Potent and selective antiplasmodial activity of marine sponges from Bahia state, Brazil

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ABSTRACT

This study evaluated the in vitro antiplasmodial and cytotoxic effects of 26 extracts from nine marine sponges collected in Salvador, Bahia state, Brazil. All assayed extracts were found to be potently active against Plasmodium falciparum W2 strain, with IC50 values ranging from 0.28 to 22.34 μg mL−1, and weakly cytotoxic against the human cell line WI-26-VA4 with CC50 values > 89 μg mL−1, thus displaying selectivity indices (SI) equal or higher than 17. Interestingly, some SI values exceeded 1,000. The highly potent and selective antiplasmodial activity of the assessed marine sponges is reported for the first time in this study.

1. Introduction

Malaria is a life-threatening protozoan disease caused by five Plasmodium species (P. falciparum, P. vivax, P. knowlesi, P. ovale and P. malariae) which are transmitted to people through the bites of infected female Anopheles mosquitoes. Among the Plasmodium species, P. falciparum is responsible for the most severe malaria and causes most of the malaria-related deaths globally. In 2019, with an estimated 229 million cases and 409,000 deaths worldwide, most cases and deaths occurred in sub-Saharan Africa and more severely affected children under 5 years old (WHO, 2020).

Artemisinin and artemisinin-based combination therapy still remain as the main treatments for malaria (Miller and Su, 2011; Tindana et al., 2021). Although China has been declared malaria-free and malaria cases and deaths have declined over the years worldwide (Zhou, 2021; Tindana et al., 2021), recent reports show that P. falciparum has been becoming resistant to artemisinin, notably in Southeast Asia (van der Pluijm et al., 2019). Therefore, the search for new antimalarial drugs continues to be urgently needed. Intensive efforts have been made to find new bioactive natural compounds that could serve as prototypes for the development of novel, potent and selective antimalarial drugs (Batista et al., 2009; Cunha et al., 2021).

In our ongoing search for novel antimalarial compounds, we disclose herein the in vitro assessment of 26 extracts from nine specimens of marine sponges collected in Salvador, Bahia state, Brazil, against Plasmodium falciparum W2 strain. We report for the first time the highly potent and selective antiplasmodial activities observed for the marine sponges selected in this study.

2. Material and methods

2.1. Sponge material

The marine sponges were collected in September 2017 at Porto da Barra’s beach, in Salvador, Bahia, Brazil, and packed in plastic bags containing seawater. Next, they were immediately moved to the LAPESBI laboratory and washed in running water prior to being stored in a freezer. All marine sponges Aplysina fulva, Cladocroce caelum, Cinachyrella apion, Calypospongia sp., Desmapsamma anchorata, Dysidea janiae, Dragmacidon reticulatum and Ircinia strobilina were identified by Prof. Dr. Emilio Lanna through comparison with exsicates deposited at the Institute of Biology, UFBA (photos available as supplementary material).
2.2. Obtaining extracts

Part of each marine sponge (20 g) was separately ground fresh in a porcelain mortar and pestle, and exhaustively extracted under sonication (water bath, room temperature, 20 min), first with ethanol (3 × 100 mL) and then with ethyl acetate (3 × 100 mL). Both ethanol and ethyl acetate extracts were combined and the resulting organic solution was evaporated under reduced pressure to obtain the corresponding crude extract (CE). In turn, CE was suspended in water (100 mL) and extracted successively with dichloromethane (3 × 100 mL) and ethyl acetate (3 × 100 mL), giving, after concentration (water bath, room temperature, 20 min), first with ethanol (50 °C), the corresponding dichloromethane (DCM) and ethyl acetate (AcOEt) extracts. The aqueous phase was then completely evaporated under reduced pressure at rotavevatorator (2 h, 50–60 °C), and the residue was extracted with methanol (100 mL) under sonication (room temperature to give the corresponding methanol extract (MeOH). Weights and yields (% w/w) of the obtained DCM, AcOEt and MeOH extracts are displayed in Table 1. Portions (1 mg) of the DCM, AcOEt and MeOH extracts from each marine sponge were assessed for their in vitro antiplasmodial properties against *Plasmodium falciparum*, as well as their cytotoxicity against human lung fibroblast cells.

2.3. In vitro antiplasmodial activity

Chloroquine (CQ)-resistant *Plasmodium falciparum* W2 strain was used for in vitro blood stage culture to test the antiplasmodial efficacy of test extracts. Parasites were maintained at 5% hematocrit using type O blood, containing 168 mg/L hypoxanthine, 40 μg/mL gentamycin, and incubated at 37 °C under approximately 5% of CO2. (Trager and Jensen, 1976) The parasites at early stages were synchronized at ring stage by sorbitol treatment (Lambrinos and Vanderberg, 1979).

In vitro antiplasmodial activity of the test extracts was done in 96 well bottom well plates (Carvalho et al., 1991). The growth inhibition of intrarerythrocytic forms and parasite morphology in culture by the microscopic observation of Giemsa-stained thin blood films. Ring stage parasites (0.5% parasitemia and 2% hematocrit) were added to each well of 96-well microculture plates. The test extracts were diluted to concentrations ranging from 0.10 to 50 μg mL⁻¹ using complete medium and stored at 4 °C. After incubation at 37 °C for 48 h, *P. falciparum* growth inhibition was assessed by Giemsa-stained smears. The culture medium was replaced with fresh medium with or without test samples/control drugs. Chloroquine (CQ) was used as a reference antimalarial (concentrations ranging from 0.001 to 10 μg mL⁻¹). The activity of the test extracts was expressed as the percentage reduction in parasitemia relative to controls without drugs. All experiments were performed in triplicate. For each blood smear, parasitemia was determined after the evaluation of 5,000 cells. The results were expressed as the mean of the IC₅₀ (the extract concentration that reduced parasite viability by 50%).

2.4. In vitro cytotoxicity

In vitro cytotoxicity of each sample was assessed on WI-26VA4

### Table 1

In vitro antiplasmodial and cytotoxic activities of marine sponges collected in Bahia state, Brazil.

| Marine sponge | Voucher code | Extract | Weight (mg) | Yield (% w/w) | IC₅₀ ± SD (μg.mL⁻¹) W2 | CC₅₀ ± SD (μg.mL⁻¹) WI-26-VA4 | SI |
|---------------|-------------|---------|-------------|---------------|------------------------|-------------------------------|----|
| *Aplysina fulva* | 1566        | DCM     | 160         | 0.80          | 2.72 ± 0.035           | >1,000                        | >368 |
|               |             | AcOEt   | 112         | 0.56          | 0.28 ± 0.022           | >1,000                        | >3,571 |
|               |             | MeOH    | 594         | 2.97          | 17.48 ± 0.032          | >1,000                        | >57  |
| *Aplysina fulva* | 2479        | DCM     | 417         | 2.09          | 17.88 ± 0.012          | >1,000                        | >56  |
|               |             | AcOEt   | 108         | 0.54          | 4.94 ± 0.033           | 330 ± 0.031                   | 67   |
|               |             | MeOH    | 816         | 4.08          | 22.34 ± 0.013          | 389 ± 0.026                   | 17   |
| *Cladorhoe caelum* | 4411        | DCM     | 134         | 0.67          | 17.28 ± 0.017          | >1,000                        | >58  |
|               |             | AcOEt   | 3           | 0.02          | 8.78 ± 0.015           | >1,000                        | >114 |
|               |             | MeOH    | 260         | 1.30          | 3.52 ± 0.010           | 310 ± 0.023                   | 88   |
| *Cinachyrella apion* | 2232        | DCM     | 60          | 0.30          | 11.62 ± 0.011          | >1,000                        | >86  |
|               |             | AcOEt   | 19          | 0.10          | 3.92 ± 0.016           | >1,000                        | >255 |
|               |             | MeOH    | 304         | 1.52          | 19.70 ± 0.013          | >1,000                        | >50  |
| *Callyspongia sp.* | 1731        | DCM     | 400         | 2.00          | 5.74 ± 0.022           | 129 ± 0.013                   | 22   |
|               |             | AcOEt   | 8           | 0.04          | 2.72 ± 0.015           | 110 ± 0.025                   | 40   |
|               |             | MeOH    | 577         | 2.89          | 2.10 ± 0.018           | 146 ± 0.028                   | 70   |
| *Desmapsamma anchorata* | 4151      | DCM     | 12          | 0.06          | 3.72 ± 0.013           | 89 ± 0.011                    | 23   |
|               |             | AcOEt   | 9           | 0.05          | 12.82 ± 0.019          | >1,000                        | >78  |
|               |             | MeOH    | 230         | 1.15          | 14.44 ± 0.021          | >1,000                        | >69  |
| *Dysidea janiata* | 461         | DCM     | 54          | 0.27          | 1.30 ± 0.029           | 247 ± 0.017                   | 190  |
|               |             | AcOEt   | 6           | 0.03          | 0.90 ± 0.012           | >1,111                        | >1,111 |
|               |             | MeOH    | 112         | 0.56          | 14.24 ± 0.011          | >1,000                        | >70  |
| *Dracoacidon reticulatum* | 4344      | DCM     | 249         | 1.25          | 5.96 ± 0.024           | >1,000                        | >168 |
|               |             | AcOEt   | <1         | <0.01        | N.D.                  | N.D.                          | N.D. |
|               |             | MeOH    | 873         | 4.37          | 2.32 ± 0.018           | 190 ± 0.045                   | 82   |
| *Ircinia strobilina* | 2477        | DCM     | 179         | 0.89          | 1.50 ± 0.016           | 394 ± 0.039                   | 263  |
|               |             | AcOEt   | 11          | 0.06          | 12.42 ± 0.015          | 320 ± 0.034                   | 26   |
|               |             | MeOH    | 128         | 0.64          | 5.74 ± 0.014           | 277 ± 0.030                   | 48   |
| Positive Control |             | Chloroquine | 0.04 ± 0.002 | >100          | >2,500                  |                               |      |

a Weight obtained from 20 g of fresh material.

b S.D., Standard Deviation.

c SI, Selectivity Index (CC₅₀ WI-26-VA4/IC₅₀ W2).

d N.D., Not Determined (insufficient amount).
U.V. Alves et al.

International Journal for Parasitology: Drugs and Drug Resistance 17 (2021) 80–83

82

ATCC CCL-95.1, USA) human pulmonary fibroblast cells. The cells were
cultured and maintained according to set conditions (Júnior et al., 2021; Denizot and Lang, 1986). The test extracts (20 μL) were diluted in
different concentrations ranging from 0.1 to 1000 μg mL⁻¹ and incu-
bated with the cells for 24 h in a 5% CO₂ atmosphere at 37 °C.

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg mL⁻¹; 20 μL/well) was added to evaluate
mitochondrial viability. After 3 h of incubation, the supernatants were
carefully removed, DMSO (100 μL) was added to each well, and the reactions were mixed to solubilize the formazan crystals. The optical
density was determined at 540 nm to measure the signal and back
ground, respectively (Spectra Max340PC®, Molecular Devices, Sun-
nynvale, California, USA) (Júnior et al., 2021).

The cell viability was expressed as a percentage of the control absorbance in the untreated cells after subtracting the appropriate
background. The minimum cytotoxic concentration for 50% of the cells
(CC₅₀) was determined as described (Céu de Madureira et al., 2002).

2.5. Selectivity index (SI)

A selectivity index (SI) corresponding to the ratio between the
cytotoxic and antiplasmodial activities has been calculated for each
electrode assayed (SI = CC₅₀/IC₅₀). Values higher than 10 were considered
indicative of lack of toxicity, while values below 10 were considered
toxic (Bell et al., 1990).

2.6. Statistical analysis

The concentrations of tested extracts able to inhibit 50% of parasite
growth (IC₅₀) were determined based on the equation of the curve ob-
tained by plotting the % of parasitemia reduction vs the log of the
concentration of extract. The coefficients of regression of these curves
were calculated using the method of least squares. The concentrations of
tested extracts able to cause the death of 50% of human cells (CC₅₀) were
determined based on the equation of the curve obtained by plotting the
% of cellular death versus the concentration of extract (Origin Lab
Corporation software, version 8.0 Northampton, MA, USA). The average
IC₅₀ and CC₅₀ were compared using ANOVA. Statistical significance was
defined at the 5% level (p < 0.05).

3. Results & discussion

The extracting procedure employed to study the marine spone
Aplysina fulva, Cladocroce caerulea, Cinachyrella apion, Callyspongia sp.,
Desmapsamma anchorata, Dysidea janiae, Dragmacidon reticulatum and
Ircinia strobilina afforded 26 extracts (Table 1). The in vitro anti-
plasmodial and cytotoxic effects against Plasmodium falciparum
resistant-chloroquine W2 strain and human lung fetal WI-26-VA4 cell
line, respectively, were assessed for all of these concentrates, except for
the AcOE extract obtained from Dragmacidon reticulatum due to its
insufficient amount, as displayed in Table 1. The experiments were carried
out in triplicate, using chloroquine as the positive control.

We found that all assayed extracts were potently active against P.
falciparum, with IC₅₀ values ranging from 0.28 to 22.34 μg mL⁻¹,
accompanied by low cytotoxicity against the human cell line WI-26-VA4
that showed CC₅₀ values > 89 μg mL⁻¹. Thus, the selectivity indices (SI)
calculated for each extract were equivalent or higher than 17, and some
of them exceeded 1,000 evidencing that the antiplasmodial properties for
all marine sponges assessed in this study are both highly potent and
selective towards P. falciparum, even though obtained from crude
extracts.

Interestingly, we can note that the two Aplysina fulva specimens
(voucher codes 1566 and 2479) were collected from the same locale, but
exhibited different antiparasomal and cytotoxic performances when compared to each other. For instance, DCM (IC₅₀ = 2.72 μg mL⁻¹;
CC₅₀ > 1,000 μg mL⁻¹; SI > 368), AcOEt (IC₅₀ = 0.28 μg mL⁻¹;
CC₅₀ > 1,000 μg mL⁻¹; SI > 3,571) and MeOH (IC₅₀ = 17.48 μg mL⁻¹;
CC₅₀ > 1,000 μg mL⁻¹; SI > 47) extracts from the first A. fulva specimen
(1566) were more potent and selective than DCM (IC₅₀ = 17.88 μg mL⁻¹;
CC₅₀ > 1,000 μg mL⁻¹; SI > 56), AcOEt (IC₅₀ = 4.94 μg mL⁻¹;
CC₅₀ = 330 μg mL⁻¹; SI = 67) and MeOH (IC₅₀ = 22.34 μg mL⁻¹;
CC₅₀ = 389 μg mL⁻¹; SI = 17) extracts from the second A. fulva specimen
(2479). These intriguing results may be explained, at least in part, by the
likely difference in the composition of both A. fulva specimens due to the
chemical variability already observed for this species, especially related
to the content of bioactive dibromotyrosine-derived metabolites (Núñez
et al., 2008). These brominated natural products are of restricted
occurrence and recognized as potent antimalarial compounds (Mani
et al., 2012).

In the development of new antimalarial drugs, it is desirable that
antimalarial compounds exhibit their activity mainly on bloodstream
parasites, since these stages are responsible for most of the clinical
sequelae of malaria. Substances must attain appropriate plasma levels,
enter the infected erythrocytes and access their intracellular targets to
inhibit one or more essential parasite activities selectively, thus pro-
ducing rapid killing of the parasite (Basore et al., 2015). Selectivity is
undoubtedly the key property, and any compound with antimalarial
potential should display a SI equal or higher than 10 (Katsuno et al.,
2015). According to this parameter, the extracts exhibited excellent SI
values in our assays. All of these results suggest that the marine sponges
listed in Table 1 are strongly promising materials as sources of anti-
malarial compounds, thus being worthy of further studies. As far as the
authors are aware, no previous studies have reported the potent and
selective antimalarial properties of these marine sponge species yet.

Curiously, Table 1 also shows that most of the AcOEt extracts were
more selective against P. falciparum than DCM and MeOH extracts, with
the exception of Callyspongia sp. and Ircinia strobilina sponges. In addi-
tion, one can note that AcOEt extracts were obtained in smaller quan-
tities/yields than their corresponding DCM and MeOH extracts. These
findings suggest that researchers should pay special attention to AcOEt
extraction in future studies with marine sponges when looking for
antiplasmodial compounds.

4. Conclusion

The present study reports for the first time the potent and selective
antiplasmodial activity of marine sponge specimens collected in Salva-
dor, Bahia state, Brazil, pertaining to the genera Aplysina, Cladocroce,
Cinachyrella, Callyspongia, Desmapsamma, Dysidea, Dragmacidon and
Ircinia.

Authors’ contributions

Collecting and extracting sponges: U.V.A., E.J.S., J.G.S., L.O.S., E.L.;
Taxonomic identification: E.L.; Performing antiplasmodial assay: A.C.S.
P., A.L.F., F.P.V.; Designing the study: R.B.; Drafting the manuscript: U.
V.A., E.L., A.L.F., F.P.V., R.B.

Declaration of competing interest

The authors declare that there is no conflict of interest. Supporting
data can be freely accessed by direct contact to the authors.

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