Biomass from Beached Algae of the Genus *Caulerpa* to Obtain the Alkaloid Caulerpin

Tiago Tosta Alves Cruz[1*], Jailson Bittencourt de Andrade[1], Sabrina Teixeira Martinez[1]
[1]SENAI CIMATEC University Center; Salvador, Bahia, Brazil

Caulerpin is a molecule that has several biological activities that are extremely important and sought after in the pharmaceutical market. It is found in algae of the genus *Caulerpa*, mainly in the species *Caulerpa racemosa*. This species of algae can be found beached in the sand along the Baía de Todos os Santos (Salvador, Bahia, Brazil). This project aims to study the use of the biomass of these marginalized algae. We extracted and analyzed the algae collected alive to evaluate the presence of caulerpin and then continue the research and processing of data with beached algae. The results showed that caulerpin was identified in all extractions. Therefore, the method for extracting and analyzing caulerpin was optimized and could be used in beached algae later.
Keywords: *Caulerpa racemosa*. Marginalized algae. Biomass. Extraction.

Introduction

Bioprospecting in the seas and oceans has been paramount for the global pharmaceutical market in recent decades [1]. The marine environment constitutes an immense biodiversity of algae rich in biologically active compounds, which makes them increasingly sought after for different purposes [2]. In the context of marine natural products with pharmacological properties, caulerpin is a molecule that stands out [3].

Caulerpin is a bisindole alkaloid with an orange-red color and molecular formula $C_{24}H_{18}O_4N_2$ [3]. This molecule has several biological activities, among which antitumor, antinociceptive, anti-inflammatory, antioxidant, antifungal, and antiviral activities stand out [4]. It is found in macroalgae of the genus Caulerpa, especially in *Caulerpa racemosa*, commonly found on the Brazilian coast [5].

In addition to being located on rocky structures and even associated with other algae in the sea, *Caulerpa racemosa* can also be found on the sand due to a natural process in which tidal activity eventually causes some algae to detach from the surface, in which they are fixed, taking them to the beach [6]. Although there may be degradation of the natural product in question caused by the time the algae is in the sand, many molecules do not change, among which we can consider the alkaloids, substances often produced as a form of defense during the marginalization of algae [7]. In this sense, investing in algae biomass as natural resources for chemical innovation is imperative, especially regarding the pharmaceutical industry, such as a highly sustainable alternative that values what is considered dysfunctional [8].

Therefore, the present work aims to investigate the possibility of using biomass and adding value by obtaining caulerpin, a natural product with diverse biological activities, in algae located in the genus *Caulerpa*, through the extraction and identification of the caulerpin alkaloid in species *Caulerpa racemosa*.

Materials and Methods

The methodological stage of the project took place at the Applied Chemistry Research Laboratory (LIPAQ), where experiments were carried out with algae of the species *Caulerpa racemosa*. The live algae were collected from the coral reef via free diving at a depth of 2 meters in Baía de Todos os Santos, Salvador, Bahia, Brazil, and transported in a thermal box to the laboratory, where they were sorted, freeze-dried and crushed with sanitized scissors.

Received on 20 May 2023; revised 17 August 2023.
Address for correspondence: Tiago Tosta Alves Cruz. Av. Rua Viviane Vieira Pedreira, 21, Ipitanga, Lauto de Freitas - BA, Brazil. Zipcode: 41.706-710. E-mail: tiago.tosta@aln.senaicimatec.edu.br.

J Bioeng. Tech. Health 2023;6(3):224-226

Next, the ultrasound extraction process was carried out, adapted from Cantarino and colleagues [9]. This method was chosen because the extraction uses just a few milligrams of sample. The extraction was carried out in micro extractor flasks, where 10 mg, 30 mg, and 60 mg of crushed algae were weighed, and then 500 µL of ethyl acetate was added. Micro extractor bottles containing algae and solvents were placed in the ultrasound bath for 25 minutes. Then, 10 mg of silica was added to each extractor as a cleanup step. The extracts were filtered into the bottles with a filter in their structure, thus obtaining samples analyzed on a gas chromatograph coupled to mass spectrometry (Figure 1).

After extraction, confirmation of the obtainment of the caulerpin alkaloid from the macroalga *Caulerpa racemosa* was carried out using a gas chromatograph coupled to a Shimatzu mass spectrometer (GCMS-QP2020 SE) equipped with an OAC-20i automatic sampler and a DB-5 column (30 m × 0.25 mm ID, 0.25 µm film, Agilent, Waldbronn, Germany). The chromatographic conditions were: after 1 minute at 220 °C the temperature was increased by 5 °C min^{-1} to 300°C and maintained at 300°C for 20 minutes. A 1 µL sample aliquot was injected in splitless mode at 290°C. High-purity helium (99.999%, White Martins, Brazil) was used as carrier gas at a column flow rate of 1.40 mL min^{-1}. The mass spectrometer was operated in electron ionization mode (EI, 70 eV). The ion source and transfer line temperature were maintained at 250 ◦C and 280 ◦C, respectively. Identification was performed by comparing the retention time and fragmentation pattern with the caulerpin pattern (Figure 2).

Results and Discussion

Initially, the extraction process took place with algae that were not beached to ensure that the caulerpin would be obtained. That being said, in all extractive processes from different masses of algae (10 mg, 30 mg, and 60 mg), it was possible to observe in the total ion chromatogram the peak referring to caulerpin, confirmed by comparing the retention time and the spectrum of masses with the standard and literature data [10]. Among the three extractions, the method starting with 10 mg of algae was chosen to continue the analysis as it uses the smallest sample (Figure 2).

In the mass spectrum, it is possible to observe the molecular ion peak as the base peak of the spectrum at m/z 398. The fragments referring to successive losses of methanol (MeOH) and carbon monoxide (CO) at m/z 366 (M - MeOH), m/z 338 (366 – CO), m/z 306 (338 – MeOH), and m/z 278 (306 - CO), referring to successive breakdowns of the caulerpin molecule [10].

Once the obtainment of caulerpin has been confirmed and the extraction process has been optimized, the methodology must be applied to

Figure 1. Microextraction of the algae *Caulerpa racemosa*.

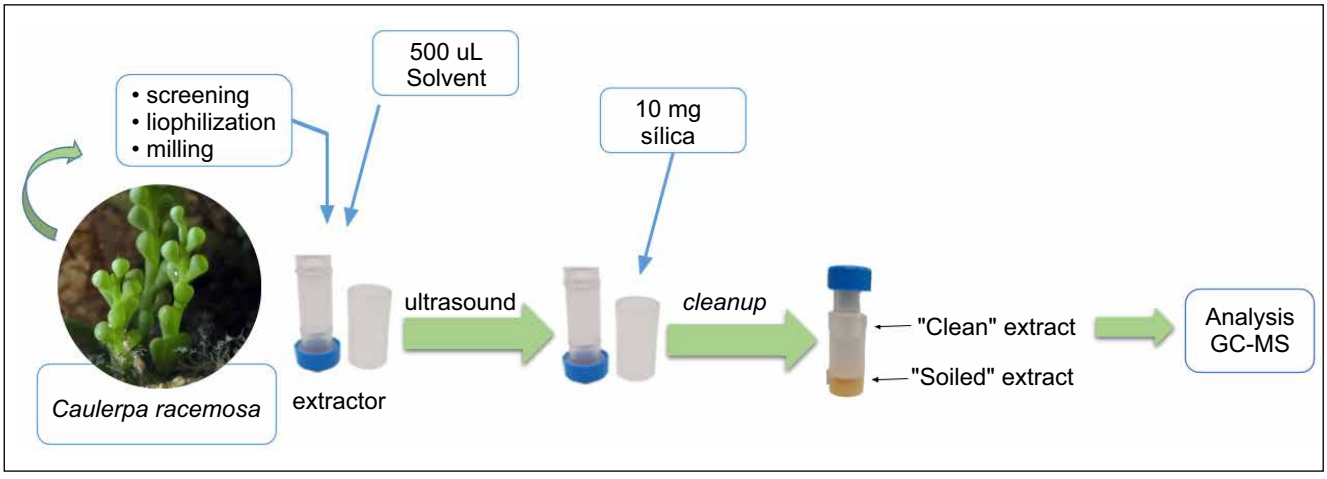
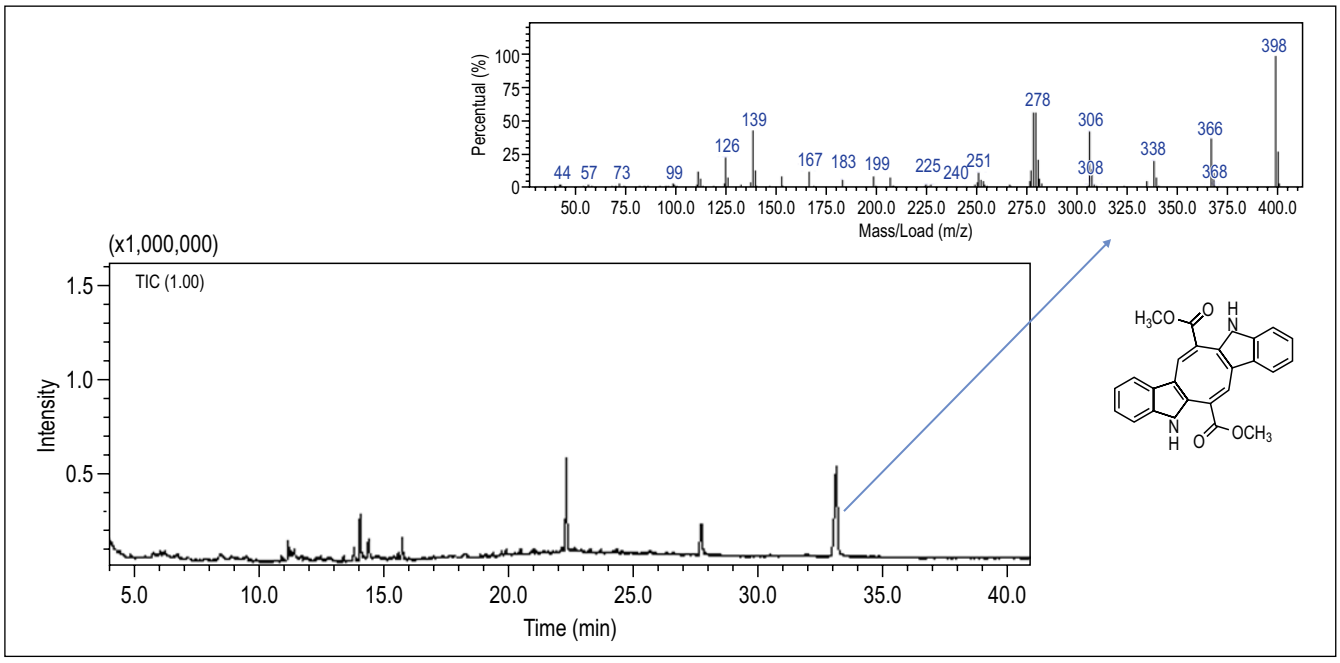


Figure 2. Total ion chromatogram and mass spectrum of caulerpin.



beached algae found along the Baía de Todos os Santos.

Final Considerations

From the analysis of the results obtained, it was possible to conclude that the methodology for extraction and analysis of caulerpin was optimized. This fact occurred by visualizing the total ion chromatogram and the mass spectrum generated. The molecule was identified in the three extractions carried out, each with different masses of algae (10 mg, 30 mg, and 60 mg), which were carried out quickly.

In this way, the project can be continued using the extraction methodology from beached algae and then research the use of the biomass of these algae.

References

1. Felício R et al. Bioprospecção a partir dos oceanos: conectando a descoberta de novos fármacos aos produtos naturais marinhos. Ciência e Cultura 2012;64:39-42.
2. De Alencar DB. Bioprospecção e atividade biológica de produtos naturais das algas marinhas vermelhas Pterocladiella capillacea e Osmundaria obtusiloba. Tese da Universidade Federal do Ceará, 2016.
3. Castro GMFA. Preparação de derivados da caulerpina e avaliação dos seus efeitos citotóxicos e microbiológicos. Tese da Universidade Federal da Paraíba, 2017.
4. Sousa JCF. Semi-síntese de novos derivados da caulerpina e avaliação da atividade leishmanicida do ácido caulerpínico. Tese da Universidade Federal da Paraíba, 2016.
5. Tavares DS. Theoretical study by PM6 of ions complexes type [M (1, 10-fen) 2 L] 2+ with biologic activity potential, where L= caulerpina. Resumo da 34a Reunião Anual da Sociedade Brasileira de Química, Florianópolis 2011.
6. Nova LLMV et al. Utilização de "Algas Arribadas" como alternativa para adubação orgânica em cultivo de moringa (*Moringa oleifera* Lam.). Revista Ouricuri 2014;4:68-81.
7. Harb TB et al. An overview of beach-cast seaweeds: Potential and opportunities for the valorization of underused waste biomass. Algal Research 2022;62:102643-102661.
8. Galembeck F et al. Aproveitamento sustentável de biomassa e de recursos naturais na inovação química. Química Nova 2009;32:571-581.
9. Cantarino SJ et al. Microwave irradiation is a suitable method for caulerpin extraction from the green algae *Caulerpa racemosa* (Chlorophyta, Caulerpaceae). Natural Product Research 2022;36:2149-2153.
10. Aguilar-Santos G. Caulperpin, a new red pigment from green algae of the genus Caulerpa. Journal of the Chemical Society C: Organic 1970:842-843.