

Article

## The Mediterranean Red Alga *Asparagopsis*: A Source of Compounds against *Leishmania*

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**Abstract:** Crude extracts and column fractions from the red algae *Asparagopsis taxiformis* and *A. armata* from the Strait of Messina (Italy) were screened for the production of antimicrobial compounds. Extracts from both species revealed remarkable antiprotozoal activity against *Leishmania*, revealing such algae as a great source of natural antiprotozoal products.

**Keywords:** antiprotozoal products; *Asparagopsis armata*; *Asparagopsis taxiformis*; halogenated metabolites; *Leishmania*; marine natural products

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### 1. Introduction

The interest in marine organisms as a potential and promising source of pharmaceutical agents has increased during the last years [1–4]. To date, many chemically unique compounds of marine origin with various biological activities have been isolated, and some of them are under investigation and are being used to develop new pharmaceuticals [5].

Some red algae were reported to produce chemicals that have potent biological effects [6,7]. Numerous natural products, including halogenated compounds, like haloforms, methanes, ketones, acetates and acrylates, were described just from the genus *Asparagopsis* [8,9].

This genus includes tropical-subtropical red seaweeds, with diplohaplontic life cycle and an heteromorphic tetrasporophyte known as the ‘*Falkenbergia*’ stage. To date the genus is represented in the Mediterranean Sea by two species, *A. armata* and *A. taxiformis*. *A. armata*, a temperate distributed species, is considered a Lessepsian immigrant, first reported from the Algerian coasts in 1923 [10]. *A. taxiformis*, a tropical to warm temperate species, is considered a pre-Lessepsian immigrant or native in the eastern Mediterranean [11], since the first record in the Mediterranean Sea was given in Egypt [12]. Both taxa exhibit a strong invasive behaviour and are included in the list of the “Worst invasive alien species threatening biodiversity in Europe” (EEA 2007) and also in the list of the 100 “Worst Invasives in the Mediterranean Sea” [13].

*A. taxiformis* and *A. armata*, as well as other species of the family Bonnemaisoniaceae are well known as sources of halogenated compounds [8,14] with strong antifungal and antibiotic activity [8,15]. Dichloromethane extracts obtained from *A. armata* exhibited a strong activity against fish pathogenic bacteria [16].

In general, the production of biologically-active metabolites is inherently linked to an ability to partition compounds into specialised storage structures in order to avoid autotoxicity [17].

Members of the Bonnemaisoniaceae form specialized cells [18–20] typically known as vesicle or gland cells [21]. In the tetrasporophyte of *A. armata*, the halogenated metabolites accumulate as a refractile inclusion inside specialized gland cells and this inclusion is no longer produced when the alga is cultured without bromine [21].

The pungent aroma of these algae is due to an essential oil that is composed mainly of bromoform with smaller amounts of other bromine, chlorine, and iodine-containing methane, ethane, ethanol, acetaldehydes, acetones, 2-acetoxypropanes, propenes, epoxypropanes, acroleins and butenones [14]. The halogenated compounds from *Asparagopsis* have a wide range of volatility and solubility and, hence, no single method of extraction and isolation can be considered entirely satisfactory [8].

In the present paper we present data on the production of antimicrobial halogenated compounds on crude extracts and column fractions from *A. taxiformis* and *A. armata* against *Leishmania*.

## 2. Results and Discussion

Three different solvents with increasing polarity were used for the extraction of freeze-dried seaweed powder. Our observations of the effects of extraction method on bioactivity revealed that the active compounds are probably non polar to moderate polar, since the highest activity was observed in ethanol crude extracts fractionated with hexane:ethyl acetate and ethyl acetate. A series of small molecular volatile halogenated compounds (halomethanes, haloethers, haloacetals) are described as responsible for the antimicrobial action of *A. armata* [8].

Hexane and dichloromethane crude extracts of *A. taxiformis* exhibited a strong inhibition of *Leishmania*. IC<sub>50</sub> (half maximal inhibitory concentration) were 17.00 µg/mL and 16.00 µg/mL, for both extracts, respectively, and IC<sub>90</sub> (90% inhibitory concentration) were 33.00 µg/mL and 32.00 µg/mL, for both extracts, respectively (Table 1).

Hexane and dichloromethane crude extracts of *A. armata* also showed a remarkable inhibition, however, both IC<sub>50</sub> and IC<sub>90</sub> values were low (over 40.00 µg/mL) at the same experimental conditions (Table 1).

The active fractions obtained from ethanol crude extracts of *A. taxiformis* were eluted with hexane-ethyl acetate and ethyl acetate. IC<sub>50</sub> values were 14.00 µg/mL and 20.00 µg/mL, for both fractions, respectively, and IC<sub>90</sub> were 32.00 µg/mL and 34.00 µg/mL, for both fractions, respectively (Table 1).

The same moderate polar fractions from *A. armata* resulted active with IC<sub>50</sub> of 10.00 and 19.00 µg/mL, IC<sub>90</sub> 30.00 and 32.00 µg/mL under the same experimental conditions (Table 1).

Pentamidine and amphotericin B were tested as control drugs. Two different inhibition assays were performed. IC<sub>50</sub> values ranged from 0.9 to 1.0 mg/mL and IC<sub>90</sub>s ranged from 1.9 to 4.0 mg/mL for pentamidine, while IC<sub>50</sub>s ranged from 0.18 to 0.19 mg/mL and the IC<sub>90</sub> was 0.32 mg/mL for amphotericin B (Table 2).

**Table 1.** Data of IC<sub>50</sub> and IC<sub>90</sub> (µg/mL) of crude extracts and fractions of *A. armata* and *A. taxiformis*. HEX: hexane; DCM: dichloromethane; EtOH: ethanol; EtOAc: ethyl acetate; MeOH: methanol.

Species	Crude extract/Fraction	IC <sub>50</sub> (µg/mL)	IC <sub>90</sub> (µg/mL)
<i>A. armata</i>	HEX	>40	>40
<i>A. armata</i>	DCM	>40	>40
<i>A. armata</i>	EtOH-Hex:EtOAc	10	30
<i>A. armata</i>	EtOH-EtOAc	19	32
<i>A. armata</i>	EtOH-EtOAc:MeOH	Inactive	Inactive
<i>A. armata</i>	EtOH-MeOH	Inactive	Inactive
<i>A. armata</i>	EtOH-H <sub>2</sub> O	Inactive	Inactive
<i>A. taxiformis</i>	HEX	17	33
<i>A. taxiformis</i>	DCM	16	32
<i>A. taxiformis</i>	EtOH-Hex:EtOAc	14	32
<i>A. taxiformis</i>	EtOH-EtOAc	20	34
<i>A. taxiformis</i>	EtOH-EtOAc:MeOH	Inactive	Inactive
<i>A. taxiformis</i>	EtOH-MeOH	Inactive	Inactive
<i>A. taxiformis</i>	EtOH-H <sub>2</sub> O	Inactive	Inactive

**Table 2.** Data of IC<sub>50</sub> and IC<sub>90</sub> (µg/mL) of tested control drugs.

Control drug	IC <sub>50</sub> (mg/mL)	IC <sub>90</sub> (mg/mL)
Pentamidine	from 0.9 to 1	from 1.9 to 4
Amphotericin B	from 0.18 to 0.19	0.32

Leishmaniasis is a disease caused by the protozoa of the *Leishmania* species and it has a worldwide distribution, especially in many tropical and sub-tropical countries. It affects as many as 12 million people worldwide, with 1.5–2 million new cases each year. There is increasing awareness that drug treatment can be complicated by variation in the sensitivity of *Leishmania* species to drugs, variation in pharmacokinetics, and variation in drug-host immune response interaction [26,27].

The LC-MS analysis of the column fraction in ethyl acetate from ethanol crude extracts of *A. taxiformis* revealed two peaks of nearly the same intensity at *m/z* 303.1 and 305.1 [M+H], which indicates presence of one bromine atom. Due to the small quantities of extracts and fractions, further characterization of this compound was not possible. The presence of a small molecular weight

brominated molecule in the active fraction confirms that the lipophilic halogenated compounds are truly the metabolites responsible for potent antimicrobial activity of this extract.

### 3. Experimental Section

Plants of *A. taxiformis* and *A. armata* were collected from the Strait of Messina (Italy), respectively at Torre Faro, Messina and Villa San Giovanni, Reggio Calabria in May 2008. Fresh plants were washed in sterile sea water and manually cleaned of epiphytes. Lyophilized and powdered plants of *A. taxiformis* and *A. armata* (dry weights: 75 g for each species) were extracted using different organic solvents with increasing polarity (hexane, dichloromethane and ethanol) at room temperature. Extracts were dried with a Rotavapor® at low temperature (35 °C) to prevent volatile compounds from evaporation.

*In vitro* antimicrobial susceptibility assays were performed on *Leishmania donovani* promastigotes cultures ( $2 \times 10^6$  cell/mL). A transgenic cell line of *L. donovani* promastigotes showing stable expression of luciferase was used as the test organism. The plates were incubated at 26 °C for 72 h, and growth of *Leishmania* promastigotes was determined by the Alamar blue assay [28]. Pentamidine and amphotericin B were tested as the standard antileishmanial agents. Microbiological assays were performed at the Microbiology laboratory of National Center For Natural Products Research of the University of Mississippi.

The hexane and dichloromethane extracts were not further fractionated because of limited amount of materials. Ethanol extracts of *A. taxiformis* and *A. armata* were submitted to fractionation using Si gel vacuum liquid chromatography eluted in order with hexane, hexane-ethyl acetate (1:1), ethyl acetate, ethyl acetate-methanol (1:1), methanol, water. Fractions were tested in antimicrobial assays.

Fractionation and isolation of compounds were further performed using HPLC, with a normal phase Silica gel column (10 mm) as stationary phase and gradient of two solvents, hexane and isopropanol, as mobile phase. Each fraction was dried in vacuum and  $^1\text{H-NMR}$  spectra in  $\text{CDCl}_3$  was recorded on a Bruker BioSpin instrument operating at 400 MHz. LC-MS analysis for each sample was carried out with a micrOTOF ESI-TOF MS.

### 4. Conclusions

Red algae of the genus *Asparagopsis* are well known as sources of halogenated compounds with strong antifungal and antibacterial activity [8,14–16], but, as far as we know, there are no published data on their activity against any protozoa. According to our results, *A. armata* and *A. taxiformis* revealed high potential as source of natural products with antiprotozoal activity *in vitro*. This first report of the effectiveness of *Asparagopsis* against *Leishmania* represents a challenge to encourage explorative research on such topic. *Asparagopsis* species merit further studies both with the aim of isolating their active metabolites on larger scale and for assaying culture methods for supplying algal biomass for industry.

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