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Original Article

Antischistosomal activity from Brazilian marine algae

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ABSTRACT

Schistosomiasis may be caused by six different species of the genus *Schistosoma*. Current treatment is based only on two drugs: oxamniquine, which is only effective against the *Schistosoma mansoni* species, and praziquantel, which is ineffective against young parasites. Therefore, research on new drugs and their targets for the treatment of this disease is urgently needed. In the present work, the efficacies of several seaweeds extracts against *S. mansoni* were tested. Worm couples were incubated with different concentration of seaweed extracts for 120 h and monitored after the first 2 h and then every 24 h to evaluate death, mobility reduction and couple detachment. The extracts of 13 different seaweed species were tested in a first trial and the active extracts were further evaluated in lower concentrations. The extracts of *Gracilaria ornata* and species belonging to the genera *Dictyota* and *Laurencia* showed activity at relatively low concentrations. The active extracts were analyzed by LC–MS, and possible candidates are proposed.

Introduction

Schistosomiasis is the most serious form of parasitism by multicellular organisms and still remains on the list of the neglected diseases prioritized by the World Health Organization (WHO, 2015). The disease, also known as bilharzia or snail fever, is caused by an infection with blood flukes of the genus *Schistosoma*. It predominantly affects the poor population, representing one of the main public health problems in more than 70 developing countries. Among the *Schistosoma* species, *Schistosoma mansoni* is the most widely spread in Africa and Latin America. The infection occurs when the host's skin is penetrated by the cercaria, the infectious form of the parasite life cycle. Once inside the host they transform into schistosomula, mature and form couples in the venous system. The egg is responsible for parasite transmission and is also the main cause of the disease symptoms (Gryssels et al., 2006).

The disease affects approximately 240 million people around the world, resulting in an annual mortality rate of 280 000 people. Disease treatment is based on two therapeutic drugs: praziquantel and oxamniquine (WHO, 2015). Praziquantel is ineffective against young parasites and larval-stage schistosomula, but is effective against one-month-old worms; while oxamniquine is only active against the *S. mansoni* species, but not against other members of the *Schistosoma* genus. Moreover, both drugs face the problem of resistance development in the parasites and side effects. Therefore, further studies on the targets and new drugs are desired.

Due to the ability to produce a wide range of chemicals with sophisticated structures, marine organisms are considered good candidates to provide new drugs with pharmaceutical activities, including chemicals to parasitic diseases (Torres et al., 2014). It is well established that marine and freshwater synthetize important metabolites with economic impact (Cardozo et al., 2006; Gressler et al., 2011a,b; Stein et al., 2011; Andreguetti et al., 2013; Machado et al., 2014; Simas-Rodrigues et al., 2015), such as low-weight hydrocarbons, tannins, fatty acids, acetogenins, saponins, phenolic compounds, lignans, alkaloids and terpenoids (Crews et al., 1978; González et al., 1982; Laus, 2001; Li et al., 2011). Specifically, the last five classes cited have been described for their anthelmintic properties.

In this paper, we show the efficacies of 13 macroalgae extracts against *S. mansoni*. The active extracts from the *Gracilaria*, *Dictyota* and *Laurencia* genera were analyzed by LC–MS, and possible candidates are proposed.

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Materials and methods

Seaweed material

The species *Chondria littoralis* Harvey – SP 428.154, *Dictyota dichotoma* (Hudson) J.V.Lamouroux SP 469.031, *Dictyota menstrualis* (Hoyt) Schnetter, Hörnig & Weber-Peukert – SP 469.032, *Plocamium brasiliense* (Greville) M.Howe & W.R.Taylor – SP 428.163 and *Spyridia hypnoides* (Bory de Saint-Vincent) Papenfuss – SP 428.171 were collected at Ubu beach, Anchieta – ES while the species of *Laurencia catarinensis* Cordeiro-Marino & Fujii – SP 400.209, *Laurencia dendroidea* J.Agardh – SP 400.198 (designated as (a)) were collected at Castelhanos beach, Guarapari – ES in October 2011. The above seaweed identification was performed by Dr. Mutue T. Fujii from Instituto de Botânica and MSc. Erika M. Stein. The vouchers of each species were placed in Herbário Maria Eneida P. Kauffman Fidalgo at Instituto de Botânica, São Paulo (SP). *Padina gymnospora* (Kützing) Sonder – 18.849, *Gracilaria ornata* Areschoug – 37.629, *L. dendroidea* J.Agardh – 18.852 (designated as (b)), *Ochtodes secundiramea* (Montagne) M.A.Howe – 18.851, *Dictyota mertensii* (Martius) Kützing – SP 469.156 and *Pterocladiella capillacea* (S.G.Gmelin) Santelices & Hommersand – 18.853 were collected at Baleia beach, Vitória – ES in July 2013, and the identification was performed by Dr. Levi P. Machado and a voucher of each specie was deposited in the herbarium VIES at the Universidade Federal do Espírito Santo.

Extraction procedure

Extracts were prepared by using different procedures. *C. littoralis*, *D. dichotoma*, *D. menstrualis*, *P. brasiliense* and *S. hypnoides* were freeze-dried, powdered and extracted with supercritical CO_2, with the addition of 4% ethyl alcohol as a modifier solvent. The extractions were performed for 3 h at 45 °C and a pressure of 280 bar with a CO_2 flow of 12 ml/min. The species *L. catarinensis* and *L. dendroidea* (b) were also freeze-dried and powdered, and different extracts were obtained by the crescent order of solvent polarity: first hexane, followed by chloroform and then methanol, three times each for 24 h. The species *P. gymnospora*, *G. ornata*, *L. dendroidea* (a), *O. secundiramea*, *D. mertensii* and *P. capillacea* were freshly extracted with a 2:1 mixture of methylene chloride: methanol, at a proportion of 10 ml/g of seaweed with stirring for one week at a temperature of 20 °C.

Schistosomicidal screening

The life cycle of *S. mansoni* (Samboon, 1907) (Trematoda: Schistosomatidae) (BH strain – Belo Horizonte, MG, Brazil) was maintained in *Biomphalaria glabrat*a (Say, 1818) (Gastropoda: Planorbidae) snails and *Mesocricetus auratus* (Waterhouse, 1839) (Mammalia: Cricetidae) hamsters. Female hamsters were infected by subcutaneous injection of 300 cercariae, and six weeks later, *S. mansoni* adult worms were recovered by perfusion of the rodent's portal and mesenteric system. The worms were washed in RPMI 1640 medium (Invitrogen), pH 7.5, supplemented with sodium bicarbonate (2000 μg/ml), penicillin (100 UI/ml), streptomycin (100 μg/ml), amphotericin B (0.25 μg/ml) and 10% fetal bovine serum (Gibco BRL). Adult worm pairs (male and female) were transferred to each well of a 24-well culture plate containing 1 ml of the medium. The seaweeds extracts were dissolved in DMSO (dimethyl sulfoxide, at 1.5%), diluted in 1 ml of RPMI medium and added to the cultured worms to achieve a final concentration of 500 μg/ml. The parasites were kept for 120 h and monitored after the first 2 h and then every 24 h under a light microscope to evaluate motor activity and the mortality rate. The worms were considered to be dead when no movement was observed. RPMI 1640 with 1.5% DMSO was used in the negative control group, and 4.8 μM (1.5 μg/ml) praziquantel (PZQ) was used in the positive control group. The experiments were carried out in five replicates and repeated at least two times. The active extracts in the assay were re-tested at a lower concentration of 100 μg/ml.

UFLC–MS analysis

The analysis were carried out using an UFLC system (Shimadzu, USA) consisting of a solvent delivery pump (Model LC-20AD) and auto sampler (SIL-20Aht) with a 100-μl loop, degasser (DGU 20A3r) and column oven (CTO-20A) followed by a Bruker ESI-microTOF II mass spectrometer. System operation was performed using otof-Control and HyStar V.3.2, while data collection and analysis were performed using Compass DataAnalysis V.4.1. Gradient elution was performed on a Phenomenex® Luna 3μ C18(2) 100A column (150 mm × 2.0 mm) at 30 °C. Mobile phase A consisted of a aqueous solution of 20 mM NH_4Ac and 0.1% formic acid adjusted to pH 6.4, and solution B consisted of acetonitrile with 0.1% formic acid. Separations were effected by a gradient elution program as follows: solution B was maintained at 0% from 0 to 2 min, followed by a linear change from 0% to 100% from 2 to 75 min, B was keep isocratic at 100% from 75 to 80 min. Solution B was changed to 0% at 80.1 min and was held constant until 90 min. The mobile phase flow rate was 0.2 ml/min, and the injection volume was 10 μl. Mass detection was performed scanning the *m*/*z* between 50 and 1100 in positive mode with the following parameters: capillary 4500 V; end plate offset −500 V; nebulizer 2.0 Bar; dry heater 180 °C; and dry gas at a flow of 8 l/min.

Results

The active extracts from the *Gracilaria*, *Dictyota* and *Laurencia* genera displayed high activity against *S. mansoni* in comparison with the other extracts. Table 1 summarizes the extraction methods and *in vitro* effects of all of positive tested seaweed extracts, which have been classified into two main groups, red algae (Rhodophyta) and brown algae (Heterokontophyta). As previously mentioned, the extracts displaying activity at 500 μg/ml exposure, were considered to be active and subjected to further testing at a lower concentration (100 μg/ml). The killing time (24, 72 and 120 h) was recorded and Table 1 shows the 100% mortality rate. The supercritical extracts of the red algae *P. brasiliense*, *C. littoralis* and *S. hypnoides* were active only at the higher concentration (500 μg/ml) tested. The macroalgae *P. capillacea*, *O. secundiramae* and *L. dendroidea* extracted with dichloromethane/methanol (2:1), *L. dendroidea* extracted with methanol, and *L. catarinensis* extracted with chloroform did not present anthelmintic activity (data not shown).

The red macroalgae *P. brasiliense*, *S. hypnoides* and *C. littoralis* submitted to supercritical extraction were active only at the higher concentrations (Table 1). Interestingly that *L. dendroidea* and *L. catarinensis* submitted to hexane extraction display distinct results against schistosoma worms. *L. catarinensis* hexanic extracts kill the worms in 24 h and *L. dendroidea*, killed the worms in females in 24 h and males in 72 h. In addition, chloroformic extract of *L. dendroidea* was also effective against male after 120 h incubation. *G. ornata* submitted to the dichlorometane/methanol extraction displays anthelmintic activity (100 μg/ml) only against male worms in after 120 h.

As observed in Table 1, the brown algae, the three *Dictyota* species showed activity at the two concentrations tested. The supercritical extract of *D. menstrualis* kills very effectively both male and female after 24 h exposure. Additionally, *D. dichotoma* submitted to supercritical extraction kills