also compared to visual observation as the negative control (aqueous solution of silver nitrate only) was colorless [19].

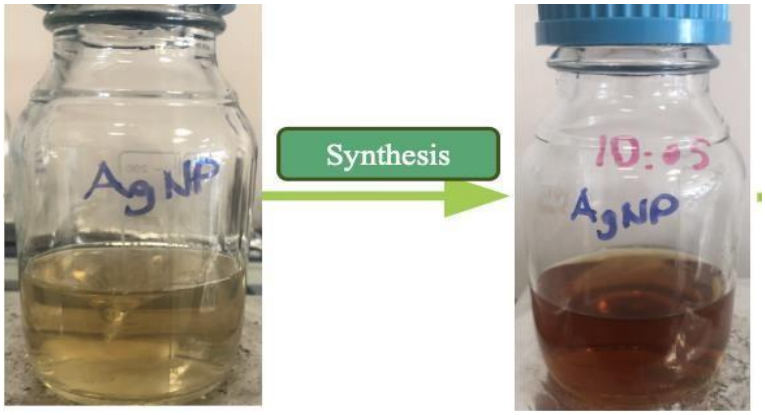

Figure 1. The change in solution color after AgNPs synthesis

3.2. Characterization of silver nanoparticles

In the characterization of AgNPs, UV-Vis spectroscopy, DLS, FTIR and XRD methods were applied. The size of these AgNPs is 49.55 nm and the polydispersity (PDI) value is 0.278 according to the DLS size distribution histogram based on three repeated readings (Figure 2). In a study related to AgNPs from *Berberis vulgaris*, the average size of the nanoparticle is within 90-100 nm while the PDI value is 0.281 [20]. The PDI value, which is an indicator of nanoparticle stability and the homogeneity of formation, also reflects the nanoparticle size distribution. Samples with a wider range of particle sizes have higher PDI values, while samples consisting of equally sized particles have lower PDI values [21]. The PDI value ranges from a value of 0.01 to 0.5 - 0.7 for monodispersed particles, and samples with very wide size distribution have PDI > 0.7 [22]. AgNPs obtained using seagrass extract in our study are within the desired size range. The fact that the PDI value of the nanoparticles is less than 0.7 indicates that the desired nanoparticle distribution is achieved. When the literature data are examined, it is seen that the nanoparticles we obtained are compatible with the literature in terms of size and distribution.

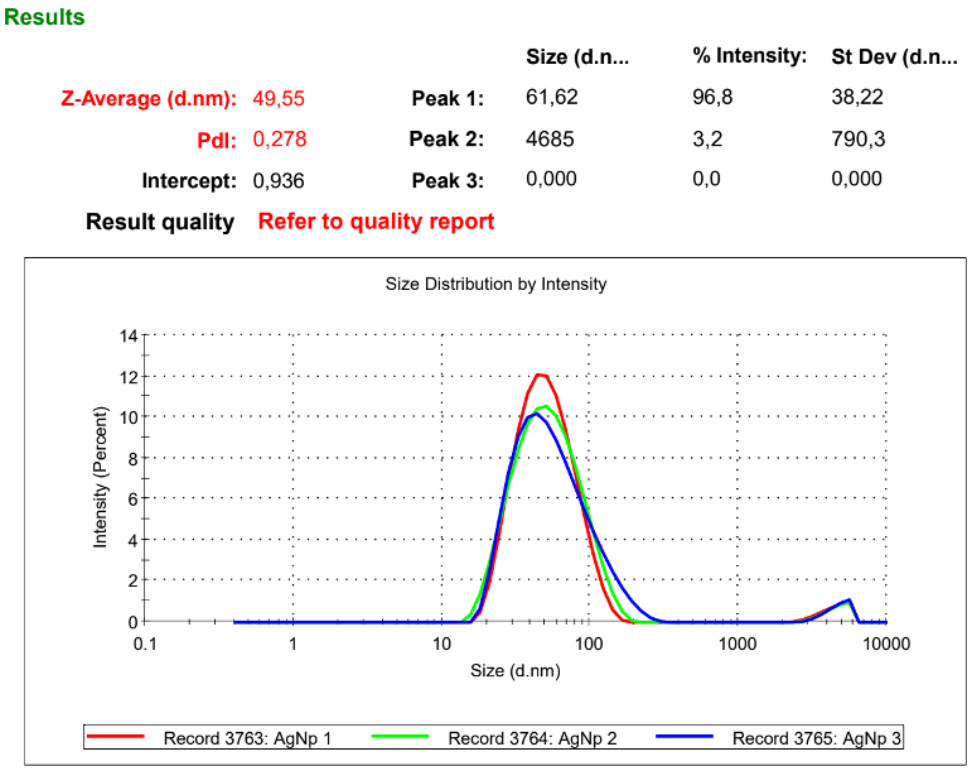

Figure 2. DLS histogram of AgNPs

UV-Vis spectrometry is a broadly used spectral tool to confirm the formation of nanoparticles through the surface plasmon resonance phenomenon of metallic nanoparticles in a colloidal solution. In the UV-vis spectrum, the surface plasmon resonance of AgNPs occurred near 420 nm (Figure 3). The reduction of silver nitrate to AgNPs is indicated by this peak. According to

Grand et al. (2019), the band corresponding to absorption by AgNPs is in the region of 400-450 nm, due to the excitation of surface plasmon vibrations [23]. In a study, for AgNPs obtained from grape and orange extracts, the typical absorption bands were observed at 430 and 390 nm, respectively [24]. Salayová et al., (2021) studied the production of AgNPs from extracts of different parts of plants such as *Berberis vulgaris, Capsella bursa-pastoris, Lavandula angustifolia, Origanum vulgare* and found the surface plasmon resonance values 421, 422, 412 and 426 nm, respectively [5].

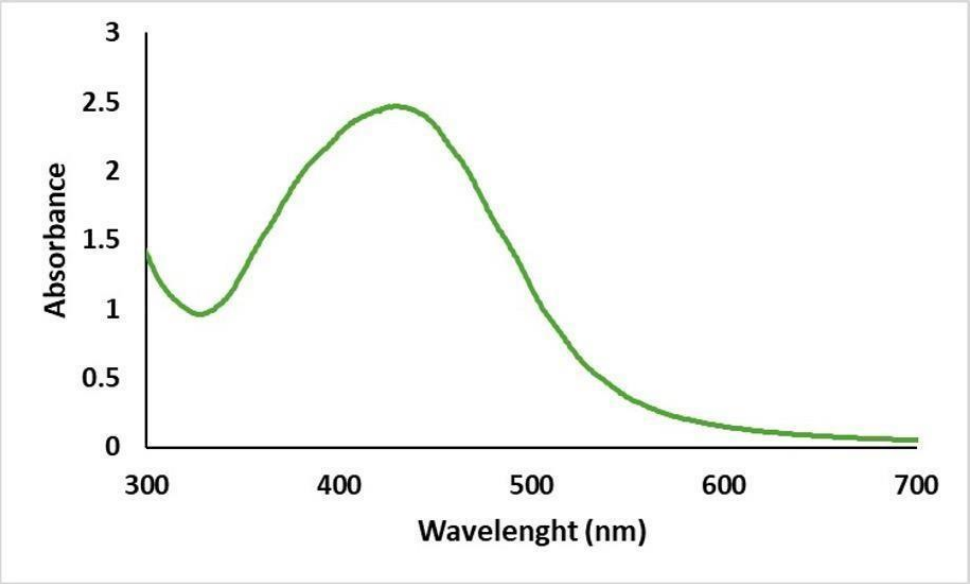

Figure 3. UV-Vis spectra of AgNPs

The extract's role as a reducing and capping agent and the presence of some functional groups were confirmed by FTIR analysis. Functional groups at different wavenumbers were observed in the AgNPs (Figure 4). The FTIR spectra of AgNPs reveal clear peaks at 3420 cm^{-1} indicating amide class with N-H stretch; 2938 cm^{-1} alkenes and alkyls by C-H stretching (presence of the polyphenolic compound), 1669 cm^{-1} amides with C=O stretch, 1384 cm^{-1} alkanes with C-H bend; 1043 cm^{-1} alcohols C-O stretching, and 761 cm^{-1} C-H bending which is in line with the literature. Based on the FTIR results, the compounds extracted from the seagrass wastes play an important role in reducing, stabilizing and capping functions [5,25,26].

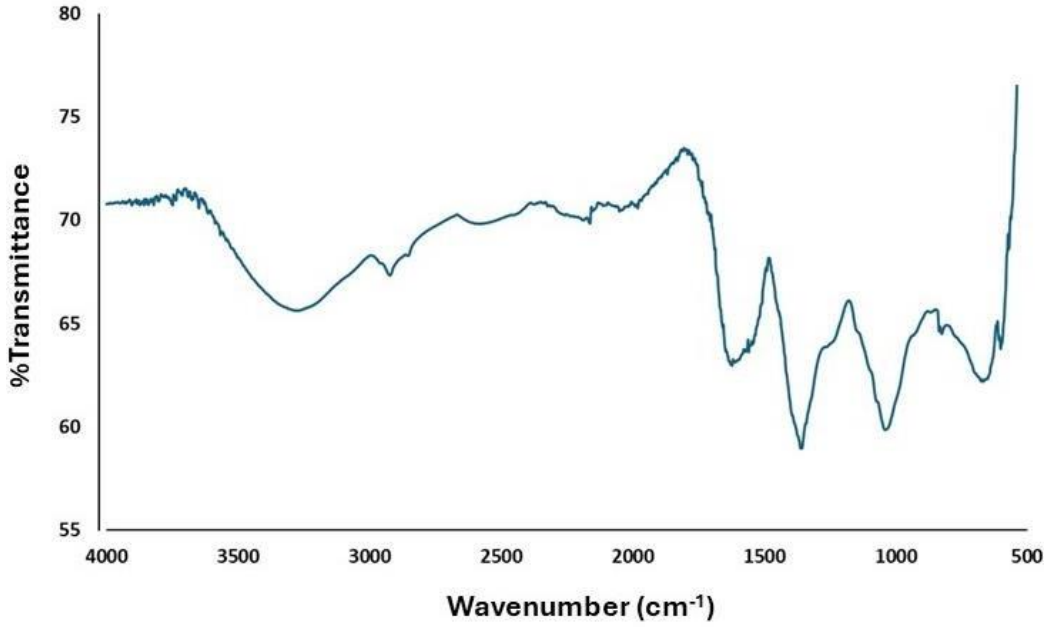

Figure 4. FTIR spectra of AgNPs

The crystalline structure of the AgNPs was determined by X-ray diffraction. Sathishkumar et al. (2019) obtained the peaks at 2θ values were 37.92°, 44.17°, 64.46°, and 77.54°, which were assigned to (1 1 1), (2 0 0), (2 2 0), and (3 1 1) planes of Bragg's reflection based on face-centered cubic structure [26]. In our study, Figure 5 shows the XRD pattern of the obtained AgNPs. The two distinct peaks of 38.3° and 76.9° could be attributed to the plane of (1 1 1) and (3 1 1), respectively. According to Debye–Scherrer equation, the size of synthesized AgNPs is almost 35 nm.

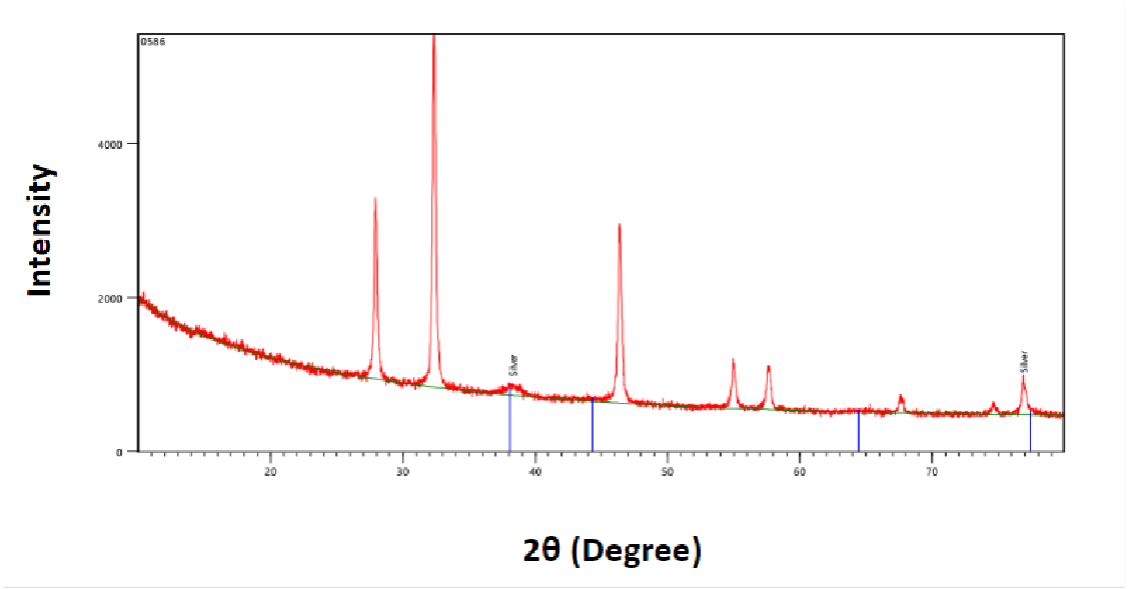

Figure 5. XRD profiles of AgNPs

3.3. Biological activity of silver nanoparticles

In addition to the seagrass waste extract, the antioxidant activity of the AgNPs produced was also evaluated according to the DPPH method. DPPH is a free and stable radical that is widely accepted as a tool for assessing the antioxidant properties of molecules [26]. These values were compared with BHA. Accordingly, seagrass extract, AgNPs and BHA were 15.13 ± 2.3 %, 18.14 ± 1.5 % and 98.02 ± 1.5 % in this method concentration, respectively (Table 2). In the green synthesis method, biometabolites from plant extracts can provide positive effects on the biological activity of AgNPs as capping agents. Therefore, the existing antioxidant activity of seagrass waste contributed to the antioxidant activity of AgNPs. The richer the extract is in antioxidant activities, the higher antioxidant scavenging activity the nanoparticles could exhibit.

Table 2. Antioxidant activities

	Antioxidant Activity (%)
Seagrass Waste-Water extract	15.13 ± 2.3
Seagrass Waste-AgNP	18.44 ± 1.5
BHA (Butylhydroxyanisole)	98.02 ± 1.5

The antimicrobial effect of AgNPs produced within the scope of the study was examined by comparing with extract and $AgNO_3$. Based on the antimicrobial effect analysis, antibacterial activity against Gram-positive *S. aureus* and Gram-negative *E. coli* was examined, while antifungal activity against *C. albicans* and *C. glabrata* fungi was examined. Secondary metabolites produced by seagrass are known to exhibit pharmacological properties. It has been proven by different studies that seagrass shows antimicrobial effects against different microorganisms [27, 28].

In the Kirby-Bauer disk diffusion performed for antibacterial tests within the scope of the analyses, seagrass extract showed no inhibition effect against *S. aureus*, while an inhibition zone of 4 mm in diameter was observed against *E. coli*. The synthesized AgNPs showed an inhibition zone of 10 ± 1 mm in diameter against *S. aureus* and *E. coli*. When the same effect was compared with $AgNO_3$, the effect against *S. aureus* was found to be 9.5 ± 1 mm and 10.17 ± 1.5 mm against *E. coli.* The zone of inhibition against gentamicin, which was used as a positive control in antibacterial trials, was 15 ± 2 mm. When the inhibition zones obtained were compared, it was determined that AgNPs synthesis increased the antimicrobial activity of seagrass wastes extract. When the inhibition zones were compared with the inhibition zone of gentamicin, it was seen that the use of seagrass waste AgNPs was potentially promising.

The value measured at 600 nm represents the exponential growth phase of bacteria with an absorbance value of 0.6 at OD600 [29]. The value of 0.6, which corresponds to approximately

1.5 x 10^8 cfu/ml for *S. aureus* and approximately 3 x 10^8 cfu/ml for *E. coli*, is frequently used in performing antibacterial tests. Determination of bacterial concentration by OD600 is a common application to determine antibacterial activity in fabric samples with different treatments [30]. McFarland 0.5 standard, which corresponds to approximately 1.5 x 10^8 cfu/ml, was used to determine the concentration in *Candida* strains, and then the cfu concentration in the medium was reduced to 10^5 by making the necessary dilutions. The main reason for this is that the basic concentration for the observation of antimicrobial resistance against *Candida* strains is 10^5 [14].

After the disk diffusion trials, the extract, AgNPs and $AgNO_3$ samples were attached to the fabrics and the trials were repeated. The zone diameters obtained on the fabrics as a result of the experiments are given in Table 3. Gentamicin was used as an antimicrobial agent in the investigation of the antibacterial effects of fabric samples against *S. aureus* and *E. coli*. Water-impregnated cotton fabrics were used as negative control. When the data obtained were analyzed, it was observed those AgNPs synthesis increased the antibacterial effect in fabric coatings according to both extract content and $AgNO_3$. When the antibacterial effect is compared with gentamicin-containing fabrics, the adhesion of the samples to the fabric surfaces and the formation of an inhibition zone is important for further studies.

The antibacterial effects of silver ions and synthesized AgNPs against *S. aureus* and *E. coli* have been widely studied to date [31]. Therefore, it is important to synthesize AgNPs using seagrass waste and thereby increase the antibacterial effect [32].

Agar well diffusion analysis was performed to investigate the antifungal activity of seagrass extract, AgNPs and $AgNO_3$ against *Candida* sp. As a result of agar well diffusion analysis, seagrass extract and $AgNO_3$ did not form a zone against *C. albicans* and *C. glabrata*. AgNPs formed an inhibition zone of 10 ± 2 mm against *C. albicans* and 14 ± 3 mm against *C. glabrata*. Antifungal agent fluconazole (2 mg/ml) was used as a positive control in antifungal analysis of *Candida* sp. In agar well diffusion analysis, fluconazole created a 14 ± 4 mm zone of inhibition against *C. albicans* and a 20 ± 5 mm zone of inhibition against *C. glabrata*.

When fabric analysis of the same samples was performed, it was observed that seagrass extract and $AgNO_3$ coated fabrics did not form an inhibition zone. AgNPs coated fabric samples did not form an inhibition zone against *C. albicans*, while a 23 ± 9 mm inhibition zone was observed against *C. glabrata*. Fluconazole-coated fabrics used as positive control during the experiments formed a 10 ± 2 mm inhibition zone against *C. albicans* and 17 ± 3 mm inhibition zone against *C. glabrata*. Detailed data are given in Table 3. It was observed that the inhibition effect of fabric samples coated with synthesized AgNPs against *C. glabrata* was higher than fluconazole. While seagrass extract and $AgNO_3$ showed no effect against *Candida* sp., the inhibition effect

of the synthesized AgNPs is an indication that the antifungal effect is formed after the synthesis step.

Table 3. Antimicrobial activities of fabric samples

		Inhibition zones (mm)			
	Microorganism	Seagrass Waste extract	AgNP	$AgNO_3$	Antimicrobial Agent
Antimicrobial activity	*S. aureus*	-	17 ± 3	12 ± 2	37 ± 5
	E. coli	-	18 ± 4	14 ± 2	38 ± 5
Antifungal activity	*C. glabrata*	-	23 ± 9	-	17 ± 2
	C. albicans	-	-	-	10 ± 2

- : The antifungal activity was not detected.

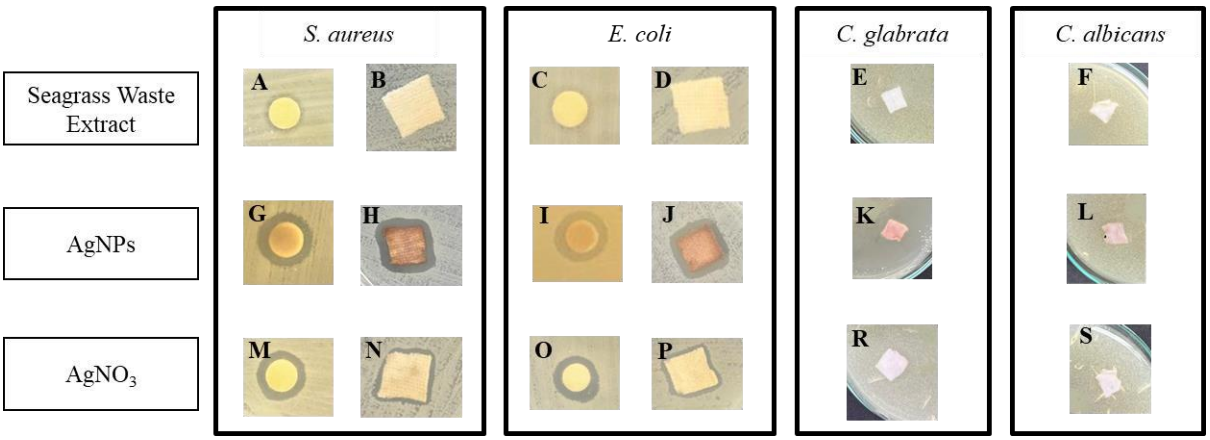

Figure 6. Antimicrobial effect of seagrass waste extract, **A.** *S. aureus* disc diffusion method, **B.** *S. aureus* fabric sample, **C.** *E. coli* disc diffusion method, **D.** *E. coli* fabric sample, **E.** *C. glabrata* fabric sample, **F.** *C. albicans* fabric sample; AgNPs **G.** *S. aureus* disc diffusion method, **H.** *S. aureus* fabric sample, **I.** *E. coli* disc diffusion method, **J.** *E. coli* fabric sample, **K.** *C. glabrata* fabric sample, **L.** *C. albicans* fabric sample; $AgNO_3$ **M.** *S. aureus* disc diffusion method, **N.** *S. aureus* fabric sample, **O.** *E. coli* disc diffusion method, **P.** *E. coli* fabric sample, **R.** *C. glabrata* fabric sample, **S.** *C. albicans* fabric sample

Nanotechnology shows promise in developing new antimicrobial agents that can kill or inhibit various microorganisms. Silver's mechanism of action is linked to its interaction with thiol-

grouped compounds found in bacterial respiratory enzymes. Silver particles inhibit the respiratory process by binding to the cell wall and membrane of the bacterial cell. The antimicrobial activity of smaller AgNPs is known to be greater [20]. Also, there are some theories about the antimicrobial actions of AgNPs. A change in the permeability of the cell membrane, which allows interaction with cell components, or the generation of free radicals, which are responsible for membrane damage and the dissipation of the proton motive force, could lead to the disruption of the membrane potential [25]. Silver nanoparticles (AgNPs) exhibit superior antibacterial activity compared to silver nitrate ($AgNO_3$) due to their unique physicochemical properties, including smaller size, larger surface area, and enhanced interaction with bacterial cells [33]. The adhesion of AgNPs to cell membranes is facilitated by electrostatic interaction between positively charged AgNPs and negatively charged cell membranes of microorganisms. It has also been shown that AgNPs have a greater antibacterial effect on Gram-negative bacteria than on Gram-positive bacteria due to cell wall thickness. The size-dependent antibacterial effect is due to the fact that smaller nanoparticles have a greater surface area in contact with bacterial cells and could reach the cytoplasm more often than larger nanoparticles [34]. Cotton fabrics treated with AgNPs exhibit significant antimicrobial activity against both Gram-positive and Gram-negative bacteria. It also shows that textiles coated with AgNPs retain their antimicrobial activity even after washing. These findings show that the use of silver nanoparticles for antimicrobial purposes in the textile industry can provide important contributions in terms of health and hygiene [35].

Ahmed et al. (2016) studied the antibacterial activity of AgNPs from *Azadirachta indica* which was 34 nm in size and found that the zone of inhibition was 9 mm for both bacteria [36]. In a study, Chakravartya et al. (2022) investigated the antibacterial effect of AgNPs from fruit extracts of *Syzygium cumini.* They found that the zone of inhibition was 16 and 17 mm in *S. aureus*, *Bacillus subtilis*, while 19 and 14 mm in *Pseudomonas aueruginosa*, *E. coli*, respectively [34].

In this study, a marine waste was used as a source for synthesizing AgNPs by green method without any chemical stabilizer. According to Soto et al. (2019), different wastes have been used for the synthesis of AgNPs, such as papaya peel, banana peel, orange peel, grape wastes, tea wastes and sugarcane bagasse [24]. Bagherzade et al. (2017) synthesized the AgNPs from extracts of saffron wastage and evaluated the antibacterial activities of these nanoparticles after characterization. AgNPs exhibited significant antibacterial activity against five bacteria [1].

4. Conclusion

Seagrass considered as waste is a potential material for nanoparticle production by green synthesis method. Higher phenolic and sugar amounts can be obtained by optimizing the extraction conditions. The obtained AgNPs production can be optimized under different conditions and their antioxidant and antimicrobial activities can be re-evaluated. In this study, seagrass wastes extract was used as a reducing agent for the synthesis of AgNPs in an environmentally friendly, low-cost and non-toxic way. UV, DLS, FTIR and XRD were used to characterize the synthesized AgNPs. The reduction of Ag^+ ions to Ag^0 was clearly observed by

secondary metabolites. These results show that seagrass wastes biomass has the potential to produce nanoparticles. Antimicrobial activities can be developed with different species and textile applications can be enriched. Among the metallic nanoparticles, AgNPs have gained attention due to their biological activities including antimicrobial activity. Thus, AgNPs could be targeted for use in biomedical, biosensors, imaging, drug delivery and textile applications.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Conceptualization and design: Aysegul Inam, Nihal Ozel, Zulal Gunay, Murat Elibol; methodology and formal analysis: Aysegul Inam, Nihal Ozel, Zulal Gunay, Murat Elibol; validation: Aysegul Inam, Nihal Ozel, Zulal Gunay, Murat Elibol; investigation: Aysegul Inam, Nihal Ozel, Zulal Gunay, Murat Elibol; Writing – original draft: Aysegul Inam, Nihal Ozel, Zulal Gunay, Murat Elibol. The submitted manuscript was accepted and reviewed by all the authors.

Acknowledgements

Nihal Ozel and Zulal Gunay thanks to 2211-C TUBITAK National Scholarship Program for PhD Students.

References

[1] Bagherzade, G., Tavakoli, M. M., & Namaei, M. H. (2017). Green synthesis of silver nanoparticles using aqueous extract of saffron (Crocus sativus L.) wastages and its antibacterial activity against six bacteria. Asian Pacific Journal of Tropical Biomedicine, 7(3), 227-233.

[2] Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. Journal of advanced research, 7(1), 17-28.

[3] Ahmad, S., Munir, S., Zeb, N., Ullah, A., Khan, B., Ali, J., ... & Ali, S. (2019). Green nanotechnology: A review on green synthesis of silver nanoparticles—An ecofriendly approach. International journal of nanomedicine, 5087-5107.

[4] Ahmad, N., Sharma, S., Alam, M. K., Singh, V. N., Shamsi, S. F., Mehta, B. R., & Fatma, A. (2010). Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. Colloids and Surfaces B: Biointerfaces, 81(1), 81-86.

[5] Salayová, A., Bedlovičová, Z., Daneu, N., Baláž, M., Lukáčová Bujňáková, Z., Balážová, Ľ., & Tkáčiková, Ľ. (2021). Green synthesis of silver nanoparticles with antibacterial activity using various medicinal plant extracts: Morphology and antibacterial efficacy. Nanomaterials, 11(4), 1005.

[6] Achamlale, S., Rezzonico, B., & Grignon-Dubois, M. (2009). Rosmarinic acid from beach waste: Isolation and HPLC quantification in Zostera detritus from Arcachon lagoon. Food Chemistry, 113(4), 878-883.

[7] Benito-González, I., López-Rubio, A., Martínez-Abad, A., Ballester, A. R., Falcó, I., González-Candelas, L., ... & Martínez-Sanz, M. (2019). In-depth characterization of bioactive extracts from Posidonia oceanica waste biomass. Marine drugs, 17(7), 409.

[8] Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical chemistry, 28(3), 350-356.

[9] Miceli, N., Trovato, A., Dugo, P., Cacciola, F., Donato, P., Marino, A., ... & Taviano, M. F. (2009). Comparative analysis of flavonoid profile, antioxidant and antimicrobial activity of the berries of Juniperus communis L. var. communis and Juniperus communis L. var. saxatilis Pall. from Turkey. Journal of agricultural and food chemistry, 57(15), 6570-6577.

[10] Biemer, J.J. (1973) Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method. Ann Clin Lab Sci 3: 135–140.

[11] Hudzicki, J. (2012) Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information. Am Soc Microbiol 1–13.

[12] Shameli, K., Ahmad, M. B., Jazayeri, S. D., Shabanzadeh, P., Sangpour, P., Jahangirian, H., & Gharayebi, Y. (2012). Investigation of antibacterial properties silver nanoparticles prepared via green method. Chemistry Central Journal, 6, 1-10.

[13] Noake, T., Kuriyama, T., White, P.L., Potts, A.J.C., Lewis, M.A.O., Williams, D.W., and Barnes, R.A. (2007) Antifungal susceptibility of Candida species using the clinical and laboratory standards institute disk diffusion and broth microdilution methods. J Chemother 19: 283–287.

[14] EUCAST (2020) Breakpoint tables for interpretation of MICs for antifungal agents.

[15] Seyrekoğlu, F. Temiz, H. Effect of Extraction Conditions on the Phenolic Content and DPPH Radical Scavenging Activity of Hypericum perforatum L. Turkish Journal of Agriculture-Food Science and Technology. 2020, 8(1), 226-229.

[16] Khanna, P., Kaur, A., & Goyal, D. (2019). Algae-based metallic nanoparticles: Synthesis, characterization and applications. Journal of microbiological methods, 163, 105656.

[17] Messina, C. M., Arena, R., Manuguerra, S., Pericot, Y., Curcuraci, E., Kerninon, F., ... & Santulli, A. (2021). Antioxidant bioactivity of extracts from beach cast leaves of Posidonia oceanica (L.) Delile. Marine Drugs, 19(10), 560

[18] Chen, J., Yang, J., Ma, L., Li, J., Shahzad, N., & Kim, C. K. (2020). Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. Scientific reports, 10(1), 2611.

[19] Valsalam, S., Agastian, P., Arasu, M. V., Al-Dhabi, N. A., Ghilan, A. K. M., Kaviyarasu, K., ... & Arokiyaraj, S. (2019). Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of Tropaeolum majus L. and its enhanced in-vitro antibacterial, antifungal, antioxidant and anticancer properties. Journal of Photochemistry and Photobiology B: Biology, 191, 65-74.

[20] Behravan, M., Panahi, A. H., Naghizadeh, A., Ziaee, M., Mahdavi, R., & Mirzapour, A. (2019). Facile green synthesis of silver nanoparticles using Berberis vulgaris leaf and root aqueous extract and its antibacterial activity. International journal of biological macromolecules, 124, 148-154.

[21] Masarudin, M. J., Cutts, S. M., Evison, B. J., Phillips, D. R., & Pigram, P. J. (2015). Factors determining the stability, size distribution, and cellular accumulation of small, monodisperse chitosan nanoparticles as candidate vectors for anticancer drug delivery: application to the passive encapsulation of [14C]-doxorubicin. Nanotechnology, science and applications, 67-80.

[22] Honary, S., Barabadi, H., Gharaei-Fathabad, E., & Naghibi, F. (2013). Green synthesis of silver nanoparticles induced by the fungus Penicillium citrinum. Tropical Journal of Pharmaceutical Research, 12(1), 7-11.

[23] Grand, J., Auguié, B., & Le Ru, E. C. (2019). Combined extinction and absorption UV–visible spectroscopy as a method for revealing shape imperfections of metallic nanoparticles. Analytical chemistry, 91(22), 14639-14648.

[24] Soto, K. M., Quezada-Cervantes, C. T., Hernández-Iturriaga, M., Luna-Bárcenas, G., Vazquez-Duhalt, R., & Mendoza, S. (2019). Fruit peels waste for the green synthesis of silver nanoparticles with antimicrobial activity against foodborne pathogens. Lwt, 103, 293-300.

[25] Widatalla, H. A., Yassin, L. F., Alrasheid, A. A., Ahmed, S. A. R., Widdatallah, M. O., Eltilib, S. H., & Mohamed, A. A. (2022). Green synthesis of silver nanoparticles using green tea leaf extract, characterization and evaluation of antimicrobial activity. Nanoscale Advances, 4(3), 911-915.

[26] Sathishkumar, R. S., Sundaramanickam, A., Srinath, R., Ramesh, T., Saranya, K., Meena, M., & Surya, P. (2019). Green synthesis of silver nanoparticles by bloom forming marine microalgae Trichodesmium erythraeum and its applications in antioxidant, drug-resistant bacteria, and cytotoxicity activity. Journal of Saudi Chemical Society, 23(8), 1180-1191.

[27] Mani, A., Author, C., Elizabeth Mani, A., Bharathi, V., and Patterson, J. (2012) Antibacterial Activity and Preliminary Phytochemical Analysis of Sea Grass Cymodocea rotundata. Int J Microbiol Res 3: 99–103.

[28] Gono, C.M.P., Ahmadi, P., Hertiani, T., Septiana, E., Putra, M.Y., and Chianese, G. (2022) A Comprehensive Update on the Bioactive Compounds from Seagrasses. Mar Drugs 20: 1–37.

[29] Othman, M., San Loh, H., Wiart, C., Khoo, T. J., Lim, K. H., & Ting, K. N. (2011). Optimal methods for evaluating antimicrobial activities from plant extracts. Journal of Microbiological Methods, 84(2), 161-166.

[30] Haase, H., Jordan, L., Keitel, L., Keil, C., & Mahltig, B. (2017). Comparison of methods for determining the effectiveness of antibacterial functionalized textiles. PLoS One, 12(11), e0188304.

[31] Woo, K.J., Hye, C.K., Ki, W.K., Shin, S., So, H.K., and Yong, H.P. (2008) Antibacterial activity and mechanism of action of the silver ion in Staphylococcus aureus and Escherichia coli. Appl Environ Microbiol 74: 2171–2178.

[32] Kannan, R.R.R., Arumugam, R., and Anantharaman, P. (2010) Antibacterial potential of three seagrasses against human pathogens. Asian Pac J Trop Med 3: 890–893.

[33] Salman, H. D. (2017). Evaluation and comparison the antibacterial activity of silver nano particles (AgNPs) and silver nitrate ($AgNO_3$) on some pathogenic bacteria. J. Glob. Pharma Technol, 9, 238-248.

[34] Chakravarty, A., Ahmad, I., Singh, P., Sheikh, M. U. D., Aalam, G., Sagadevan, S., & Ikram, S. (2022). Green synthesis of silver nanoparticles using fruits extracts of Syzygium cumini and their bioactivity. Chemical Physics Letters, 795, 139493.

[35] Jain, A., Kongkham, B., Puttaswamy, H., Butola, B. S., Malik, H. K., & Malik, A. (2022). Development of wash-durable antimicrobial cotton fabrics by in situ green synthesis of silver nanoparticles and investigation of their antimicrobial efficacy against drug-resistant bacteria. Antibiotics, 11(7), 864.

[36] Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. Journal of radiation research and applied sciences, 9(1), 1-7.