Revista Virtual de Química
ISSN 1984-6835

Artigo

Antinociceptive and Anti-inflammatory Effects of *Caulerpa kempfii* (Caulerpaceae)

Matta, C. B. B.; Cavalcante-Silva, L. H. A.; Araújo-Júnior, J. X.; Miranda, G. E. C.; Bastos, K. X.; Souza, J. C. F.; Barbosa-Filho, J. M.; Santos, B.V.O, Alexandre-Moreira, M. S.*

Rev. Virtual Quim., **2015**, *7* (2), 730-743. Data de publicação na Web: 6 de janeiro de 2015

http://www.uff.br/rvq

Efeito Antinociceptivo e Anti-inflamatório de *Caulerpa kempfii* (Caulerpaceae)

Resumo: Os organismos marinhos são uma fonte rica para a pesquisa de novos protótipos de fármacos, entre eles, as algas são um grupo muito diverso, com diferentes características morfológicas, estruturais e metabólicas. O presente trabalho teve como objetivo avaliar as propriedades anti- inflamatória e antinociceptiva das frações hexânica (HE), acetato de etila (AE) e hidroalcoólico (HA) de *Caulerpa kempfii* em camundongos. Para tanto, foram utilizados os modelos de contorção abdominal induzida por ácido acético, teste de placa quente e nocicepção induzida por formalina para avaliar o potencial antinociceptivo das frações, enquanto o teste de peritonite induzida por carragenina foi utilizado para investigar o efeito anti-inflamatório de *C. kempfii.* No ensaio de contorções abdominais, o HE, AE, HA e dipirona induziram uma inibição de 76,7, 83,9, 90,8 e 89,3%, respectivamente. Já no teste da placa quente, os extratos de *C. kempfii* não aumentaram o tempo de latência dos animais em todos os tempos avaliados. Na fase neurogênica do teste de formalina, as frações induziram uma inibição de 28,0% (HE), 37,4% (AE) e 35,9% (HA). Enquanto na fase inflamatória, a inibição foi de 55,1% (HE), 44,5% (AE) e 54,9% (HA), enquanto a indometacina inibiu 62,6%. Além disso, na peritonite induzida por carragenina, foi observada uma redução na migração celular após o tratamento com todas as frações. Dessa forma, com o presente estudo, conclui-se que HE, AE e HA de *C. kempfii* possuem atividade antinociceptiva e anti-inflamatória e poderiam ser utilizados no desenvolvimento de fitoterápicos e na busca por novos protótipos de fármacos.

Palavras-chave: *Caulerpa kempfii*; antinociceptivo; anti-inflamatório; algas verdes.

Abstract

Marine organisms are a rich source of new prototype drugs, and among them, the seaweeds are a very diverse group with different morphological, structural and metabolic features. The present work was designed to evaluate the anti-inflammatory and antinociceptive properties of the hexane (HE), ethyl acetate (EA) and hydroalcoholic (HA) fractions of *Caulerpa kempfii* in mice. The acetic acid-induced writhing, hot plate and formalin-induced nociception tests were carried out to evaluate the antinociceptive potential of these fractions, while the carrageenan-induced peritonitis test was used to investigate the anti-inflammatory activity of *C. kempfii*. In the acetic acid-induced writhing test, HE, EA, HA and dipyrone reduced the number of writhings by 76.7, 83.9, 90.8 and 89.3%, respectively. In the hot plate test, *C. kempfii* fractions did not increase the latency time of the animals in the time evaluated. In the neurogenic phase of the formalin test, the fractions significantly inhibited the pain response by 28.0% (HE), 37.4% (EA), and 35.9% (HA). While in the inflammatory phase, the inhibition was 55.1% (HE), 44.5% (EA) and 54.9% (HA), and indomethacin caused a 62.6% decrease in response. Moreover, in the carrageenan-induced peritonitis test, a reduction in cell migration was seen with all fractions evaluated. The results of this study suggest that HE, EA and HA fractions of *C. kempfii* have anti-inflammatory and antinociceptive properties. Further studies should be conducted to ensure the safety of *C. kempfii* as a natural medicine.

Keywords: *Caulerpa kempfii*; antinociceptive; anti-inflammatory; green algae.

* Federal University of Alagoas, Laboratory of Pharmacology and Immunity, Institute of Biological Sciences and Health, CEP 57020-720, Maceió-AL, Brazil.
suzana.magna@gmail.com; magnasuzana@pq.cnpq.br
DOI: 10.5935/1984-6835.20150033

Volume 7, Número 2 Março-Abril 2015

Revista Virtual de Química
ISSN 1984-6835

Antinociceptive and Anti-inflammatory Effects of *Caulerpa kempfii* (Caulerpaceae)

Carolina B. B. da Matta,[a] Luiz Henrique A. Cavalcante-Silva,[a] João Xavier de Araújo Júnior,[b] George Emmanuel C. de Miranda,[c] Katherine X. Bastos,[d] Jessica Celestino F. Sousa,[d] Bárbara Viviana O. Santos,[e] José Maria Barbosa-Filho,[e] Magna Suzana Alexandre-Moreira[a,*]

[a] Federal University of Alagoas, Laboratory of Pharmacology and Immunity, Institute of Biological Sciences and Health, CEP 57020-720, Maceió-AL, Brazil.

[b] School of Nursing and Pharmacy, Av. Lourival de Mello Motta, s/n Tabuleiro dos Martins, CEP 57072-970, Maceió-AL, Brasil.

[c] Federal University of Paraíba, Laboratory of Marine Algae, Department of Systematics and Ecology, CEP 58051-900, João Pessoa-PB, Brazil.

[d] Federal University of Paraíba, Postgraduate Program in Natural Products and Synthetic Bioactive, CEP 58051-900, João Pessoa-PB, Brazil.

[e] Federal University of Paraíba, Department of Pharmaceutical Sciences, CEP 58051-900, João Pessoa-PB, Brazil.

* suzana.magna@gmail.com; magnasuzana@pq.cnpq.br

Recebido em 5 de janeiro de 2015. Aceito para publicação em 5 de janeiro de 2015

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen, naproxen, and indomethacin constitute a family of drugs that taken as a group represent some of the most frequently prescribed around the world. They are primarily used as pain killers as well as anti-inflammatory agents, and their potential adverse effects are well known. Most of these effects are the direct result of their mode of action, i.e., inhibition of cyclooxygenase (COX), a key enzyme in the biosynthesis of prostaglandins (PGs).[1,2] There are two well-identified isoforms of COX, COX-1 and COX-2. COX 1 isoform is expressed in most tissues, where it serves as a housekeeping enzyme responsible for normal cell homeostasis. On the other hand, COX-2 has little or no expression in most tissues but is rapidly induced in response to inflammatory stimuli, such as lipopolysaccharides.[3,4]

Traditional NSAIDs are nonselective inhibitors of both isoforms of COX and these drugs produce various side-effects, such as gastrointestinal irritation or ulceration and suppression of renal function, due to inhibition of the constitutive COX-1, which is responsible for the production of prostaglandins, responsible for gastroprotection and vascular homeostasis.[5] Furthermore, inhibition of COX-1 blocks platelet thromboxane production, which increases the chances of bleeding.[6] Thus, the development of NSAIDs that selectively inhibit COX-2 (coxibs) was initiated to produce anti-inflammatory agents and analgesics with reduced toxicity compared to traditional NSAIDs. However, the cardiovascular side-effects associated with selective COX-2 inhibitors highlight the pitfalls that may be encountered in the drug discovery paradigm.[5,7] Thus, the search for new sources of substances with anti-inflammatory and analgesic activity is needed.

Marine organisms are believed to be a potential source of novel biologically active substances and have been extensively studied in search of promising drug candidates for the treatment of various pathologies. In recent years, the Food and Drug Administration (FDA) in the United States has approved three marine-derived drugs: cytarabine (isolated from sponges), vidarabine (isolated from sponges), and ziconotide (isolated from cone snails). In addition, 13 marine-derived compounds are either in phase I, phase II or phase III clinical trials, and several hundred novel marine compounds are in the preclinical pharmaceutical pipeline.[8,9]

Among the marine organisms of interest, macroalgae constitute a very diverse group differing in morphological, structural and metabolic features. The versatility of the functions of algae may derive from their abundant bioactive metabolites, which have attracted a great deal of attention due to their potential effects in promoting health and reducing disease risk. In the past years, there was a remarkable increase in macroalgal pharmacology research.[10-12] Somchit and colleagues described the antipyretic and anti-inflammatory properties of extracts of two blue-green algae, *Spirulina platensis* and *S. lonar*, in rats treated orally with 2 or 4 mg/kg, respectively.[13] Two publications yielded potentially novel compounds targeting anti-inflammatory activities: an ethanolic extract of *Sargassum horneri* and 8,8'-bieckol, isolated from brown algae, inhibited LPS-induced murine macrophage RAW 264.7 cells by affecting NFκB signaling activity.[14,15] Ahn and colleagues described a new phlorotannin, dieckol, from the brown algae *Ecklonia cava*, which potently induced apoptosis of ovarian cancer cells and inhibited tumor xenograft growth.[16] These and other studies, such as those found in the most current review by Mayer and colleagues, show that these organisms possess promising activities.[17]

Chlorophytes represent the most diverse group among all green algae, comprising approximately 17,000 species widely distributed in several coastal environments in

the world. Among the chlorophytes, in turn, are algae of the genus *Caulerpa* (Caulerpaceae), recognized by Lamouroux in 1809, with about 75 species distributed in tropical and subtropical waters worldwide.[18] Macroalgae *Caulerpa* spp. are morphologically unusual, because they are unicellular and differentiated giant cells.[19] Algae of this family produce several secondary metabolites including sesquiterpenoids and diterpenoids to protect themselves from predators.[20] It seems that caulerpenyne, a sesquiterpene, plays a major role in chemical defense.[21] In addition, triterpenes, squalene, squalene epoxides, sterols, di-indole pigments, caulerpin and its analogues, caulersin,[22] a mixture of ceramide derivatives, and caulerpecin[23] are the other secondary metabolites that have been isolated from different *Caulerpa* species.

Several activities of preparations from different species of *Caulerpa* have been reported, including immunomodulatory,[24] hemagglutinating,[25] hypolipidemic[26] and antioxidant.[27] However, no study about biological activities related to the species *Caulerpa kempfii* has been reported to date, probably due to its restricted distribution along the Brazilian coast.[28,29] Thus, given that other species of *Caulerpa*[30-32] have shown antinociceptive and anti-inflammatory activities, the aim of this work was to evaluate these pharmacological properties in *C. kempfii*.

2. Material and Methods

2.1. Plant material

Specimens of the alga *C. kempfii* were collected along the coast of Pitimbu, Paraíba – Brazil, during spring tides (-0.2 to 2.0 m) in 2009. The sample was classified by Dr. George Emmanuel C. de Miranda (Department of Systematics and Ecology). The voucher specimen (JPB 13986) was deposited in Herbarium Lauro Pires Xavier of Federal University of Paraiba. After collection, the material was washed with distilled water, cleaned of epiphytes, weighed (1 kg) and subsequently dried at room temperature (~25°C).

2.2. Preparation of extracts

The fresh algae material (dry weight at room temperature, 0.5 kg) was exhaustively extracted three times with 95% aqueous EtOH (30 Leach) for 24 h each time, at room temperature. The combined EtOH extracts were filtered and concentrated *in vacuo*. The resulting brown residue (50 g) was suspended in 2.5 L H_2O, which was then partitioned successively with hexane (three times with 1.5 L each) and EtOAc (three times with 1.5 L each). After removal of solvent, 5, 20 and 25 g of hexane, ethyl acetate and hydroalcoholic fractions were obtained, respectively.

2.3. Drugs and reagents

The following drugs and chemicals were used: sodium chloride, trypan blue, carboxymethylcellulose (CMC), carrageenan, Tween 80 and dipyrone were purchased from Sigma (St. Louis, MO, USA). Formaldehyde and acetic acid were obtained from Vetec Química Farm Ltda (Rio de Janeiro, RJ, BR). Indomethacin was purchased from Merck (Darmstadt, Germany) and morphine sulfate from Cristália (Rio de Janeiro, RJ, BR). The hexane (HE), ethyl acetate (EA) and hydroalcoholic (HA) fractions obtained from *C. kempfii* were diluted with CMC+0.1% Tween 80 as a suspension (vehicle) and were administered by the oral route (p.o.) at a dose of 100 mg/kg. Dipyrone, morphine and indomethacin were used as reference drugs. The doses were chosen based on previous studies.[30,31]

2.4. Animals

Swiss mice of either sex (20–25 g) maintained at the Central Vivarium (BIOCEN) at the Alagoas Federal University in Brazil were used throughout the experiments. They were housed in single-sex cages under a 12-h light/dark cycle in a controlled temperature room (22 ± 2°C) with free access to water and pellet food. Eight hours before each experiment, animals received only water, to avoid food interference with substance absorption. The experiments were performed after the approval of the protocol by the Ethics Committee for Animal Handling – UFAL (No: 23065.002260/2011-21).

2.5. Acetic acid-induced writhing test

The test was carried out using the method previously described by Collier *et al*.[33] The animals were divided into five groups, with six mice in each group. Each mouse was injected intraperitoneally (i.p.) with 0.1 mL/10 g body weight of 0.6% v/v acetic acid 40 min after p.o. administration of the test fractions (100 mg/kg) or vehicle (negative control). Dipyrone (40 mg/kg, p.o.) was administered to mice as a positive control. The writhing response, which consists of a contraction of the abdominal muscle together with a stretching of the hind limbs, was evaluated for 20 min after a latency period of 5 min, recording the number of writhings.

2.6. Hot-plate test

The test was performed as described by Kuraishi *et al*.[34] The temperature was regulated at 54° ± 1°C. Mice were divided into five groups consisting of six animals each. The mice of each group were placed in the beaker (on the hot plate) to observe their response to electrical heat-induced pain for a 30-min period. The baseline was considered the mean reaction time obtained at 30 and 60 min before administration of the fractions (100 mg/kg, oral), vehicle (oral) or morphine (5 mg/kg, subcutaneous) and was defined as the normal reaction of the animal to temperature. After treatment, the reaction time (in seconds) was recorded when the animals licked their fore and hind paws and jumped at 30, 60, 90 and 120 min, as an indicator of the animal's response to heat-induced pain. Animals showing a reaction time greater than 15 s were discarded.

2.7. Formalin-induced nociception test

The procedure described by Hunskaar and Hole[35] was followed with slight modifications. The mice were divided into five groups each containing six mice and were administered distilled water (1 mL/kg, i.p.), fractions of *C. kempfii* (100 mg/kg, p.o.) or indomethacin (35.7 mg/kg, p.o.). After 40 min of treatment, 2.5% formalin (20 µl) solution was injected into the right hind paw. The response was the amount of time the animals spent licking the injected paw. Two distinct periods of high licking activity can be identified, a neurogenic phase lasting the first 5 min and an inflammatory phase lasting from 15 to 30 min after the injection of formalin.

2.8. Carrageenan-induced peritonitis test

This test was conducted as described by Ferrándiz and Alcaraz.[36] The mice were divided into five groups each containing six mice. Carrageenan was freshly prepared (10 mg/mL) in sterile normal saline and 0.25 mL was injected i.p. Four hours later, the animals were killed by cervical dislocation. The peritoneal cavity was washed with 1.5 mL cold phosphate buffered saline (PBS), and after gentle manual massage, the exudate was extracted. The peritoneal exudate was used for total leukocyte counts. The fractions (100 mg/kg), indomethacin (35.7 mg/kg) or vehicle were administered p.o. 40 min before the carrageenan injection.

2.9. Statistical analysis

Data obtained from animal experiments were expressed as mean and standard error of the mean (mean ± S.E.M.) for six animals. Statistical differences between the treated and the control groups were evaluated by ANOVA and Dunnett's test. $p < 0.05$ was considered statistically significant.

3. Results and Discussion

In this study, the antinociceptive effects of HE, EA and HA fractions of *C. kempfii* were evaluated using classical *in vivo* models of nociception induced by chemical stimuli as in the acetic acid-induced writhing test[33] and formalin-induced nociception test[35] as well as by thermal stimulus as in the hot plate test.[34] In addition, we used the carrageenan-induced peritonitis by test,[36] which is a model of cell migration, also to investigate the anti-inflammatory activity of these fractions.

The first test conducted was the acetic acid-induced writhing. In this test, the writhings induced by i.p. injection of 0.6% acetic acid (44.7 ± 2.6) were markedly reduced by pre-treatment with *C. kempfii*, given p.o. (100 mg/kg) 40 min beforehand, exhibiting significant inhibition of the nociceptive response: 76.7% - HE (10.40 ± 2.4; $p< 0.01$), 83.9% - EA (7.2 ± 2.1; $p < 0.01$), and 90.8% - HA (4.1 ± 0.7; $p< 0.01$). These results are similar to the inhibition resulting from treatment with the standard drug dipyrone (89.3%; 4.8 ± 1.6; $p < 0.01$) (Figure 1).

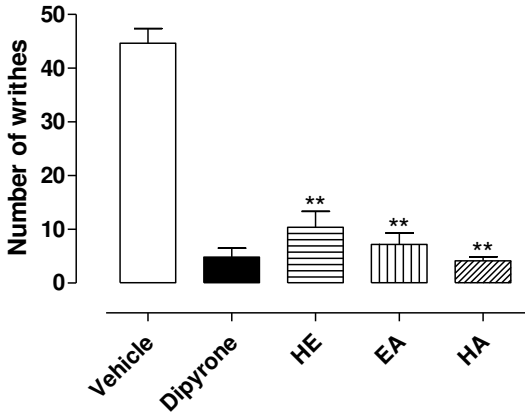

Figure 1. Antinociceptive effect of *C. kempfii* (100 mg/kg, p.o.) in acetic acid-induced writhing. Each column represents the mean ± S.E.M. of 6 animals. Statistical differences between the treated and the control groups were evaluated by ANOVA and Dunnett's test and the asterisks denote the levels of significance in comparison with control groups, $**p < 0.01$

There are previous studies that describe the antinociceptive activity of other species of the genus *Caulerpa* using this same model. These studies include works that demonstrated that a lectin and sulfated polysaccharides from *C. cupressoides* exhibited antinociceptive effects.[32,37] Souza *et al.*[30] showed that a bisindole alkaloid, caulerpin, isolated from genus *Caulerpa* had antinociceptive and anti-inflammatory activities. Furthermore Matta *et al.*[31] evaluated the antinociceptive activity of *C. mexicana* and *C. sertularioides* and obtained similar results as ours.

In the acetic acid-induced abdominal writhing test (visceral pain model), endogenous mediators that sensitize nociceptors are released, and prostaglandins

(PGs) are the major inflammatory mediators that cause algesia. Thus, this model is generally associated with the release of prostanoids, resulting in increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid, as well as of products of the lipoxygenase pathway,[38] where activity in this model may be related to greater inhibition of peripheral COX.[33] As a result, the abdominal pain induced by acetic acid can be prevented by NSAIDs. Moreover, other agents such as sedatives and neuromuscular blockers may also act in this model, which could result in a misinterpretation of the results.[40,41]

Considering this and the results obtained in this work, it is possible that the antinociceptive effect of the *C. kempfii* fractions may be due to direct inhibition of the release of mediators induced by acetic acid by inhibiting the migration of cells that would exacerbate the painful process, or even to central modulation of nociceptive transmission.

To distinguish between central and peripheral antinociceptive actions, the hot plate test was carried out to evaluate the profile of *C. kempfii* fractions. However, no frac of *C. kempfii* evaluated showed an effect in the hot-plate test (data not shown).

To confirm and better understand the antinociceptive activity of these fractions, we performed the formalin-induced nociception test. In this assay, the fractions of *C. kempfii* significantly reduced paw licking time, after subplantar injection of formalin, in both phases of the test as shown in Figure 2.

In the neurogenic phase, the time the animal spent licking the paw in response to formalin in the group treated only with vehicle was 70.6 ± 2.3 s. This time was reduced after pre-treatment with fractions of *C. kempfii* (100 mg/kg, p.o.) 40 min beforehand, exhibiting a significant decrease of 28.0% - HE (50.8 ± 5.0 s; $p < 0.05$), 37.4% - EA (44.2 ± 3.5 s; $p < 0.01$), and 35.9% - HA (45.2 ± 5.7 s; $p < 0.01$). While the standard drug, indomethacin (p.o) reduced the time by 24.9% (53.0 ± 6.1; $p < 0.05$) (Figure 2A). Souza *et al*.[30] assessed the crude methanolic extract and n-butanol and chloroform phases of *C. racemosa* in that same assay, and observed a inhibition of nociceptive response of 51.8, 35.1 and 32.7%, respectively[30]. In another study conducted by our group, treatment with the hexane, chloroform, ethyl acetate and methanolic extracts of *C. mexicana* produced a decrease in paw-licking time of 39.7, 31.1, 60.2 and 50.2%, respectively, in the neurogenic phase of the formalin test.[31]

In the inflammatory phase of this test, the control group (vehicle) spent 250.9 ± 21.6 s licking the paw in response to formalin, while pre-treatment with the fractions of *C. kempfii* reduced this time significantly by 55.1% - HE (112.8 ± 21.1 s; $p< 0.01$), 44.5% - EA (139.2 ± 12.0 s, $p < 0.01$) and 54.9% - HA (113.1 ± 12.4 s; $p < 0.01$). In addition, indomethacin decreased the time by 62.6% (93.8 ± 19.1; $p < 0.01$) (Figure 2B). These data corroborate those obtained by Matta *et al*.,[31] where extracts of *C. mexicana* and *C. sertularioides* significantly reduced paw-licking time in the inflammatory phase of formalin test.

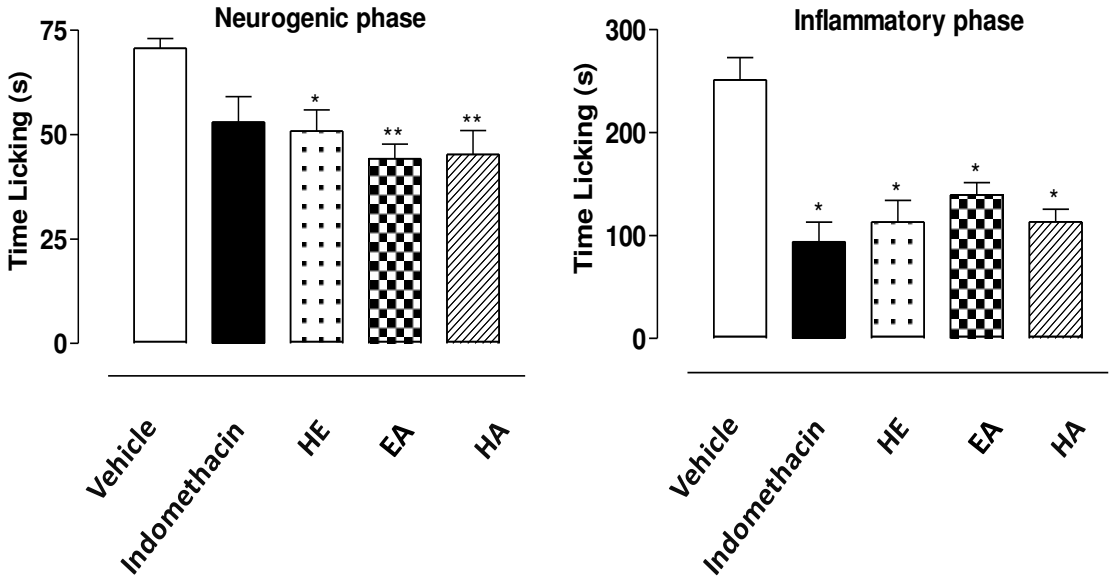

Figure 2. Antinociceptive effect of *C. kempfii* (100 mg/kg, p.o), against early phase (0–5 min, panel A) or late phase (15–30 min, panel B) of formalin-induced nociception in mice. Each column represents the mean ± S.E.M. of 6 animals. Statistical differences between the treated and the control groups were evaluated by ANOVA and Dunnett's test and the asterisks denote the levels of significance in comparison with control groups, $^{*}p < 0.05$ and $^{**}p < 0.01$

In formalin-induced nociception, it is possible to evaluate two different types of pain for the same stimulus, central and peripheral.[42] Neurogenic nociception (early phase) starts immediately after formalin injection, resulting in the release of neuropeptides such as substance P and CGRP in central terminals and peripheral mediators such as bradykinin in peripheral endings.[43,44] After a period of 5 to 10 min, the inflammatory phase starts; in this late phase, inflammatory mediators are formed in peripheral tissues, such as cytokines, bradykinin, prostaglandins, substance P, nitric oxide, serotonin and histamine, inducing functional changes in dorsal horn neurons, which over time, promote awareness of transmission at the spinal level.[45]

It is described in the literature that NSAIDs and corticosteroids only inhibit the inflammatory phase of the test.[46,47] However, selective inhibitors of COX-1 (SC-560) inhibit both phases of the formalin test, indicating that non-selective NSAIDs are capable of acting in both the neurogenic and inflammatory phase of formalin test.[42] Santos *et al*.[49] proved that meloxicam, a non-selective NSAID, can inhibit the neurogenic phase at concentrations required to inhibit the inflammatory phase of the formalin test.

Note that *C. kempfii* fractions showed an inhibition profile similar to that of indomethacin in the neurogenic and inflammatory phases. The data show that these fractions contain substances that appear to be acting on the inflammatory process and peripheral mechanisms modulating the nociceptive response.[50,51]

To evaluate the ability of the fractions of *C. kempfii* to inhibit cell migration, one of the steps in the inflammatory process, carrageenan-induced peritonitis was evaluated. In this assay, cell migration was markedly reduced by pre-treatment with *C. kempfii* extracts (100 mg/kg, p.o.) 40 min beforehand: 13.3 ± 0.4 x 10^6 cells/mL (HE), 8.4 ± 0.9 x 10^6 cells/mL (EA) and 13.5 ± 1.0 x

10^6 cells/mL (HA), exhibiting significant inhibition of 27.0% ($p < 0.01$), 53.8% ($p < 0.01$), and 26.0% ($p < 0.01$), respectively, in comparison with the carrageenan alone group (18.2 ± 0.6 x 10^6 cell/mL). In addition, indomethacin inhibited cell migration by 67.1% (6.0 ± 0.4 x 10^6 cells/mL, $p < 0.01$) (Figure 3).

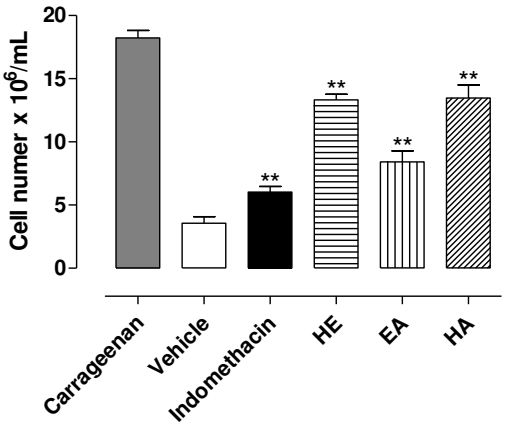

Figure 3. Anti-inflammatory effect of *C. kempfii* extracts (100 mg/kg, p.o) on carrageenan-induced peritoneal inflammation. Each point represents the mean ± S.E.M. of 6 animals. Statistical differences between the treated and the control group were evaluated by ANOVA and Dunnett's test and the asterisks denote the levels of significance in comparison with control groups, $^{**}p < 0.01$

Bitencourt *et al*.[52] demonstrated in an *in vivo* study on the anti-inflammatory activity of *C. mexicana* in mice that aqueous and methanolic extracts were able to suppress cell migration to the peritoneal cavity in a time-dependent but not dose-dependent manner. Furthermore, these extracts reduced cell migration to different sites as well as decreasing edema formation induced by chemical irritants. These results corroborated our findings of an anti-inflammatory effect of *C. kempfii* fractions.

Carrageenan-induced peritonitis is an experimental model of acute inflammation characterized and employed to test new anti-inflammatory therapies to allow quantification of cell migration and its correlation with different inflammatory mediators.[53] It is believed that carrageenan-induced inflammation can be inhibited by pre-treatment with anti-inflammatory drugs such as NSAIDs, which inhibit COX, thereby reducing prostaglandin biosynthesis.[54] Thus, the data presented in this paper corroborate previous results obtained with the writhing and formalin tests (inflammatory phase), indicating that active compounds present in fractions of *C. kempfii* may exert anti-inflammatory activity, probably by inhibition of cell migration, COX activity, or other inflammatory mediators.

In summary, the data obtained in this study show that fractions of *C. kempfii* have peripheral antinociceptive activity with an anti-inflammatory profile. It is possible to assign this activity to secondary metabolites present in these fractions, which could act synergistically. Some examples are the characteristic metabolites of the genus *Caulerpa*, such as caulerpin, a bisindolic alkaloid with antinociceptive and anti-inflammatory activity in murine models, described by our group.[30] In addition, recent studies conducted in our laboratory demonstrated that caulerpin is able to inhibit COX (unpublished data).

Another dominant secondary metabolite in the genus *Caulerpa* is caulerpenyne, a sesquiterpene that has lipoxygenase

inhibitory activity.[55,56] Furthermore, a recent study revealed that caulerpenyne inhibits xanthine oxidoreductase (XOD) irreversibly. XOD catalyzes the final steps of purine catabolism leading to uric acid formation and has a significant role in many diseases such as inflammations.[57,58]

Terpenes are one of the most abundant classes of metabolites in the genus *Caulerpa* and have anti-inflammatory and analgesic activity already identified in other plant species.[59,60] Alkaloids also could be responsible for these effects of *C. kempfii*, since many of this class show analgesic and anti-inflammatory activity.[61] Besides these secondary metabolites, certainly other substances may also be responsible for the effects found in these fractions.

Thus, the present study contributes to our knowledge of the therapeutic potential of *C. kempfii*, suggesting its applicability as a marine natural product for the preparation of herbal medicines or as a source of biologically active compounds that can serve as prototype drugs or phytochemicals after more pharmacological and toxicological tests. Furthermore, this work contributes to our research with marine natural products.[30,31,62,63]

4. Conclusions

The results of this work show that extracts of the macroalga *C. kempfii* exhibit an antinociceptive and anti-inflammatory profile. However, further studies are needed to better identify the mechanisms of action of these extracts.

Acknowledgments

The authors are grateful to the CAPES, CNPq, MCT, FINEP, FAPEAL (PRONEM 20110722-006-0018-0010), CNPQ (479822/2013-1), CNPQ (404344/2012-7), INCT-INOFAR/CNPq (573.564/2008-6) and INCT Amb Tropic and the Ministry of Environment (License MMA/CGEN 18/2007) for the joint funding of this research project. The authors wish to thank several of their colleagues working at the Federal University of Alagoas for their constructive criticism and assistance in carrying out this project. Dr. A. Leyva helped with English editing of the manuscript.

References

[1] Ungprasert, P.; Kittanamongkolchai, W.; Pri ce, C. D. What is the “safest” non-steroidal anti-inflammatory drugs? *American Journal of Medicine* **2012**, *3*, 115. [CrossRef]

[2] Patrignani, P.; Tacconelli, S.; Bruno, A.; Sostres, C.; Lanas, A. Managing the adverse effects of nonsteroidal anti-inflammatory drugs. *Expert Review of Clinical Pharmacology* **2011**, *4*, 605. [CrossRef]

[3] Patrignani, P.; Patrono, C. Cyclooxygenase inhibitors: From pharmacology to clinical read-outs. *Biochimica et Biophysica Acta* **2014**, *9*, 29. [CrossRef] [PubMed]

[4] Ricciotti, E.; FitzGerald, G. A. Prostaglandins and Inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2011**, *31*, 986. [CrossRef]

[5] Asif, M. General study of pyridazine compounds against cyclooxygenase enzyme and their relation with analgesic, anti-inflammatory and anti-arthritic activities. *Chronicles of Young Scientists* **2010**, *1*, 3. [CrossRef]

[6] Sostres, C.; Gargallo, C. J.; Arroyo, M. T.; Lanas, A. Adverse effects of non-steroidal anti- inflammatory drugs (NSAIDs, aspirin, and coxibs) on upper gastrointestinal tract. *Best Practice and Research Clinical Gastroenterology* **2010**, *24*, 121. [CrossRef]

[7] Rao, P. N. P.; Knaus, E. E. Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond. *Journal of Pharmaceutical Sciences* **2008**, *11*, 81s. [PubMed]

[8] Mayer, A. M.; Glaser, K. B; Cuevas, C.; Jacobs, R. S.; Kem, W.; Little, R. D.; McIntosh, J. M.; Newman, D. J.; Potts, B. C.; Shuster, D. E. The odyssey of marine pharmaceuticals: a current pipeline perspective. *Trends in Pharmacological Sciences* **2010**, *31*, 255. [CrossRef] [PubMed]

[9] Molinski, T. F. Drug development from marine natural products. *Nature Reviews Drug Discovery* **2009**, *8*, 69. [CrossRef] [PubMed]

[10] Liu, M.; Hansen, P. E.; Lin, X. Bromophenols in marine algae and their bioactivities. *Marine Drugs* **2011**, *9*, 1273. [CrossRef] [PubMed]

[11] Ngo, D .H.; Vo, T. S.; Ngo, D. N.; Wijesekara, I.; Kim, S. K. Biological activities and potential health benefits of bioactive peptides derived from marine organisms. *International Journal of Biological Macromolecules* **2012**, *51*, 378. [CrossRef] [PubMed]

[12] Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H.; Prinsep, M. R. Marine natural products. *Natural Product Reports* **2012**, *29*, 144. [CrossRef] [PubMed]

[13] Somchit, M. N.; Mohamed, N. A.; Ahmad, Z.; Zakaria, Z. A.; Shamsuddin, L.; Omar-Fauzee, M. S.; Kadir, A. A. Anti-inflammatory and anti-pyretic properties of *Spirulina platensis* and *Spirulina lonar*: a comparative study. *Pakistan Journal of Pharmaceutical Sciences* **2014,** *27*, 1277. [Link]

[14] Kim, M. E.; Jung, Y. C.; Jung, I.; Lee, H. W.; Youn, H. Y.; Lee, J. S. Anti-inflammatory effects of ethanolic extract from *Sargassum horneri* (Turner) C. Agardh on lipopolysaccharide-stimulated macrophage activation via NF-κB pathway regulation. *Immunological Investigations* **2014**, 1. [CrossRef] [PubMed]

[15] Yang, Y. I.; Jung, S. H.; Lee, K. T.; Choi, J. H. 8,8'-Bieckol, isolated from edible brown algae, exerts its anti-inflammatory effects through inhibition of NF-κB signaling and ROS production in LPS-stimulated macrophages. *International Immunopharmacology* **2014**, *14*, S1567. [CrossRef] [PubMed]

[16] Ahn, J. H.; Yang, Y. I.; Lee, K. T.; Choi, J. H. Dieckol, isolated from the edible brown algae *Ecklonia cava*, induces apoptosis of ovarian cancer cells and inhibits tumor xenograft growth. *Journal of Cancer Research and Clinical Oncology* **2014**, 1. [CrossRef] [PubMed]

[17] Mayer, A. M. S; Rodríguez, A. D.; Taglialatela-Scafati, O; Fusetani, N. Marine pharmacology in 2009–2011: marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action. *Marine Drugs* **2013**, *11*, 2510. [CrossRef] [PubMed]

[18] Fama, P.; Wysor, B.; Wiebe, H. C.; Kooistra, F.; Zuccarello, G. C. Molecular phylogeny of the genus *Caulerpa* (Caulerpales Chlorophyta) inferred from chloroplast tuf A gene. *Journal of Phycology* **2002**, *38*, 1040. [CrossRef]

[19] Menzel, D. How do giant plant cells cope with injury? The wound response in siphonous green alga. *Protoplasma* **1988**, *144*, 73. [CrossRef]

[20] Capon, R. J.; Ghisalberti, E. L.; Jefferies, P. R. Metabolites of the green algae, *Caulerpa* species. *Phytochemistry* **1983**, *22*, 1465. [CrossRef]

[21] Box, A.; Sureda, A.; Tauler, P.; Terrados, J.; Marba, N.; Pons, A. Seasonality of caulerpenyne content in native *Caulerpa prolifera* and invasive *C. taxifolia* and *C. racemosa* var. cylindracea in the western Mediterranean Sea. *Botanica Marina* **2010**, *53*, 367. [CrossRef]

[22] Guven, K. C.; Percot, A.; Sezik, E. Alkaloids in marine algae. *Marine Drugs* **2010**, *8*, 269. [CrossRef] [PubMed]

[23] Vidal, J. P.; Laurent, D.; Kabore, S. A.; Rechencq, E.; Boucard, M.; Girard, J. P.; Escale, R; Rossi, J. C. Caulerpin, caulerpicin, *Caulerpa scalpelliformis*: comparative acute

toxicity study. *Botanica Marina* **1984**, *2*, 533. [Link]

[24] Shen, W. Z.; Wang, H.; Guo, G.; Tuo, J. Immunomodulatory effects of *Caulerpa racemosa* var. peltata polysaccharide and its selenizing product on T lymphocytes and NK cells in mice. *Science in China. Series C, Life sciences* **2008**, *51*, 795. [CrossRef] [PubMed]

[25] Benevides, N. M. B.; Holanda, M. L.; Melo, F. R.; Pereira, M. G.; Monteiro, A. C. O.; Freitas, A. L. P. Purification and partial characterization of the lectin from the marine green alga *Caulerpa cupressoides* (Vahl) C. Agardh. *Botanica Marina* **2001**, *44*, 17. [CrossRef]

[26] Ara, J.; Sultana, V.; Qasim, R.; Ahmad, V. U. Hypolipidaemic activity of seaweed from Karachi coast. *Phytotherapy Research* **2002**, *16*, 479. [CrossRef] [PubMed]

[27] Santoso, J.; Yoshie-Stark, Y.; Suzuki, T. Anti-oxidant activity of methanol extracts from Indonesian seaweeds in an oil emulsion model. *Fisheries Science* **2004**, *70*, 183. [CrossRef]

[28] Joly, A. B.; Pereira, S. M. B. *Caulerpa kempfii* Joly et Pereira, a new *Caulerpa* from northeastern Brazil. *Ciência e Cultura* **1975**, *27*, 417. [Link]

[29] Barata, D. Taxonomia e filogenia do gênero *Caulerpa* J.V. Lamour. (Bryopsidales, Chlorophyta) no Brasil. *Doctoral Thesis*. São Paulo Botanical Institute: São Paulo, SP, BR, 2008. [Link]

[30] Souza, E. T.; Queiroz, A. C.; Miranda, G. E. C.; Lorenzo, V. P.; Silva, E. F.; Freire-Dias, T. L. M.; Cupertino-Silva, Y. K.; Melo, G. M. A.; Santos, B. V. O.; Chaves, M. C. O.; Alexandre-Moreira, M. S. Antinociceptive activities of crude methanolic extract and phases, n-butanolic, chloroformic and ethyl acetate from *Caulerpa racemosa* (Caulerpaceae). *Revista Brasileira de Farmacognosia* **2009**, *19*, 115. [CrossRef]

[31] Matta, C. B. B.; Souza, E. T.; Queiroz, A. C.; Lira, D. P.; Araújo, M. V.; Cavalcante-Silva, L. H. A.; Miranda, G. E. C.; Araújo-Júnior, J. X.; Barbosa-Filho, J. M.; Santos, B. V. O.; Alexandre-Moreira, M. S. Antinociceptive and anti-Inflammatory activity from algae of the genus *Caulerpa*. *Marine Drugs* **2011**, *9*, 307. [CrossRef] [PubMed]

[32] Rodrigues, J. A.; Vanderlei, E. S.; Silva, L. M., Araújo, I. W., Queiroz, I. N.; Paula, G. A.; Abreu, T. M.; Ribeiro, N. A.; Bezerra, M. M.; Chaves, H. V.; Lima, V.; Jorge, R. J.; Monteiro, H. S.; Leite, E. L.; Benevides, N. M. Antinociceptive and anti-inflammatory activities of a sulfated polysaccharide isolated from the green seaweed *Caulerpa cupressoides*. *Pharmacological Reports* **2012**, *64*, 282. [CrossRef]

[33] Coolier, H. O. J.; Dinneen, L. C.; Johnson, C. A.; Schneider, C. The abdominal constriction response and its suppression by analgesic drugs in mice. *British Journal of Pharmacology* **1968**, *32*, 285. [PubMed]

[34] Kuraishi; Y.; Harada, Y.; Aratani, S.; Satoh, M.; Takagi, H. Involvement of the spinal noradrenergic and serotonergic systems in morphine analgesia: the differences in mechanical and thermal algesic tests. *Brain Research* **1983**, *273*, 245. [CrossRef]

[35] Hunskaar, S.; Hole, K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain* **1987**, *30*, 103. [CrossRef]

[36] Ferrándiz, M. L.; Alcaraz, M. J. Antiinflammatory activity and inhibition of arachidonic acid metabolism by flavonoids. *Inflammation Research* **1991**, *32*, 283. [PubMed]

[37] Vanderlei, E. S.; Patoilo, K. K.; Lima, N. A.; Lima, A. P.; Rodrigues, J. A.; Silva, L. M.; Lima, M. E.; Lima, V.; Benevides, N. M. Antinociceptive and anti-inflammatory activities of lectin from the marine green alga *Caulerpa cupressoides*. *International Immunopharmacology* **2010**, *10*, 1113. [CrossRef] [PubMed]

[38] Parveen, Z.; Yulin, D.; Saeed, M. K.; Dai, R.; Ahamad, W.; Yu, Y. H. Antiinflammatory and analgesic activities of *Thesium chinese* Turcz extracts and its major flavonoids, kaempferol and kaempferol-3-O-glucoside. *Yakugaku Zasshi* **2007**, *127*, 1275. [CrossRef] [PubMed]

[39] Ballou, L. R.; Botting, R. M.; Goorha, S.; Zhang, J.; Vane, J. R. Nociception in cyclooxygenase iso-enzyme-deficient mice. *Proceedings of the National Academy of Sciences* **2000**, *97*, 10272. [CrossRef] [PubMed]

[40] Reichert, J. A.; Daughters, R. S.; Rivard, R.; Simone, D. A. Peripheral and preemptive opioid antinociception in a mouse visceral pain model. *Pain* **2001**, *89*, 221. [CrossRef]

[41] Miranda, H. F.; Prieto, J. C.; Puig, M. M.; Pinardi, G. Isobolographic analysis of multimodal analgesia in an animal model of visceral acute pain. *Pharmacology Biochemistry & Behavior* **2008**, *88*, 481. [CrossRef] [PubMed]

[42] Tjølsen, A.; Berge, O.G.; Hunskaar, S.; Rosland, J.H.; Hole, K. The formalin test: an evaluation of the method. *Pain* **1992**, *51*, 5. [CrossRef]

[43] Goncalves, J. C. R.; Oliveira, F. D. E. S.; Benedito, R. B.; Sousa, D. P.; Almeida, R. N.; Araújo, D. A. Antinociceptive activity of (-)-carvone: evidence of association with decreased peripheral nerve excitability. *Biological & Pharmaceutical Bulletin* **2008**, *31*, 1017. [CrossRef] [PubMed]

[44] Murno, G. Pharmacologcal assessment of the rat formalin test utilizing the clinically used analgesic drugs gabapentin, lamotrigin, morphine, duloxetine, tramadol and ibuprofen: influence of low and high formalin concentrations. *European Journal of Pharmacology* **2009**, *605*, 95. [CrossRef] [PubMed]

[45] Oliveira, F. S.; Sousa, D. P.; Almeida, R. N. Antinociceptive effect of hydroxydihydrocarvone. *Biological & Pharmaceutical Bulletin* **2008**, *31*, 588. [CrossRef]

[46] Miranda, F. G. G.; Vilar, J. C.; Alves, I. A.; Cavalcanti, S. C.; Antoniolli, A. R. Antinociceptive and antiedematogenic properties and acute toxicity of *Tabebuia avellanedae* Lor. ex Griseb. inner bark aqueous extract. *BMC Pharmacology* **2001**, *1*, 1. [CrossRef] [PubMed]

[47] El Habazi, K.; Aboufatima, R.; Benharref A, Z. A.; Chait, A.; Dalal, A. Study on the antinociceptive effects of *Thymus broussonetii* Boiss extracts in mice and rats. *Journal of Ethnopharmacology* **2006**, *107*, 406. [CrossRef] [PubMed]

[48] Tegeder, I.; Niederberger, E.; Israr, E.; Guhring, H.; Brune, K.; Euchenhofer, C.; Grösch, S.; Geisslinger, G. Inhibition of NF-{kappa}B and AP-1 activation by R- and S-flurbiprofen. *FASEB Journal* **2001**, *15*, 2. [PubMed]

[49] Santos, A. R. S.; Vedana, E. M. A.; Freitas, G. A. G. Antinociceptive effects of meloxicam, in neurogenic and inflammatory nociceptive models in mice. *Inflammation Research* **1998**, *47*, 302. [CrossRef] [PubMed]

[50] Calixto, J. B.; Cabrini, D. A.; Ferreira, J.; Campos, M. M. Kinins in pains and inflammations. *Pain* **2000**, *87*, 1. [CrossRef]

[51] Chichorro, J. G.; Lorenzetti, B. B.; Zampronio, A. R. Involvement of bradykinin, cytokines, sympathetic amines and prostaglandins in formalin-induced orofacial nociception in rats. *British Journal of Pharmacology* **2004**, *141*, 1175. [CrossRef] [PubMed]

[52] Bitencourt, M. A.; Dantas, G. R.; Lira, D. P.; Barbosa-Filho, J. M.; De Miranda, G. E.; Santos, B. V. O.; Souto, J. T. Aqueous and methanolic extracts of *Caulerpa mexicana* suppress cell migration and ear edema induced by inflammatory agents. *Marine Drugs* **2011**, *9*, 1332. [CrossRef] [PubMed]

[53] Montanher, A. B.; Zucolotto, S. M.; Schenkel, E. P.; Fröde, T. S. Evidence of anti-inflamatory effects of *Passiflora edulis* in an inflammation model. *Journal of Ethnopharmacology* **2007**, *109*, 281. [CrossRef] [PubMed]

[54] Farsam, H.; Amanlou, M.; Reza Dehpour, A.; Jahaniani, F. Antiinflammatory and analgesic activity of *Biebersteinia multifida* DC. root extract. *Journal of Ethnopharmacology* **2000**, *71*, 43. [CrossRef]

[55] Cavas, L.; Pohnert, G. In *Seaweeds and their role in globally changing environments*;

Israel, A.; Einav, R.; Seckbach, J., eds.; Springer, 2010, *15*, 385. [CrossRef]

[56] Cengiz, S.; Cavas, L.; Yurdakoc, K.; Pohnert, G. The sesquiterpene caulerpenyne from *Caulerpa* spp. is a lipoxygenase Inhibitor. *Marine Biotechnology* **2011**, *13*, 1. [CrossRef] [PubMed]

[57] Cengiz, S.; Cavas, L.; Yurdakoc, K.; Aksu, S. Inhibition of xanthine oxidase by caulerpenyne from *Caulerpa prolifera*. *Turkish Journal of Biochemistry* **2012**, *37*, 445. [CrossRef]

[58] Kelley, E. E.; Khoo, N. K. H.; Hundley, N. J.; Malik, U. Z.; Freeman, B. A.; Tarpey, M. M. Hydrogen peroxide is the major oxidant product of xanthine oxidase. *Free Radical Biology and Medicine* **2010**, *48*, 493. [CrossRef] [PubMed]

[59] Su, S.; Wang, T.; Duan, J.A.; Zhou, W.; Hua, Y. Q.; Tang, Y. P.; Yu, L.; Qian, D. W. Anti-inflammatory and analgesic activity of different extracts of *Commiphora myrrha*. *Journal of Ethnopharmacology* **2011**, *134*, 251. [CrossRef] [PubMed]

[60] Lukhoba, C. W.; Simmonds, M. S. J.; Paton, A. J. Plectranthus: A review of ethnobotanical uses. *Journal of Ethnopharmacology* **2006**, *103*, 1. [CrossRef] [PubMed]

[61] Barbosa-Filho, J. M.; Piuvezam, M. R.; Moura, M. D.; Silva, M. S.; Lima, K. V. B.; Cunha, E. V. L.; FechineIII, I. M.; Takemura, O. S. Anti-inflammatoy activity of alkaloids: A tweenty- century review. *Revista Brasileira de Farmacognosia* **2006**, *16*, 109. [CrossRef]

[62] Cavalcante-Silva, L. H. A.; Matta, C. B. B.; Araújo, M. V.; Barbosa-Filho, J. M.; Lira, D. P.; Santos, B. V. O; Miranda, G. E. C.; Alexandre-Moreira, M. S. Antinociceptive and anti-inflammatory activities of crude methanolic extract of red alga *Bryothamnion triquetrum*. *Marine Drugs* **2012**, *10*, 1977. [CrossRef] [PubMed]

[63] Cavalcante-Silva, L. H. A.; Correia, A. C. C.; Barbosa-Filho, J. M.; Silva, B. A.; Santos, B. V. O; Lira, D. P.; Sousa, J. C. F.; Miranda, G. E. C.; Cavalcante, F. A.; Alexandre-Moreira, M. S. Spasmolytic effect of caulerpine involves blockade of Ca^{2+} influx on guinea pig ileum. *Marine Drugs* **2013**, *11*, 1553. [CrossRef] [PubMed]