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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 IC_{50} values ranging from 0.28 to 22.34 μg mL^{-1}, and weakly cytotoxic against the human cell line WI-26-VA4 with CC_{50} 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, Callyspongia* 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

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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 in a rotary evaporator (40 °C), the corresponding dichloromethane (DCM) and ethyl acetate (AcOEt) extracts. The aqueous phase was then completely evaporated under reduced pressure at rotaevaporator (2 h, 50–60 °C), and the residue was extracted with methanol (100 mL) under sonication at 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^+ human erythrocytes in RPMI 1640 medium (Sigma-Aldrich®, St. Louis, Missouri, USA) supplemented with 25 mM NaHCO3, 1.0% albumax, 45 mg/L hypoxanthine, 40 μg/mL gentamycin and incubated at 37 °C under approximately 5% of CO_2 (Trager and Jensen, 1976) The parasites at early stages were synchronized at ring stage by sorbitol treatment (Lambros and Vanderberg, 1979).

In vitro antiplasmodial activity of the test extracts was done in 96 round bottom well plates (Carvalho et al., 1991). The growth inhibition of intraerythrocytic 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 smears, parasitemia was determined after the evaluation of 5.000 cells. The results were expressed as the mean of the IC_{50} (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)[a]	Yield (% w/w)	IC_{50} ± SD[b] (μg.mL^{-1}) W2	CC_{50} ± SD[b] (μg.mL^{-1}) WI-26-VA4	SI[c]
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
Cladocroce 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 janiae	461	DCM	54	0.27	1.30 ± 0.029	247 ± 0.017	190
		AcOEt	6	0.03	0.90 ± 0.012	>1,000	>1,111
		MeOH	112	0.56	14.24 ± 0.011	>1,000	>70
Dragmacidon reticulatum	4344	DCM	249	1.25	5.96 ± 0.024	>1,000	>168
		AcOEt	<1	<0.01	*N.D.*[d]	*N.D.*[d]	*N.D.*[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 (CC_{50} WI-26-VA4/IC_{50} W2).
[d] *N.D.,* Not Determined (insufficient amount).

(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 incubated with the cells for 24 h in a 5% CO_2 atmosphere at 37 °C.

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg mL^{-1}; 20 μL.$well^{-1}$) 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 background, respectively (Spectra Max340PC^{384}, Molecular Devices, Sunnyvale, 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 (CC_{50}) 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 extract assayed ($SI = CC_{50}/IC_{50}$). 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 (IC_{50}) were determined based on the equation of the curve obtained by plotting the % of parasitemia regression 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 (CC_{50}) 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 IC_{50} and CC_{50} 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 sponges *Aplysina fulva, Cladocroce caelum, Cinachyrella apion, Callyspongia* sp., *Desmapsamma anchorata, Dysidea janiae, Dragmacidon reticulatum* and *Ircinia strobilina* afforded 26 extracts (Table 1). The in vitro antiplasmodial 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 AcOEt 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 IC_{50} 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 CC_{50} 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 antiplasmodial and cytotoxic performances when compared to each other. For instance, DCM (IC_{50} = 2.72 μg mL^{-1}; CC_{50} > 1,000 μg mL^{-1}; SI > 368), AcOEt (IC_{50} = 0.28 μg mL^{-1}; CC_{50} > 1,000 μg mL^{-1}; SI > 3,571) and MeOH (IC_{50} = 17.48 μg mL^{-1}; CC_{50} > 1,000 μg mL^{-1}; SI > 47) extracts from the first *A. fulva* specimen (1566) were more potent and selective than DCM (IC_{50} = 17.88 μg mL^{-1}; CC_{50} > 1,000 μg mL^{-1}; SI > 56), AcOEt (IC_{50} = 4.94 μg mL^{-1}; CC_{50} = 330 μg mL^{-1}; SI = 67) and MeOH (IC_{50} = 22.34 μg mL^{-1}; CC_{50} = 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 (Nuñez et al., 2008). These brominated natural products are of restricted occurrence and recognized as potent antiplasmodial 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 producing 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 antimalarial compounds, thus being worthy of further studies. As far as the authors are aware, no previous studies have reported the potent and selective antiplasmodial 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 addition, one can note that AcOEt extracts were obtained in smaller quantities/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 Salvador, 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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