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

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Caulerpin is an alkaloid with various biological activities, such as antinociceptive, anti-inflammatory, and antitumoral. This natural product is the majority in algae of the genus *Caulerpa*, which are found fixed to rocks on the seabed and beached to the sands. The objective of the research was to evaluate the possibility of using the biomass of these marginalized algae to obtain the alkaloid. Using ultrasound extraction and analysis by gas chromatography coupled with mass spectrometry, the results showed that it was possible to identify caulerpin in the beached algae. Furthermore, the alkaloid showed good resistance to photodegradation since caulerpin remained practically unchanged after 7 days of exposure to sunlight.
Keywords: Biological Activities. Marginalized Algae. Extraction, Analysis. Photodegradation.

Introduction

The seas and oceans are vital resources for the global economy, which justifies the growth of their productive activities in recent decades [1]. The bioprospecting of natural products in the seas has been increasingly valuable for the pharmaceutical industry since they stand out as a source and inspiration for several drugs currently marketed [2]. Macroalgae play an essential role in the market since they are one of the largest producers of chemically active metabolites with valuable cytotoxic properties, which can treat diseases like cancer [3]. Thus, regarding biological activities in marine natural products found in algae, caulerpin is a molecule that stands out [4].

Caulerpin is a bisindolic alkaloid, reddish-orange in color and molecular formula $C_{24}H_{18}O_4N_2$ (Figure 1) [5]. This molecule plays a crucial role in medicinal chemistry and as a chemical agent, possessing several therapeutic potentials such as antidiabetic, antinociceptive, anti-inflammatory, antitumoral, anti larvicidal, antiherpes, antitubercular, antimicrobial and immunostimulant, among other activities [4]. It is found in macroalgae of the genus *Caulerpa* [5], especially in the species *Caulerpa racemosa*, found on the Brazilian coast [6]. In addition to being attached to rocks and even associated with other algae on the ocean floor, algae of the genus *Caulerpa* may also be beached to the sand due to a natural process in which tidal activity eventually causes the algae to detach from the surface, where they are attached, leading them to the beach [7].

These residual algae can undergo various degradation processes, mainly due to heat and ultraviolet light, because of the high exposure to sunlight on the shores of beaches.

However, how some molecules respond to light exposure may differ, they do not undergo as much change and are more stable than others algae [8,9]. The utilization of the biomass of these algae emerges as an extraordinarily sustainable and highly productive initiative for giving utility to these resources, given as dysfunctional, by obtaining powerful natural products with biological actions.

This work aimed to extract the caulerpin of the biomass of the beached algae genus *Caulerpa*. In addition, the photostability of caulerpin was evaluated for more economical and sustainable extraction.

Materials and Methods

The collection of live algae of the species *Caulerpa racemosa* occurred on the ocean floor,

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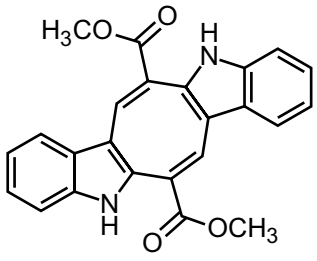
Figure 1. Caulerpin.



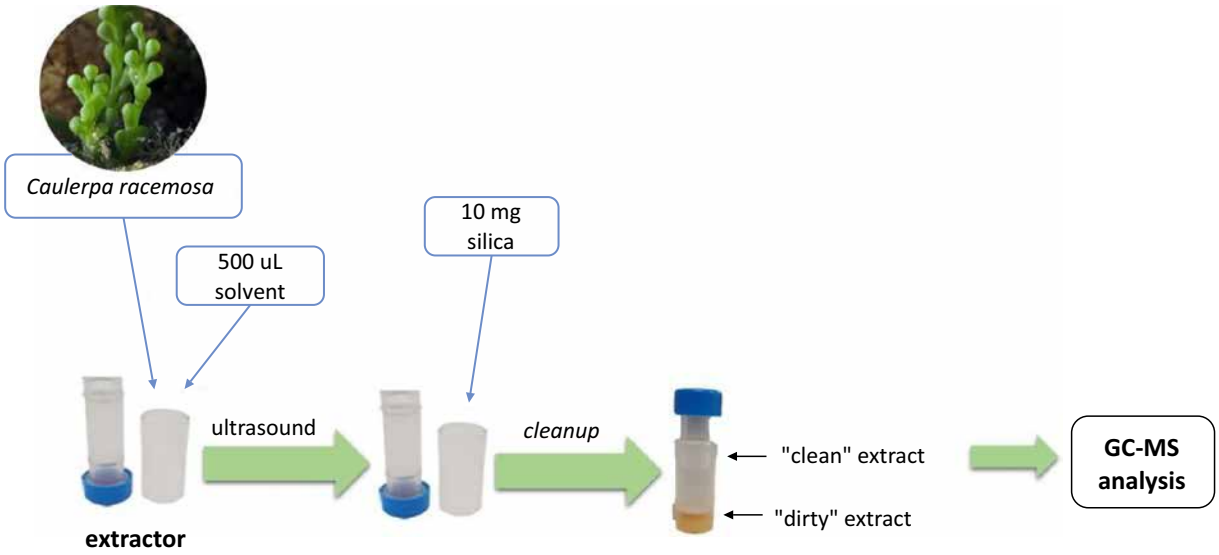
on the coral reef via scuba diving at a depth of 8-16 meters in the Baía de Todos os Santos. After this step, the algae were transported in a thermal box containing ice bags to the Federal University of Bahia (UFBA), where they were sorted and freeze-dried in an SL-404 Solab lyophilizer (Piracicaba, Brazil) for 42 hours and then ground in a ball mill Cienlab (Campinas, Brazil). The ground samples were transferred to clean 50 mL glass flasks, covered by aluminum foil, and transported to the Integrated Laboratory of Applied Research in Chemistry (LIPAQ) of SENAI CIMATEC, where the procedures continued. The Caulerpin standard was obtained according to Cantarino and colleagues [10]. After isolation, caulerpin was analyzed in a Shimatzu gas chromatography coupled to a mass spectrometer (GCMS-QP2020 SE) equipped with OAC-20i autosampler and DB-5 column (30 m × 0.25 mm DI, 0.25 μm film, Agilent, Waldbronn, Germany) into the SCAN mode. The chromatographic conditions were: after 1 min at 220 °C the temperature was increased by 5 °C min^{-1} to 300 °C and held at 300 °C for 20 min. 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), starting at 40 m/z and ending at 400 m/z. The ion source and transfer line temperature were maintained at 250 ◦C and 280 ◦C, respectively. To extract the caulerpin from the live algae collected, they were crushed with sanitized scissors to begin the ultrasonic extraction, adapted from Cantarino and colleagues [10]. This method was chosen due to the extraction capacity in a short period with the use of a small amount of sample mass. The extraction occurred in a micro extractor flask (Whatman Mini-Uniprep Syringeless Filters, Cytiva, USA), adding 10 mg of crushed algae and 500 μL of ethyl acetate as solvent. The flask was directed to the ultrasonic bath (Nova Instruments, model NI 1208, 220 Volts), which remained for 20 minutes. Then, 10 mg of silica was added as a cleanup step to the extract, which was manually stirred and filtered in the micro extractor, which has a filtering membrane. After the extraction, the sample was analyzed by gas chromatography coupled to mass spectrometry under the same conditions and method as the standard was analyzed. After performing on the algae collected alive, the same methodology of extraction and analysis of caulerpin (Figure 2) was applied to the beached algae collected in Praia do Forte, a region located in the municipality of Mata de São João, Bahia. However, unlike those collected alive, these algae underwent a natural drying process with sunlight instead of being lyophilized.

For the caulerpin photostability assays, a freeze-dried algae sample collected alive was placed in a watch glass and exposed to sunlight for 7 days in a ventilated, covered environment with solar solid incidence. After that, the sample was crushed with scissors and subjected to a granulometric sieve of 80 mesh, where the granules were homogenized. Finally, the algae sample exposed to sunlight underwent the extraction and analysis process, together with an algae sample that was not exposed to the sun (Figure 1), for comparative purposes, in which the latter underwent the same grinding and homogenization process as the sample exposed to the sun.

Results and Discussion

Figure 3a presents the GC-MS chromatogram for the isolated standard caulerpin from the algae

Figure 2. Microextraction of the algae *Caulerpa racemosa*.



collected alive, with a retention time of 33.09 minutes. Moreover, the mass spectrum (Figure 3b) generated by the peak referring to caulerpin corresponded with the literature data, in which it is possible to observe the molecular ion peak as the base peak of the spectrum of m/z 398. The fragments referring to successive losses of methanol (MeOH) and carbon monoxide (CO), of m/z 366 (M - MeOH), m/z 338 (366 - CO), m/z 306 (338 - MeOH) and m/z 278 (306 - CO), reveal the breakdown of the caulerpin molecule [11].

According to the method, caulerpin was extracted and analyzed from 10 mg of live algae collected. The extraction process was initially carried out with algae that were not beached to ensure that caulerpin would be obtained. In the total ion chromatogram, it was possible to observe the peak referring to the alkaloid (Figure 4), confirmed by comparing the retention time and mass spectrum with the standard (Figure 3b) and with data from the literature [11].

Once the caulerpin was confirmed and the extraction and analysis methodology were optimized, the same process was carried out on 10 mg of beached algae. Once this procedure was carried out, a total ion chromatogram was generated, whose peak referring to caulerpin was also identified (Figure 5), with a mass spectrum generated identically to that found previously.

When analyzing the chromatogram (Figure 5), it was noted that the peaks not referring to caulerpin were less intense than that of the alkaloid, i.e., the extract was purer than that evidenced in Figure 4. As previously presented, the algae marginalized in the beach sand suffer from the incidence of sunlight, in addition to the fact that the beached algae sample was dried naturally with sunlight instead of freeze-dried. Thus, these results served as a basis for studying the photostability of caulerpin. In this sense, a fraction of algae collected alive and lyophilized was exposed to sunlight for 7 days to show the difference in the total ion chromatogram. Thus, when performing the extraction and analysis of this sample, simultaneously with a fraction of the same algae without having been exposed to the sun, to generate a more reliable comparison, it was found that the peak referring to caulerpin practically did not change after 1 week of exposure to the sunlight (Figure 6).

The results from Figure 6 and Figure 7 allowed a qualitative analysis that the peak referring to caulerpin remained practically unchanged, and the extract was cleaner after 7 days of exposure to sunlight. It suggests that caulerpin has a high resistance to photodegradation.

Figure 3. Results of the analysis of the caulerpin standard. (a) Total ion chromatogram of the standard. (b) Mass spectrum of caulerpin.

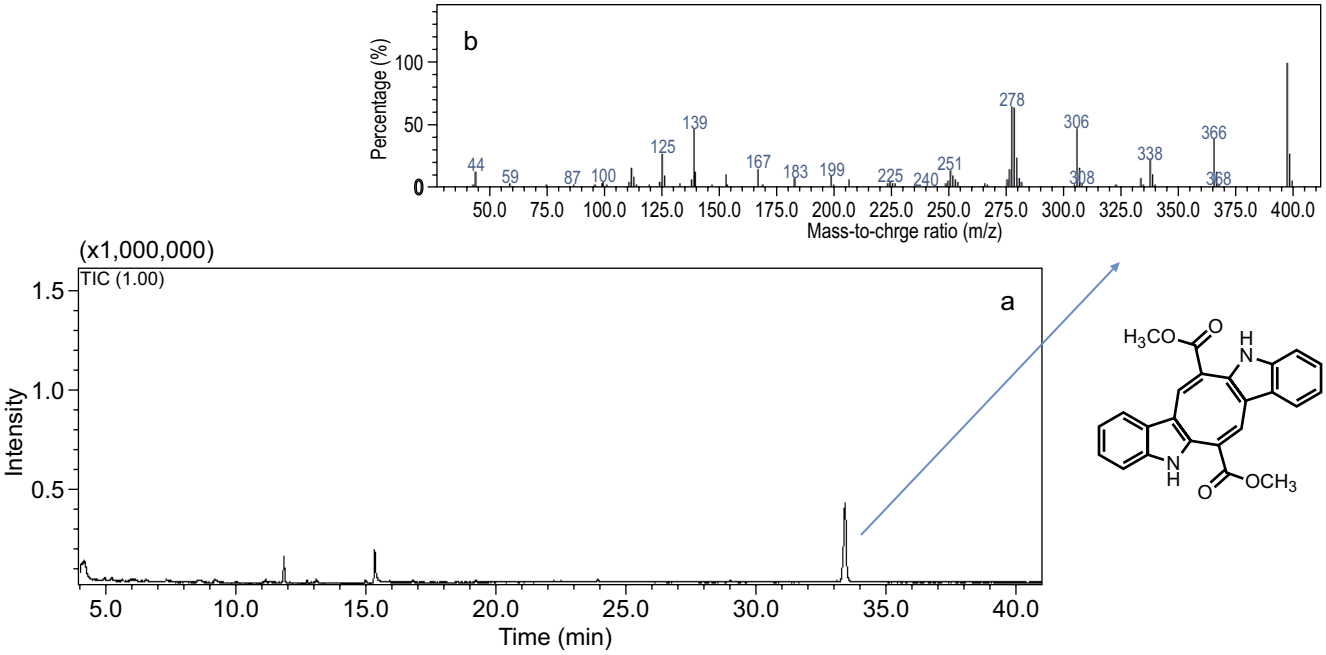


Figure 4. Total ion chromatogram of the algae sample collected alive.

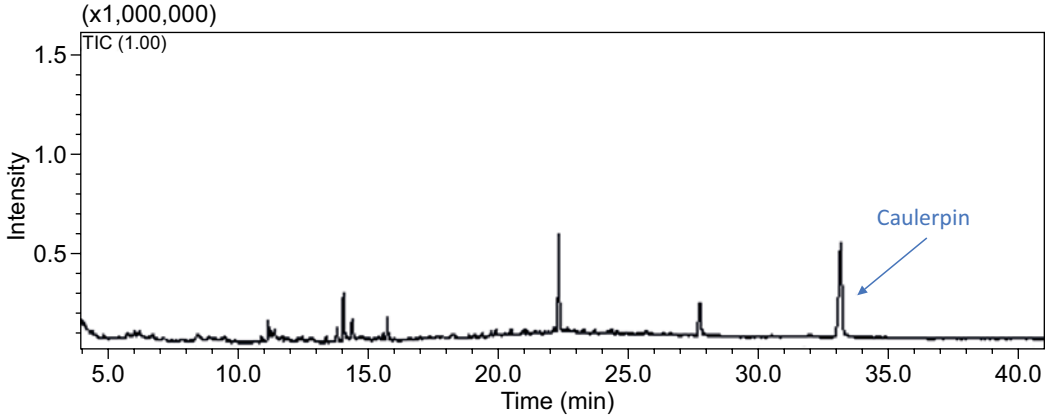


Figure 5. Total ion chromatogram of the beached algae sample.

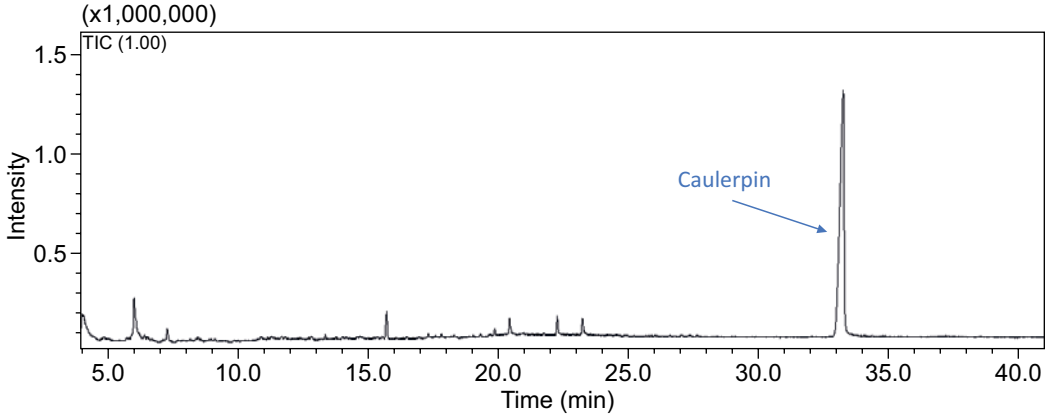


Figure 6. Total ion chromatogram of the freeze-dried live algae sample.

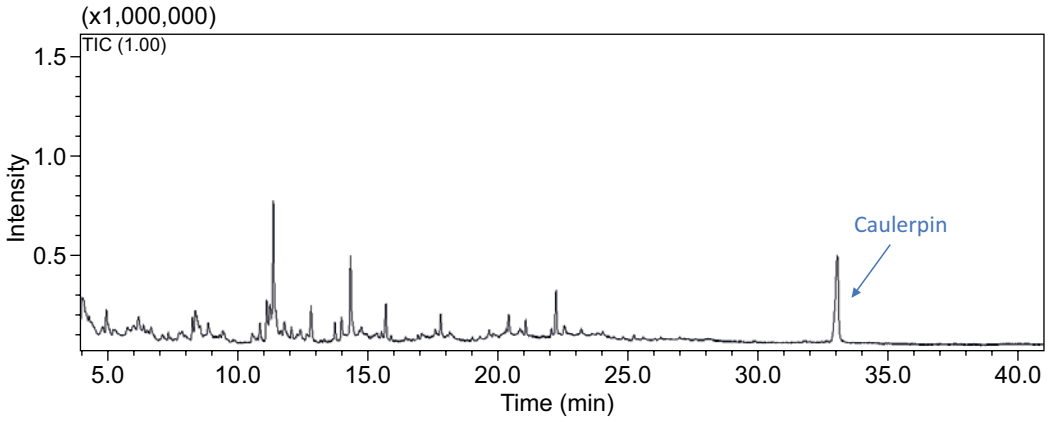
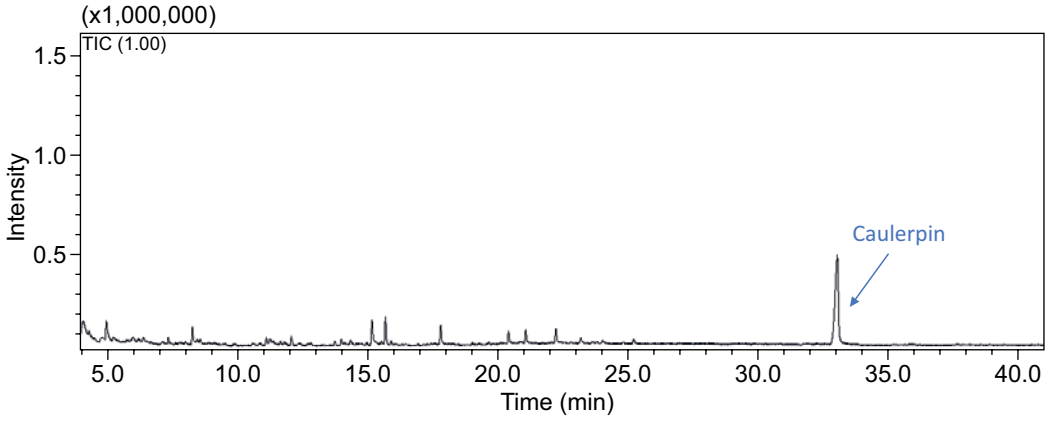


Figure 7. Total ion chromatogram of the freeze-dried live algae sample exposed to the sun for 7 days.



Conclusion

Finally, it was possible to verify that the residual algae, although degraded, can be helpful since, by applying the extraction and analysis methodology on beached algae, the presence of caulerpin was confirmed by identifying the corresponding peak and its mass spectrum. This result prompted photostability studies of the alkaloid, which proved remarkably sunlight resistant. Therefore, the results presented propose a much more dignified destination for the large biomass of algae harbored in the sands of the beaches since these can contain essential natural products with various biological activities, which can be helpful for the most diverse areas of the industry. Hence, using beached algae of the genus *Caulerpa* is a very sustainable and intelligent alternative.

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