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Synthesis and Characterization of Silver Nanoparticles Using Alginate from the Brown Seaweed *Laminaria ochroleuca*: Structural Features and Antibacterial Activity

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Abstract: In this study, inexpensive and eco-friendly production of the silver nanoparticles (AgNPs) was explored using *Laminaria ochroleuca* sodium alginate as stabilizing and reducing agent. The synthesized nanoparticles were characterized by various analytical techniques such as UV–Vis spectroscopy, Energy dispersive X-ray spectrometer (EDX), X-ray diffraction (XRD), transmission electron microscopy (TEM), TEM selected area electron diffraction (SAED), Fourier transform infrared (FTIR) spectroscopy, and thermogravimetric analysis (TGA). The synthesis of AgNPs was elucidated by characteristic absorption peaks in the UV–Vis test. The FTIR analysis revealed the involvement of many functional groups in the bioreduction and the stabilization of AgNPs, while TEM images illustrated the spherical shape with maximum particles found in the size range of 10–20 nm. Prominent peaks and silver diffraction rings shown by the XRD spectrum and SAED pattern, respectively, confirmed the crystalline nature (fcc) of the synthesized AgNPs, which were found to be thermally stable based on TGA analysis. The AgNPs exhibited strong antibacterial activity against Gram+ and Gram– bacteria. The synthesized silver nanoparticles using *Laminaria ochroleuca* sodium alginate revealed interesting properties that could be potentially used for pharmaceutical applications.

Keywords: silver nanoparticles; biosynthesis; sodium alginate; *Laminaria ochroleuca*.

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1. Introduction

Nanotechnology has progressively more expanded as a promising field of applied science due to its various applications, which involve many aspects of human life using nanoparticles [1–3]. Research on nanomaterials mainly emphasizes the synthesis of nanoparticles of various sizes, shapes, and structures for desired applications [4]. Among the various nanoparticles, silver nanoparticles have established more interest due to their properties such as size, shape, antimicrobial and antioxidant activity [5]. Because of their distinguishing physico-chemical attributes [6], silver nanoparticles (AgNPs) are now one of the inorganic noble metal nanoparticles that have potential application in the areas of chemistry, physics,

environmental remediation, optoelectronics, material science, biomedical devices, and renewable energies [7].

Several methods have been explored for the synthesis and stabilization of AgNPs. However, many of them were proved to have adverse effects in applications with potential environmental toxicity, as well as biological risks [8]. Hence, biological methods were established as a valuable, inexpensive, and less cumbersome alternative.

Currently, there is increasing attention to the synthesis of metal nanoparticles from marine sources [9]. The use of marine macroalgae in the biosynthesis of nanoparticles arises as an ecofriendly and thrilling approach [10]. By taking advantage of its biochemical properties, including its biocompatibility, biodegradability, immunogenicity, and non-toxicity in drug delivery system and cell encapsulation [11, 12], alginate from brown seaweeds is one of the various bio-molecules often used as an organic matrix to produce metallic nanoparticles [13–16].

In the present study, silver nanoparticles (AgNPs) were synthesized using sodium alginate extracted from the kelp *Laminaria ochroleuca* harvested from the Atlantic coast of Morocco. The characterization of the AgNPs was performed by UV–VIS spectroscopy, X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, transmission electron microscopy (TEM), TEM selected area electron diffraction (SAED), and thermogravimetric analysis (TGA). The synthesized AgNPs were tested for their antibacterial activity.

2. Materials and Methods

2.1. Alginate extraction.

Laminaria ochroleuca samples were collected from the Atlantic coast of Morocco (33°14'47.5"N 8°32'31.9"W). The kelp biomass was washed with water to remove impurities and dried in an oven at 50°C. Dried samples were soaked in 2% formaldehyde for 24 h at room temperature, washed with distilled water, and added to 0.2 M HCl afterward for 24 h, then washed again with distilled water before extraction using 2% sodium carbonate (Na₂CO₃) during 24 h. The soluble fraction was collected by centrifugation. The sodium alginate was precipitated by ethanol and washed by acetone, then dried at 50°C.

2.2. Synthesis of silver nanoparticles.

Sodium alginate solution (1.5 %) extracted from *Laminaria ochroleuca* was added to AgNO₃ (0.05 M) at a ratio 1:1. The mixture was stirred for 10 min at 60 °C, and then incubated at room temperature in the dark for 2h. After the centrifugation at 5000 rpm for 45 min and the precipitation, the product was dried at 40 °C to obtain the dry powder of the silver nanoparticles.

2.3. Characterization of silver nanoparticles.

UV–Vis spectrophotometer was performed to monitor the absorption pattern of synthesized silver nanoparticles using a spectrophotometer Metashe 5200 HPC. The samples were periodically recorded between 250 and 700 nm. An energy-dispersive X-ray spectrometer (EDX) was used to determine the silver concentration of the synthesized nanoparticles (AgNPs).

X-ray diffraction pattern of AgNPs was carried out on an XRD instrument operating at room temperature in a 2θ range of 0 to 70° with a scan step width of 0.02° using a D8 AdvanceBruker AXS diffractometer (Cu $K\alpha$ radiation, $\lambda = 1.5418 \text{ \AA}$). The resulting XRD pattern profile was treated with the EVA program.

Transmission electron microscopy (TEM) was used to determine the size and shape of the synthesized silver nanoparticles. The analysis was performed using an FEI Tecnai G2 20 by suspending the nanoparticles in distilled water. After being homogenized, a drop was placed on a copper grid, then air-dried and observed in TEM. The crystalline nature of the silver nanoparticles was examined using the selected area electron diffraction (SAED) pattern.

FTIR spectroscopy analysis of biosynthesized AgNPs was performed with Thermo Scientific Nicolet IS50 FT-IR Spectrometer at room temperature over the wavenumber range $4000\text{--}400 \text{ cm}^{-1}$ in an attenuated total reflectance (ATR) mode. A total of 64 scans were averaged for each sample at 4 cm^{-1} resolution, and the IR spectra were then plotted and analyzed with the Omnic 9.1 software.

Thermogravimetric analysis (TGA) was performed using TGA Seteram 92. The TGA thermograms were recorded at a heating rate of 10°C per min in the temperature range of 50°C to 800°C under a nitrogen atmosphere.

2.4. Antibacterial activity.

Antibacterial activity of the AgNPs was evaluated against different human pathogenic strains whose three Gram-negative bacteria *Klebsiella pneumoniae* (clinically isolated), *Pseudomonas aeruginosa* and *Escherichia coli*, and three Gram-positive bacteria *Staphylococcus aureus*, *Bacillus cereus*, and *Micrococcus luteus* using the technique of wells with a diameter of the beads of 6 mm [17]. In fact, $80 \mu\text{L}$ of the synthesized AgNPs were deposited in wells formed on the Muller-Hinton agar medium previously inoculated with 0.1 mL of bacterial suspensions (10^6 CFU/mL) of the tested pathogenic microorganisms. The zones of inhibition that appeared around the wells after 18 hours of incubation at 37°C were subsequently measured [18].

3. Results and Discussion

3.1. Synthesis of silver nanoparticles.

When the extracted alginates solution is mixed with AgNO_3^- aqueous solution, the color of the reaction began immediately turn from colorless to yellowish and then to brownish. It has been reported that Ag-NPs solutions have brown coloration due to their characteristic excitation of surface plasmons in the range of $400\text{--}414 \text{ nm}$ [19, 20]. The UV–Visible spectrum of the synthesized Ag-NPs (Figure 1) showed a prominent peak at 400 nm . This peak is consigned to the excitation of surface plasmon resonance (SPR) and the reduction of AgNO_3 for the thermodynamically favored formation of the AgNPs using sodium alginate [21, 22]. This heteropolysaccharide is a structural component of marine brown algae (Phaeophyceae) made up of linked $\beta\text{-D}$ mannuronic acid (M) and $\alpha\text{-L}$ guluronic acid (G) residues and operates as a controller of nucleation, as well as a stabilizer due to its functional groups (COOH and OH) with oxygen-rich structures. The latter binds strongly with nanoparticles via electrostatic interactions [23] due to the high charge density of the anionic sodium alginate that allows the replacement of sodium ions (existing in the sodium alginate structure) by the silver ions and contributes to the release of nitrate in the form of sodium nitrate

[24]. This one-electron transfer from the carboxyl groups of alginates to the bound Ag^+ reduces the silver ion to Ag^0 by dint of the hydroxyl groups provided by sodium alginate used as an organic matrix to produce metallic nanoparticles [25].

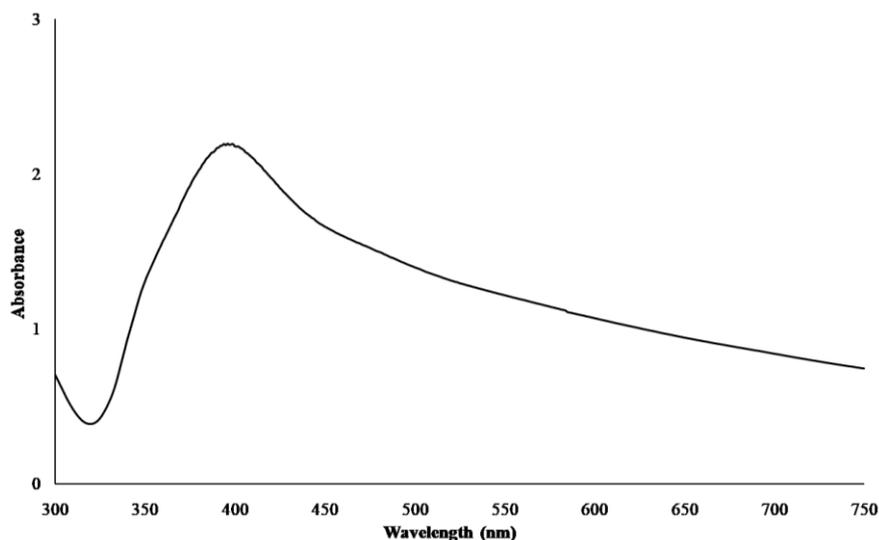


Figure 1. UV–Vis spectrum of AgNPs synthesized with *Laminaria ochroleuca* Na-alginate.

3.2. TEM-SAED analysis.

Transmission electron microscopy (TEM) images (Figure 2) clearly illustrate the homogeneity and spherical shape of the synthesized silver nanoparticles with predominant size in the range of 10–20 nm and a mean diameter of 14.89 ± 6.1 nm. It was reported that reducing and stabilizing agents strongly influence the metallic ion solution and incubation conditions the morphologies of the obtained nanoparticles [25, 26], being commonly quasi-spherical when produced for biopolymer particles [27, 28]. The obtained particle size is in accordance with previously reported green-synthesized AgNPs [29]. The TEM-SAED pattern revealed significant silver diffraction rings, suggesting the face cubic centered (fcc) crystalline nature of the AgNPs (Figure 3).

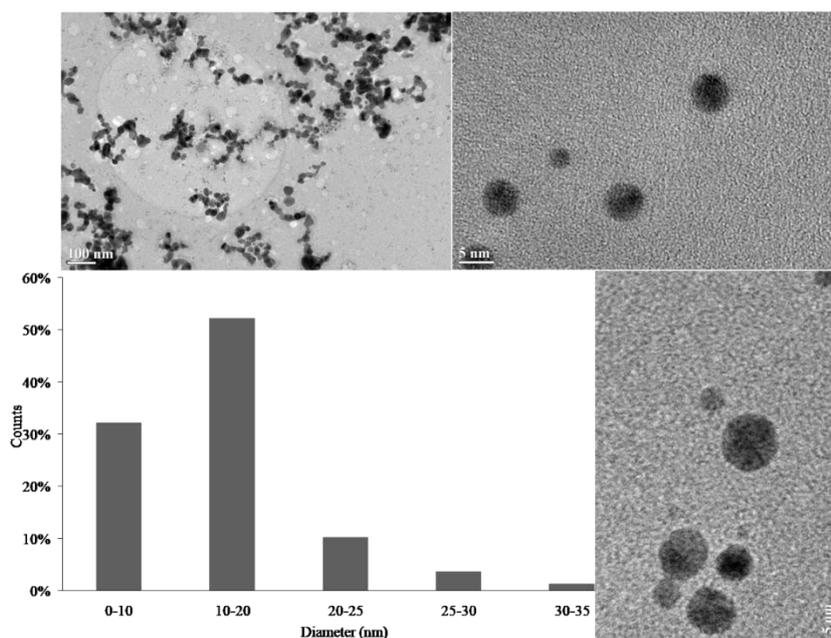


Figure 2. TEM micrographs and size distribution analysis of AgNPs synthesized with *Laminaria ochroleuca* Na-alginate.

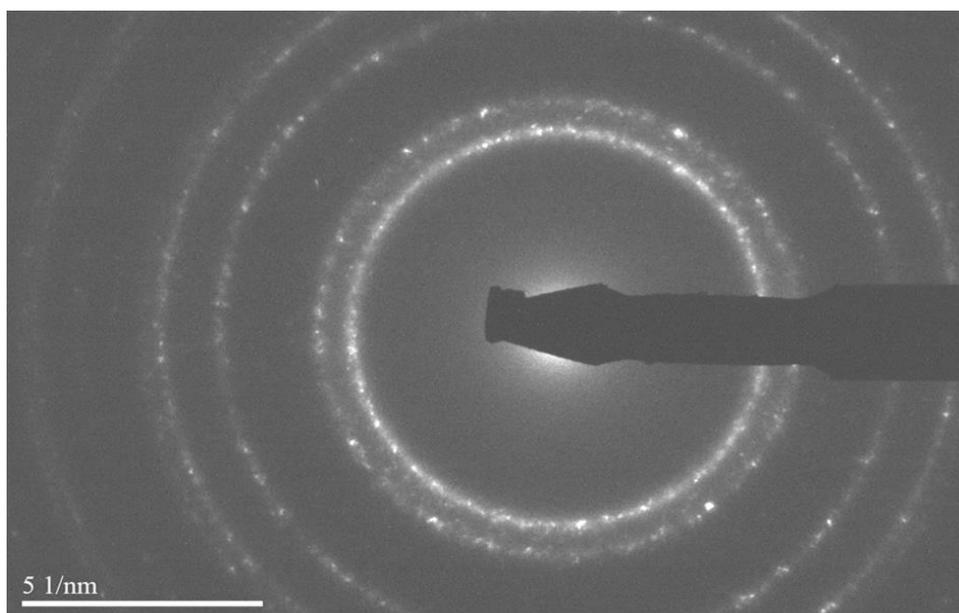


Figure 3. TEM-SAED ring pattern of the synthesized AgNPs.

3.3. XRD analysis.

The XRD pattern of the AgNPs (Figure 4) indicates four strong Bragg reflections exhibiting the characteristic peaks of the silver crystallites observed at 2θ values of 38.06° , 44.21° , and 64.43° corresponding to the (111), (200) and (220) planes of silver crystals, respectively, indexed to the crystallographic planes of the face-centered cubic structure of silver (fcc) according to ICDD/ICSD from X'Pert High Score Plus (reference code: 01-087-0719). XRD pattern clearly illustrates the crystalline structure of the synthesized AgNPs with fcc form. The weak diffraction peaks detected at 2θ values of 28° , 29° , 32.5° , and 34° (Figure 4) can be assigned to Na-alginates due to the reflection of the (110) plane from polyguluronate unit, (200) plane from polymannuronate unit [30, 31]. This biopolymer used as an organic matrix to synthesize the silver nanoparticles is generally crystalline due to a strong interaction between chains through intermolecular hydrogen bonding [30].

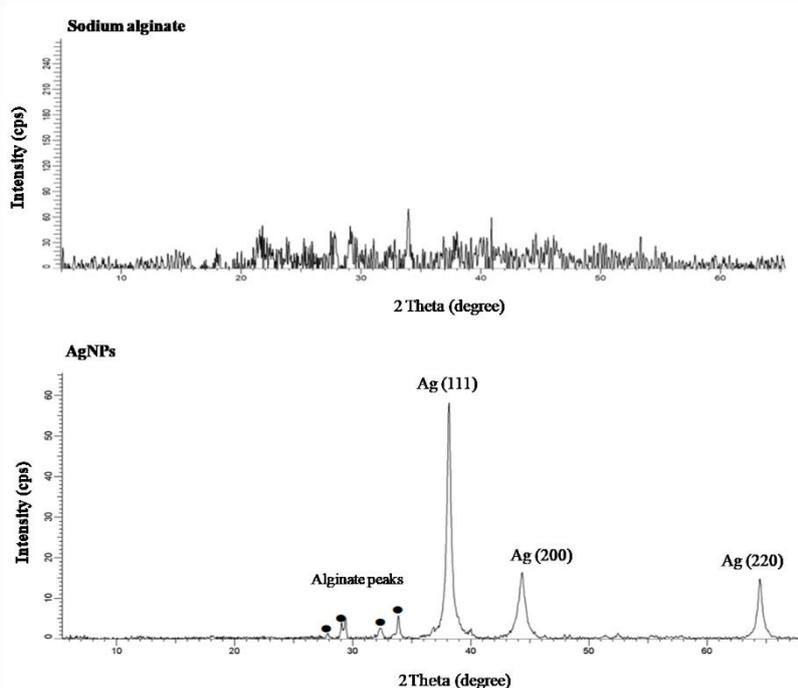


Figure 4. XRD spectra of *Laminaria ochroleuca*' Na-alginate and the synthesized AgNPs.

3.4. EDX Analysis.

Energy-dispersive X-ray spectrometer (EDX) was employed to reveal the presence of silver nanoparticles and their elemental nature. As shown in Figure 5, the presence of elemental silver along with Na, O, C, Si, and Cu in the synthesized nanoparticles are confirmed by a prominent peak detected at 3 keV linked to the typical absorption peak of AgNPs due to the surface plasma resonance (SPR) phenomenon [32].

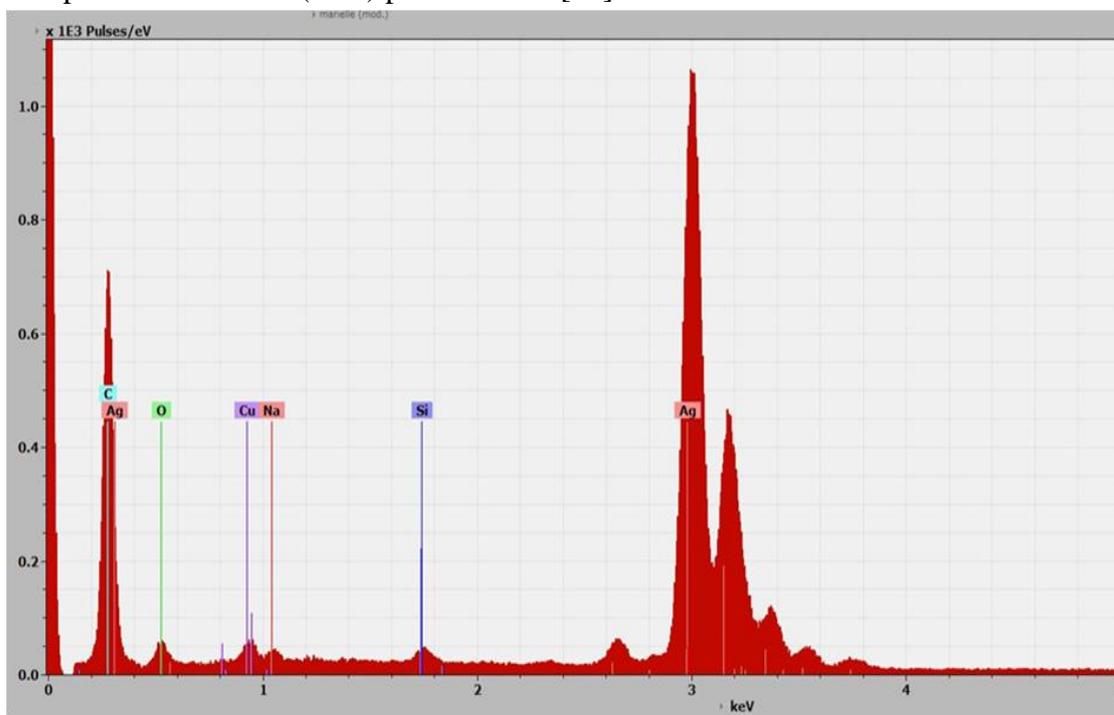


Figure 5. EDX spectrum of the synthesized AgNPs.

3.5. FTIR Analysis.

The FTIR spectra of *Laminaria ochroleuca* Na-alginate and synthesized AgNPs were performed in the range of 4000 to 500 cm^{-1} , as shown in Figure 6. Broadband appeared at 3204.68 cm^{-1} could be assigned to the stretching vibration of $-\text{OH}$ group [33]. The peak noted at 2919.75 cm^{-1} is related to carboxylate $\text{O}=\text{C}-\text{O}$ asymmetric stretching vibrations [34, 35]. According to Fenoradosoa *et al.* [36] and Leal *et al.* [37], the characteristic peak of alginate found at 1599.21 cm^{-1} can be attributed to asymmetric stretching vibrations of carboxylate salt ion. The strong peak at 1404.91 cm^{-1} may be assigned to $\text{C}-\text{OH}$ deformation vibration with the contribution of $\text{O}-\text{C}-\text{O}$ symmetric stretching vibration of the carboxylate group, while the weak band at 1023 cm^{-1} may be assigned to $\text{C}-\text{O}$, and $\text{C}-\text{C}$ stretching vibrations of pyranose ring [38]. The following bands are important for the alginate characterization as they correspond to the anomeric region (950 to 750 cm^{-1}), in which two peaks were reported. The first one was observed at 877.95 cm^{-1} which corresponds to the $\text{C}1-\text{H}$ deformation vibration of β -D-mannuronic acid residues. The second absorption band at 810,93 cm^{-1} was often reported for alginates, due to mannuronic acid residues [39]. Silver nanoparticles spectrum showed significant differences compared to that of Na-alginate (Figure 8). In fact, the band assigned to $\text{O}-\text{H}$ group in Na-alginate absorption region did not appear on the AgNPs spectrum while that attributed to $\text{C}-\text{OH}$ deformation shifted to 1329.22 cm^{-1} . Similarly, the peak assigned to $\text{C}-\text{O}$ and $\text{C}-\text{C}$ stretching vibrations of pyranose ring shifted to 978.71 cm^{-1} . These results

indicated that, hydroxyl and carboxylate groups could be involved in the synthesis of silver nanoparticles.

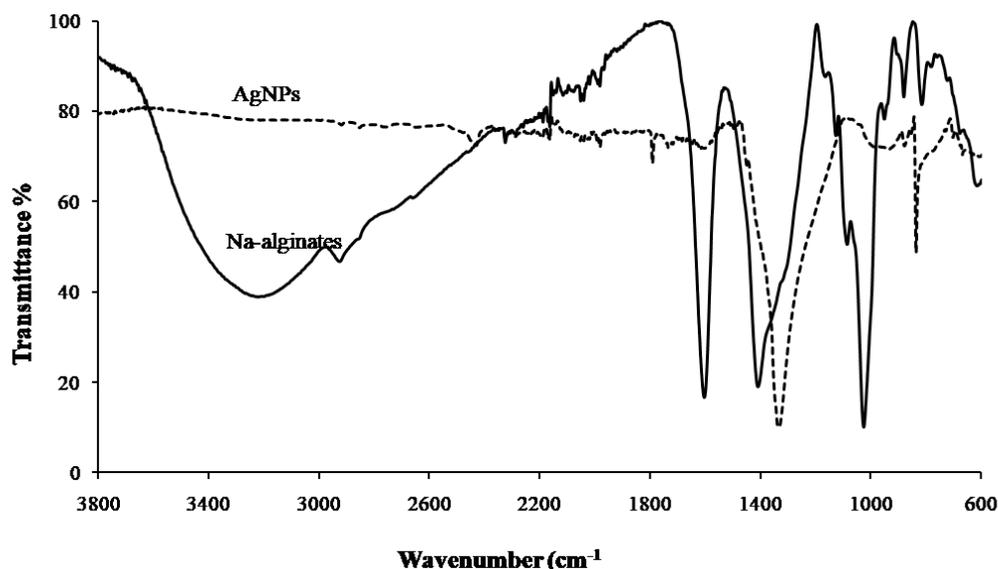


Figure 6. FTIR spectra of *Laminaria ochroleuca* Na-alginate and the synthesized AgNPs.

3.6. Thermogravimetric Analysis.

TGA curves of AgNPs and sodium alginate (Figure 7) depicted three main degradation steps. The initial slight weight loss (100 °C) is due to moisture adsorbed on particles surface. After that, the main thermal degradation step was recorded between 120-220 °C for AgNPs, and it occurred between 120-280 °C for sodium alginate. During the third degradation step, the percentage of the residual mass decreased quietly to reach 35 and 70 % at 700°C for sodium alginate and AgNPs, respectively. The synthesized nanoparticles appear to be thermally stable.

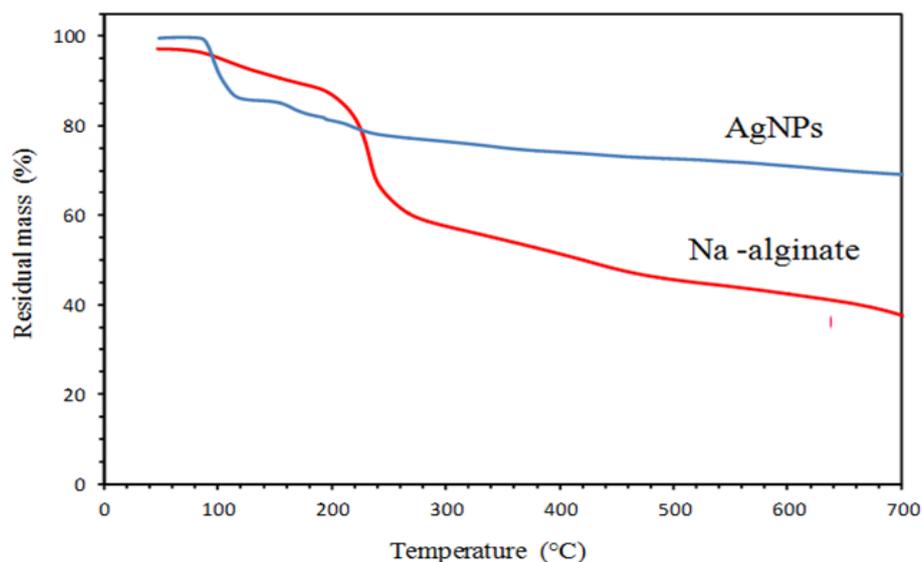


Figure 7. TGA curves of *Laminaria ochroleuca* Na-alginate and the synthesized AgNPs.

3.7. Antibacterial properties.

The antibacterial assay was tested against different human pathogenic strains of Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*) and Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, and *Micrococcus luteus*). It is apparent that the synthesized nanoparticles showed important inhibition zones against two

of the tested bacterial microorganisms, Gram-negative *K. pneumonia* and Gram-positive *S. aureus* (Table 1).

A maximum zone of inhibition was detected against Gram-positive *S. aureus* (21 mm) and a minimum zone of inhibition was obtained against Gram-negative *E. coli* (15 mm), while no inhibition zone appeared for the rest of the tested pathogenic strains. This might be explained by the large contact areas of small-sized synthesized AgNPs [40] and the different structure and composition of the cell wall in both bacteria types [41, 42]. Gram-negative bacteria with their thick peptidoglycan layer allow the entrance of only macromolecules. In contrast, the thinner layer of peptidoglycan and the abundant pores existing in the cell wall of Gram-positive bacteria allow foreign molecules to penetrate easily. This contributes to facilitating the absorption of the AgNPs into the bacterial cytoplasm, which leads to the disruption of its membrane, loss of cytoplasmic constituents, and ultimately cell death [43]. Additionally, Gram-positive bacteria have a high negative charge on the cell wall surface which possibly improves the antibacterial activity by attracting NPs [44]. Thus, the inhibition of the microbial processes could be made in the cell and on its surface. As reported in Kvittek *et al.* [45], nanoparticles may attach to the surface of the bacterial membrane, influencing the shape and function of the cell membrane and the disturbance of the bacteria respiration and permeability functions. AgNPs could also penetrate the bacteria to prevent protein synthesis and DNA replication through interaction with phosphorus-containing molecules [46], generating Reactive Oxygen Species by deactivating cellular enzymes [47].

Table 1. Antibacterial activity of biosynthesized silver nanoparticles compared with literature data.

	Zone of inhibition ¹ (mm)						References
	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Micrococcus luteus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>	
AgNPs – <i>Caulerpa serrulata</i> extract	16	nt	nt	21	14	nt	[48]
AgNPs – <i>Caulerpa racemosa</i> extract	12	nt	nt	nt	nt	nt	[49]
AgNPs – <i>Chlorella pyrenoidosa</i> extract	12	nt	nt	nt	nt	18	[50]
AgNPs – <i>Enteromorpha flexuosa</i> extract	10	nt	nt	12	0	9	[51]
AgNPs – <i>Pithophora oedogonia</i> extract	13.6	nt	15	16.8	17.2	nt	[52]
AgNPs – <i>Sargassum ilicifolium</i> extract	16.8	nt	nt	18.2	nt	16.2	[53]
AgNPs - <i>Sargassum muticum</i> Na-alginate	7	11	11	3	8	3	[19]
AgNPs - <i>Padina tetrastromatica</i> Na-alginate	6	nt	nt	nt	4	nt	[54]
AgNPs – <i>Laminaria ochroleuca</i> Na-alginate	21	0	0	15	0	0	This study

¹ Zone of inhibition does not include the diameter of wells; nt: not tested

4. Conclusions

In the present study, an inexpensive, eco-friendly, and efficient method was developed for the green synthesis of silver nanoparticles (AgNPs).

Sodium alginate extracted from the kelp *Laminaria ochroleuca* from the Atlantic coast of Morocco was used as a stabilizing and reducing agent for the synthesis of the silver nanoparticles. Their characterization was determined by various analytical techniques such as

UV–VIS spectroscopy, X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, transmission electron microscopy (TEM), TEM selected area electron diffraction (SAED), and thermogravimetric analysis (TGA).

The resulting particles exhibited interesting characteristics, including the spherical shape of the synthesized AgNPs, with maximum particles in the size range of 10–20 nm. The results also showed the face cubic centered (fcc) crystalline nature of the thermally stable synthesized silver nanoparticles (AgNPs). This study suggests that the synthesized silver nanoparticles possess significant antibacterial activity against Gram-negative and Gram-positive bacteria. The use of Na-alginate extracted from *Laminaria ochroleuca* seems to be an interesting environmentally friendly procedure for synthesizing bioactive AgNPs, which makes it potentially interesting for medical uses.

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Conflicts of Interest

The authors declare no conflict of interest

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