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Original Article

Antinociceptive activity of *Sargassum polyceratium* and the isolation of its chemical components

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ABSTRACT

Marine algae have been the focus of important studies over the past fifty years, with a considerable number of components important to chemists and taxonomists having been isolated and characterized. The scientific data available on *Sargassum polyceratium* are extremely limited. The objective of the present study was to evaluate the antinociceptive activity of an ethanol extract of *S. polyceratium* and to isolate its components. Intraperitoneal treatment with ethanol extract of *S. polyceratium* reduced the number of acetic acid-induced writhes and the amount of time spent in paw-licking in the second phase of the formalin test. Ethanol extract of *S. polyceratium* also reduced the amount of time spent in paw-licking in the glutamate test; however, there was no difference in the reaction time in the hot plate test at any of the doses tested. The chemical components isolated from ethanol extract of *S. polyceratium* were identified using one- and two-dimensional spectroscopic methods such as infrared spectroscopy, mass spectrometry and ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. The analytical results were also compared with data obtained in the literature. The following porphyrin derivatives were isolated from *S. polyceratium*: 13^2-hydroxy-(13^2-*R*)-pheophytin-*a*, 13^2-hydroxy-(13^2-*S*)-pheophytin-*a*, pheophytin-*a*, and the steroid fucosterol. The present results indicate that the ethanol extract of *S. polyceratium* has antinociceptive activity. In addition, four new substances were isolated from the species evaluated.

Introduction

Algae represent one of the most biologically active resources in nature, since they are rich in primary bioactive compounds and secondary metabolites (O'Sullivan et al., 2010). Marine algae have captured the attention of the scientific community due to their great potential as producers of chemical substances that interest the industrial, economic and medical/pharmaceutical sectors. These substances exert a variety of pharmacological activities that have already been described in the literature. These include antibacterial (Lima-Filho et al., 2002; Freile-Pelegrín and Morales, 2004), antitumoral (Yamamoto et al., 1974; Mayer and Hamann, 2005), antiangiogenic (Dias et al., 2005), hemagglutinating (Nishino and Nagumo, 1991; Freitas et al., 1997) and antiviral activities (Damonte et al., 1996; Carlucci et al., 1999; Romanos et al., 2002).

The importance of the genus *Sargassum* C. Agardh, family Sargassaceae, as a component of the marine flora in tropical and subtropical regions of the world is indisputable. It is one of the most representative of the 41 genera of the order Fucales, with an estimated 485 species (Coimbra, 2006). The species of the *Sargassum* genus are distributed predominantly in coastal areas of consolidated substrate, both in tropical and subtropical regions, frequently forming what is known as *Sargassum* banks (Széchy and Paula, 2000). Some recent studies suggest that the polysaccharides, alginates and fucoidans isolated from marine algae of the genus *Sargassum* exert important biological activities with therapeutic relevance due to their antioxidant activity in endothelial cells and immunoregulatory activity in natural killer (NK) cells, macrophages and T-cells (Chen et al., 2007). This genus appears promising from a biological point of view; however, studies are sparse and initial results require greater scientific support.

Sargassum polyceratium is a benthic microalgae species found in regions that stretch from the coastal waters of the Caribbean in southwest Florida to northeastern Brazil (Engelen et al., 2001). Its ecological importance relies on the fact that it acts as a biofilter, reducing pollution in the marine environment, accumulating toxic metals and thus reducing their harmful effects on the local ecosystem of coastal regions (Murugadas et al., 1995). *S. polyceratium* is

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also used for human consumption in some countries such as Cuba where it is eaten fresh, cooked or preserved (Engelen et al., 2001).

Although there are various drugs that act by modulating nociceptive response, the scientific community has been seeking more powerful drugs with fewer toxic effects (Le Bars et al., 2001). Therefore, the present study was developed to evaluate the antinociceptive activity of *S. polyceratium* and to identify its chemical components, as well as to contribute to the chemotaxonomy of the species in question.

Material and methods

Plant materials

Sargassum polyceratium was collected on Bessa beach in the municipality of João Pessoa in the state of Paraíba, Brazil in March 2009. The marine material was identified by Professor George Emmanuel Cavalcanti de Miranda of the Systematics and Ecology Department of the Federal University of Paraíba and three exsiccates were deposited in the *Professor Lauro Pires Xavier* herbarium of the Federal University of Paraíba under the following codes: JPB 13994, JPB 13995 and JPB 13996.

Preparation of the crude ethanol extract

The marine material collected (30 kg) was kept in an ice bath and later frozen at a temperature below 0 °C to conserve its chemical components. Prior to extracting its compounds, the algae were washed with distilled water and dried in the shade. After drying, 3 kg of marine alga were obtained, corresponding to 10% of the material collected. Next, the material was macerated and the chemical components of the alga were extracted with 95% ethanol (EtOH) over seven days. This process was repeated three times to obtain the extracted solution, which was then concentrated in a rotating evaporator under reduced pressure at 40 °C. This resulted in 210 g of crude ethanol extract (SpEE), a yield of 7% in relation to the dry weight of the algae.

Animals

Male Swiss mice weighing 25–35 g, obtained from the Thomas George animal laboratory of the Federal University of Paraíba, were maintained under controlled temperature conditions (21 ± 2 °C) with free access to water and feed pellets (Purina). Prior to initiating the study, all the experimental procedures were reviewed and approved by the Federal University of Paraíba's Ethics Committee on Animal Research under approval certificate number 1006/13.

Drugs

Glutamate and MK-801 were acquired from Sigma–Aldrich (St. Louis, MO, USA). A solution of 37% formaldehyde and morphine hydrochloride was purchased from Vetec, Brazil. All the drugs were diluted in distilled water except for the crude ethanol extract of *S. polyceratium* (SpEE), for which Tween 80 was required in addition to distilled water. All the other reagents used in this study were of the highest analytical grade available or of high-performance liquid chromatography (HPLC) grade.

Acetic acid writhing tests

The animals were divided into five groups ($n = 8$) and allocated to receive the vehicle, SpEE (50, 100 and 200 mg/kg, *i.p.*), or morphine (6 mg/kg, *i.p.*) as a positive control. Thirty minutes after treatment, the animals received an intraperitoneal injection of 1% acetic acid (10 μl/g of weight) (Koster et al., 1959). The number of writhes was counted over a 10 min period for each animal. A writhe is characterized by a contraction of the abdominal muscle and stretching of the hind paws.

Hot plate test

Thirty, 60 and 120 min after receiving their respective treatment with the vehicle, SpEE (50, 100 and 200 mg/kg, *i.p.*) or morphine (10 mg/kg, *i.p.*), the mice were placed individually on a hot plate at a temperature of 55 ± 1 °C. The parameter recorded was the latency period until the animal began licking its hind paw or jumping from the plate. The cut-off time was defined at 15 s to avoid tissue injury.

Formalin test

The procedure was performed as described by Hunskaar and Hole (1987), with some modifications. Thirty minutes after treatment with the vehicle, SpEE (50, 100 and 200 mg/kg, *i.p.*) or morphine (10 mg/kg, *i.p.*), formalin (20 μl, 2.5%) was administered into the plantar region of the animal's right hind paw. The total amount of time spent in paw-licking was recorded in two phases: the early phase (0–5 min after the formalin injection) and the late phase (15–30 min after the formalin injection).

Glutamate test

The role of the glutamatergic system was investigated in accordance with the descriptions of Beirith et al. (2002). The mice were divided into five groups ($n = 8$) and treated intraperitoneally with the vehicle, SpEE (50, 100 and 200 mg/kg) or MK-801 (0.03 mg/kg) 30 min prior to the injection of 20 μl of glutamate solution (30 μmol) into the animal's right hind paw. Immediately after the injection of the phlogistic agent, the mice were individually observed in a transparent box for 15 min and the amount of time that the animal spent licking the glutamate-injected paw was considered the nociceptive reaction.

Rotarod test

With the objective of evaluating the sedative or muscle relaxant effects of SpEE, the mice were submitted to the rotarod test (Duham and Miya, 1957; Santos et al., 2011). The device consists of a rod that rotates at 7 r.p.m. The animals were pretreated with the vehicle, with SpEE (50, 100 and 200 mg/kg, *i.p.*) or with diazepam (4 mg/kg, *i.p.*). The total time they remained on the rotating bar was evaluated at 30, 60 and 120 min following treatment, up to a maximum of 3 min.

Isolation and purification of the chemical components

Part of the SpEE (180 g) was dissolved in a solution of methanol (MeOH):H_2O (7:3, v/v) and homogenized under mechanical agitation for 60 min, obtaining a hydroalcoholic solution that was partitioned in a separation funnel with 3000 ml of hexane, 1500 ml of dichloromethane, 1500 ml of ethyl acetate (AcOEt) and 1000 ml of *n*-butanol (*n*-BuOH). Four phases were obtained, with the following yields: 83.5 g from the hexane phase, 15.5 g from the dichloromethane phase, 3.7 g from the ethyl acetate phase and 12.4 g from the *n*-butanol phase. The isolation, purification and analysis of the chemical components of *S. polyceratium* were performed using chromatographic methods: column chromatography (CC), preparative thin layer chromatography (PTLC) and analytical thin layer chromatography (TLC), respectively. A 20 g aliquot from the hexane phase was submitted to CC using silica gel 60 as the stationary phase, eluted initially with hexane and finalizing with a mixture of AcOEt and MeOH in an increasing gradient of