Pterocladiella capillacea–Stabilized Silver Nanoparticles as a Green Approach toward Antibacterial Biomaterials

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Electronic Supplementary Information

Summary

1. Materials and Methods

Collection and treatment of the alga. *Pterocladiella capillacea* red macroalga was collected directly from rock formations not exposed to intense maritime conditions, on the sea cost, at Poá Beach, at the city of Penha, Santa Catarina/Brazil (26°78'S; 48°58'W). The algae were washed with tap water for screening, and selected material was then washed with distilled water before storage at -10ºC. Moisture content was determined based on literature procedures, as follows: 1g of sample was transferred to a MA037 Marconi drying oven with natural air convection, and the sample was weighted at appropriate time intervals till no weight variation was observed. The procedure was performed in triplicate.

Preparation of the algal extract. *Pterocladiella capillacea* extract was prepared adapting a literature methodology.[1] Dried and blender-crushed alga (1g) was added to 100 mL of distilled water, and the system was stirred for 15 minutes with heating (60 °C). Then, the extract was filtrated and freezed for storage (-10 °C).

Synthesis of silver nanoparticles (AgNPs). Silver nanoparticles (AgNPs) were synthesized adapting procedures from the literature.[2] Typically, an aliquot of an aqueous stock solution of silver nitrate (1 mM) was mixed with an aliquot of *Pterocladiella capillacea* extract (10^4 mg L^{-1}), resulting in a final volume of 100 mL of reaction medium, and the system was stirred for a given time interval at specific conditions of temperature and pH. The obtained nanoparticles were subjected to characterization analyses, as described below. In order to optimize the AgNP synthesis, the experimental variables were analyzed in the experimental screening shown in Table S1.

Table S1. Experimental conditions for different assays in the synthesis of AgNPs with *Pterocladiella capillacea*.

#	$AgNO_3$ / mg L^{-1}	Ag / mg L^{-1}	Algal extract / mg L^{-1}	Time / min	Temperature / ºC	pH
1	161.4	102.5	500	180	60	5.0 – 6.0
2	152.9	97.1	1000	180	60	5.0 – 6.0
3	144.4	91.7	1500	180	60	5.0 – 6.0
4	135.9	86.3	2000	180	60	5.0 – 6.0
5	152.9	97.1	1000	240	60	5.0 – 6.0
6	152.9	97.1	1000	240	25	5.0 – 6.0
7	152.9	97.1	1000	180	60	9.0

For assay 2, silver concentration was confirmed by an analysis of Microwave Plasma Atomic Emission Spectroscopy (MP-AES) using an Agilent 4100 MP-AES equipment. Sample digestion was performed employing hydrochloric and nitric acids with heating. Silver concentration determined was 94.3 mg L^{-1} from triplicate measurements, closely related to the targeted 97.1 mg L^{-1} (Table S1 above).

Characterization of AgNPs. Silver nanoparticles were characterized by UV/Vis spectroscopy and transmission electron microscopy (TEM). UV/Vis data of colloidal samples of AgNPs were recorded at a Shimadzu UV Spectrophotometer in the wavelength range of 200–600 nm. TEM analyses were performed to evaluate morphology and size distribution of the nanoparticles, employing a JEOL JEM-1011 transmission electron microscope, operated at 100 kV. Samples were prepared by deposition of 10 μL of AgNP dispersion on a cupper-coated carbon grid, and dried at ambient atmosphere for 24 h. Image J software (version 1.52a) was used to calculate particle diameter.

Antibacterial activity of AgNP. The antibacterial activity of the *Pterocladiella capillacea*-stabilized AgNPs against gram-positive *Staphylococcus aureus* (ATCC 6538P), was evaluated by means of the determination of the minimal inhibitory concentration (MIC), employing the nanoparticles synthesized in the optimized conditions. The nanoparticles were used as obtained in the optimized assay and no previous purification was performed. MIC was determined by broth microdilution following the methodology described by the CLSI (2018)[3] with minor changes. For this, microtitration plates of 96 well were employed, and 50 μL of Mueller-Hilton broth were transferred to each well individually, except for the first column (1A to 1H), in which wells 80 μL of broth were added. In the first column, 20 μL of AgNPs were added, and successive dilutions were performed with stepwise transfer of 50 μL to the consecutive well. Inoculum of gram-positive *S. aureus* was standardized with the aid of the 0.5 scale of McFarland ($1.5x10^8$ cells mL^{-1}), and diluted to reach the value of $5.0x10^5$ cells mL^{-1}. In each well, 50 μL of the diluted inoculum were added, completing a 100 μL final volume in each. After homogenization, the plate was sealed and incubated at 35 ºC for 18 hours. Then, the plate was evaluated in order to attest visible growth of the microorganism, and the MIC was determined as the lowest silver concentration capable of causing total inhibition of the microbial growth.

2. Characterization of *Pterocladiella capillacea* Extract

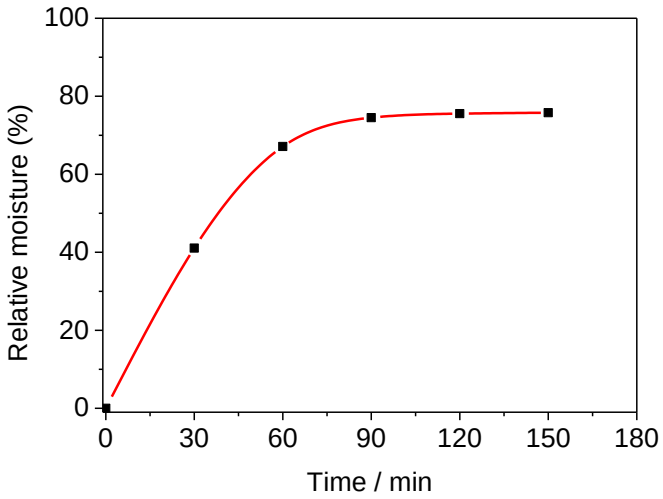

Figure S1. Evaluation of moisture content of *Pterocladiella capillacea*.

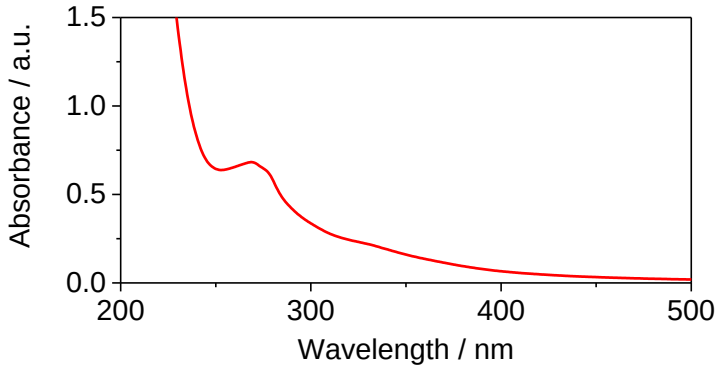

Figure S2. UV/Vis spectrum of *Pterocladiella capillacea* extract in aqueous medium.

3. Synthesis of Silver Nanoparticles

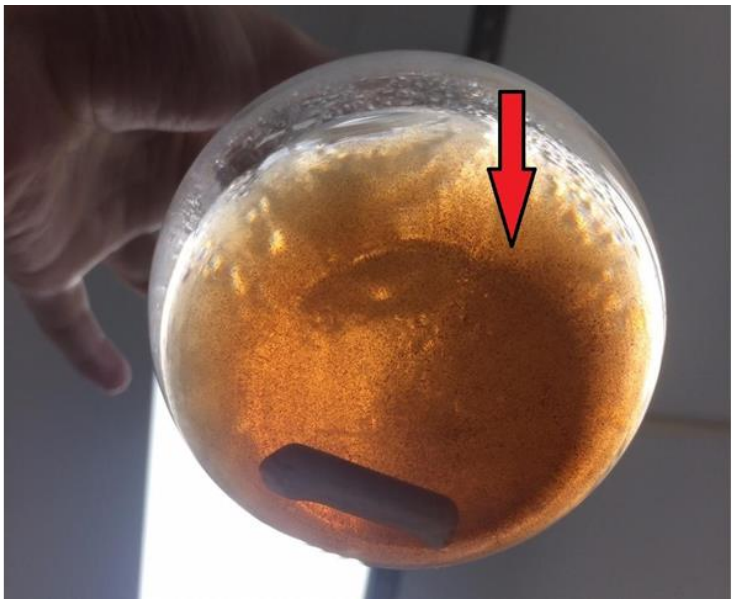

Figure S3. Photo of *Pterocladiella capillacea*-stabilized AgNPs showing irreversible aggregation after 240 min in the synthesis procedure at 60ºC and pH 5.0–6.0, employing 152.9 mg L^{-1} of $AgNO_3$ and 1000 mg L^{-1} of algal extract.

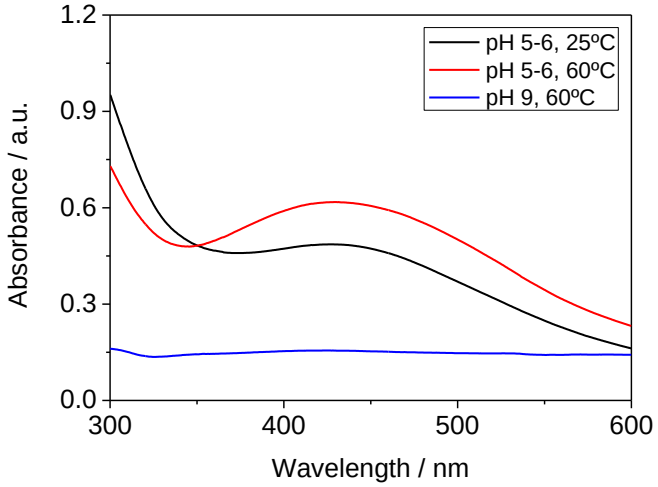

Figure S4. UV/Vis spectra of *Pterocladiella capillacea*-stabilized AgNPs in different conditions, all employing 152.9 mg L^{-1} of $AgNO_3$ and 1000 mg L^{-1} of algal extract: at pH 5.0–6.0 and 60ºC after 120 min (red), at pH 5.0–6.0 and 25ºC after 240 min (black), and at pH 9.0 and 60ºC after 180 min (blue).

4. Storage Studies

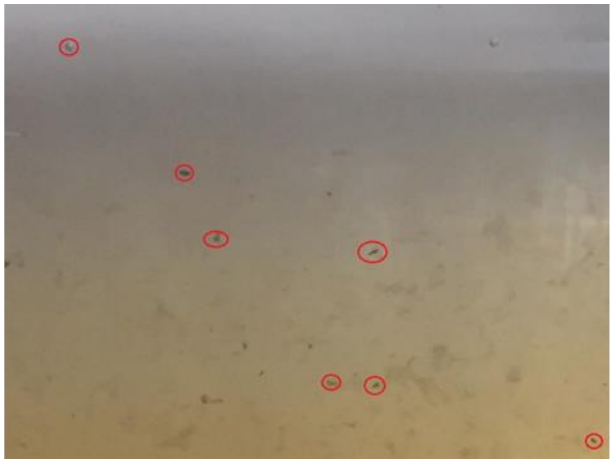

Figure S5. Photo of *Pterocladiella capillacea* extract after one day of storage at 12ºC, in which it is possible to observe macroscopic particles (some examples are circled in red).

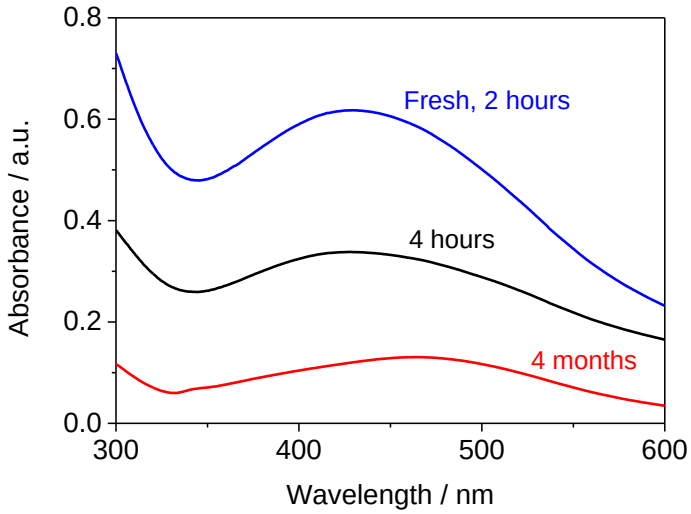

Figure S6. UV/Vis spectra of *Pterocladiella capillacea*-stabilized AgNPs synthesized at 60ºC, pH 5.0–6.0, employing 152.9 mg L^{-1} of $AgNO_3$ and 1000 mg L^{-1} of algal extract. In blue and black colors, spectra collected 2 and 4 hours after the reaction beginning, respectively. In red, spectrum of the AgNPs of assay 2 after a storage period of 4 months at 12ºC.

5. References

(1) de Aragão, A. P.; de Oliveira, T. M.; Quelemes, P. V.; Perfeito, M. L. G.; Araújo, M. C.; Santiago, J. de A. S.; Cardoso, V. S.; Quaresma, P.; de Souza de Almeida Leite, J. R.; da Silva, D. A. Green Synthesis of Silver Nanoparticles Using the Seaweed Gracilaria Birdiae and Their Antibacterial Activity. *Arabian Journal of Chemistry* **2019**, *12* (8), 4182–4188.

(2) Venkatpurwar, V.; Pokharkar, V. Green Synthesis of Silver Nanoparticles Using Marine Polysaccharide: Study of in-Vitro Antibacterial Activity. *Materials Letters* **2011**, *65* (6), 999–1002.

(3) Clinical and Laboratory Standards Institute; Weinstein, M. P. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*; 2018.