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Asian Pacific Journal of Tropical Disease

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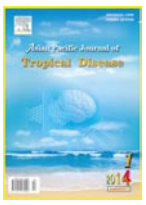

Document heading doi: 10.1016/S2222-1808(14)60433-3

In vitro antiplasmodial activity of marine sponge *Clathria vulpina* extract against chloroquine sensitive *Plasmodium falciparum*

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PEER REVIEW

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Comments

This is a good study in which the authors mentioned about the sponge *C. vulpina* possesses a significant suppressive effect on *in vitro* cultures of chloroquine sensitive *P. falciparum*. The results are interesting that *P. falciparum* could be used as a potential antiplasmodial drug.
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ABSTRACT

Objective: To explore the antiplasmodial potential of marine sponge *Clathria vulpina* (*C. vulpina*) against chloroquine sensitive *Plasmodium falciparum* (*P. falciparum*).
Methods: The marine sponge *C. vulpina* was collected from Thondi coast, authenticated and subjected for extraction by soaking in ethanol:water mixture (3:1 ratio). The percentage of extract was calculated. Filter sterilized extracts (100, 50, 25, 12.5, 6.25, 3.125 μg/mL) were screened for antiplasmodial activity against chloroquine sensitive *P. falciparum*. The extract was also tested for its hemolytic activity.
Results: The percentage yield of extract of *C. vulpina* was found to be 4.8%. The crude extract of *C. vulpina* showed excellent antiplasmodial activity (IC_{50}=14.75 μg/mL) which was highly comparable to the positive control chloroquine (IC_{50}=7 μg/mL). Statistical analysis reveals that the significant antiplasmodial activity (P<0.05) was observed between the concentrations and the time of exposure. The chemical injury to erythrocytes was also carried out, which showed that there were no morphological changes in erythrocytes by the ethanolic extracts of sponges after 48 h of incubation. The extract showed slight hemolytic activity which almost equal to chloroquine at 100 μg/mL concentration (1.023%).
Conclusions: The marine sponge *C. vulpina* can be used as a putative antiplasmodial drug after completing successful clinical trials.

KEYWORDS

Clathria vulpina, Antiplasmodial activity, Chloroquine sensitive *Plasmodium falciparum*, Sponges

1. Introduction

Malaria is an infectious disease caused by the genus *Plasmodium* such as *Plasmodium falciparum* (*P. falciparum*), *Plasmodium ovale*, *Plasmodium vivax* and *Plasmodium malariae*. Among them, *P. falciparum* is the parasite responsible for most severe diseases and fatal cases, which may kill over one millions of people per annum. The parasite, *P. falciparum* is genetically diverse and has multiple independent origins of mutations in genes that confer resistance to widely used antimalarial drugs like chloroquine[1]. A major breakthrough of the past decades is the discovery of artemisinin by Chinese researchers. Artemisinin combination treatments for *P. falciparum* are currently used only first line antimalarial drugs ameanable to widespread use against all chloroquinone resistant malarial parasites. However, artemisinin resistant strains were recently found in Cambodia[2]. So, there is an urgent need for the new antimalarial drugs. Living organisms are recognized as a source of potential bioactive molecules

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Foundation Project: Supported by Indian Council of Medical Research, New Delhi.

Article history:
Received 3 Dec 2013
Received in revised form 10 Dec, 2nd revised form 17 Dec, 3rd revised form 22 Dec 2013
Accepted 20 Jan 2014
Available online 28 Jan 2014

which are commonly more effective than those obtained through the combinatorial synthetic chemistry. Hopefully, the new breakthrough in the malaria treatment will come with the development of a marine lead compound. The incredible potential of marine organisms (mostly invertebrates, such as sponges, tunicates, and soft corals) known to produce a large array of secondary metabolites can be interpreted by considering the common features of secondary metabolism in all living organisms as well as some peculiar features of the marine environment. The marine ecosystem is the biggest source for the development of new drugs against *P. falciparum*. Pharmaceutical interest in sponges was aroused in the early 1950′s by the discovery of number of unknown nucleosides, such as spongothymidine and spongouridine in the marine sponge *Cryptotheca crypta*[3,4]. Most of the bioactive compounds from sponges consist of anti-inflammatory, antitumor, immunosuppressive (or) neurosuppressive, antiviral, antibiotics, antifouling and antimalarial properties[5]. In this connection, the present study was made an attempt to find out the antimalarial compounds from the crude extract of marine sponge *Clathria vulpina* (*C. vulpina*) collected from Thondi coast of Palk Strait region, Tamil Nadu, India.

2. Materials and methods

2.1. Collection of marine sponges

Marine sponge *C. vulpina* was collected by using by-catch at Thondi (lat. 9°44′10″N, lon. 79°10′12″E) of Palk Strait region, Tamil Nadu, India, and authenticated by Dr. S. Lazarus, emeritus fellow (retired), Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam, Kanyakumari district, Tamil Nadu, India. The collected samples were washed thrice with tap water and twice with distilled water to remove the adhering associated animals. A sample voucher specimen was deposited in the herbarium facility (sponsored by the Indian Council of Medical Research, New Delhi) maintained in the Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Tamil Nadu, India.

2.2. Extraction of bioactive principles

The samples were cut into pieces and kept for shade drying. After the complete removal of moisture, the samples were subjected for percolation by soaking in ethanol:water mixture (3:1 ratio). After 21 d of dark incubation, the filtrate was concentrated separately by rotary vacuum evaporator (>45 °C) and then freeze dried (-80 °C) to obtain solid residue. The percentage of extraction was calculated by using the following formula:

$$\text{Percnet of extraction (\%)} = \frac{\text{Weight of the extract (g)}}{\text{Weight of the sponge material (g)}} \times 100$$

The ethanolic extracts was dissolved in dimethyl sulphoxide (Hi media Laboratories Private Limited, Mumbai, India) and filtered through sterile millipore filters (mesh 0.20 μm, Sartorious Stedim Biotech GmbH, Germany). The filtrate was used for testing at different concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 μg/mL[6].

2.3. Culture maintenance

The *in vitro* antiplasmodial activity of marine sponge extract was assessed against *P. falciparum* (obtained from the Jawaharlal Nehru Centre for Advanced Scientific Research, Indian Institute of Science, Bangalore, India). *P. falciparum* were cultivated in human O Rh^+ red blood cells (donated by the volunteers) using RPMI 1640 medium (Hi Media Laboratories Private Limited, Mumbai, India)[7]. Hi Media Laboratories Private Limited, Mumbai, India, supplemented with O Rh^+ serum (10%), 5% sodium bicarbonate and 40 μg/mL of gentamycin sulphate. Haematocrits were adjusted at 5% and parasite cultures were used when they exhibit 2% parasitaemia[8].

2.4. In vitro antiplasmodial activity

Different concentrations of filter-sterilized crude extract from *C. vulpina* (100, 50, 25, 12.5, 6.25 and 3.125 μg/mL) were incorporated in 96-well tissue culture plate containing 200 μL of *P. falciparum* culture with fresh red blood cells diluted to 2% haematocrit. Negative control was maintained with fresh red blood cells and 2% parasitized *P. falciparum* diluted to 2% haematocrit and positive control was maintained with parasitized blood culture treated with chloroquine[9]. Parasitaemia was evaluated after 48 h by giemsa stain and the average percentage suppression of parasitaemia was calculated by the following formula:

$$\text{Average suppression of parasitaemia (\%)} = \frac{\text{Ac}-\text{At}}{\text{Ac}} \times 100$$

Where Ac is average percentage of parasitaemia in control (%); At is average percentage of parasitaemia in test (%).

2.5. Antiplasmodial activity calculation and analysis

The antiplasmodial activity of marine sponge *C. vulpine* were expressed by the inhibitory concentrations (IC_{50}) of the drug that induced 50% reduction in parasitaemia compared to the control (100% parasitemia). The IC_{50} values were calculated (concentration of extract in X-axis and percentage of inhibition in Y-axis) using office XP (SDAS) software. This activity was analyzed in accordance with the

norms of antiplasmodial activity of Rasoanaivo *et al*[10]. It was suggested that an extract was very active if IC_{50}<5 μg/mL, active IC_{50}<50 μg/mL, weakly active IC_{50}<100 μg/mL and inactive IC_{50}>100 μg/mL.

2.6. Chemical injury to erythrocytes

To assess any chemical injury to erythrocytes that might attributed to the extract, 200 μL of erythrocytes was incubated with 100 μg/mL of the extract at a dose equal to the highest used in the antiplasmodial assay. The conditions of the experiment were maintained as in the case of antiplasmodial assay. After 48 h of incubation, thin blood smears were stained with giemsa stain and observed for morphological changes under high-power light microscope. The morphological findings were compared with those erythrocytes that were uninfected and not exposed to extract[11].

2.7. Hemolytic activity

Hemolytic activity was evaluated as described by Andra *et al*. with a slight modifications[12]. Human erythrocyte suspensions were washed with phosphate buffer saline (PBS) (1.5 mmol/L KH_2PO_4, 2.7 mmol/L KCl, 8.1 mmol/L Na_2HPO_4, 135 mmol/L NaCl, pH 7.4) and then centrifuged at 7 000 r/min for 10 min. After washing four times with PBS (or until the supernatant was colourless), the human erythrocytes were re-suspended and diluted to 10 times of the original volume with PBS, referred as stock erythrocyte suspension. The suspension (2% v/v) was incubated with different concentrations (3.125 to 100 μg/mL) of *C. vulpina* sponge extracts at 37 °C for 1h. After the incubation period, the reaction mixture was centrifuged at 3 000 r/min for 10 min to remove intact erythrocytes. The supernatant was collected and the absorbance was determined at 450 nm by using spectrophotometer (Cyber UV-1, Mecasys Co. Ltd.) using PBS as negative control and Triton X-100 as positive control. The percent hemolysis was calculated using the formula:

$$\text{Haemolysis (\%)} = 100 - \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

3. Results

The percentage yield of extract of *C. vulpina* was found to be 4.8%. The extract of *C. vulpina* showed excellent antiplasmodial activity (IC_{50}=14.75 μg/mL) (Table 1) which was one fold higher when compare to the positive control chloroquine (IC_{50}=7 μg/mL). The uninfected erythrocytes incubated with the ethanolic extract of marine sponge *C. vulpina* and uninfected erythrocytes from the blank column of the 96-well plate showed no morphological differences after 48 h of incubation. The extract showed slight hemolytic activity of 1.023% at 100 μg/mL concentration (Figure 1).

Table 1

Percentage suppression of parasitaemia and IC_{50} value in different concentrations of crude extract of marine sponge *C. vulpina* (μg/mL).

Samples	Yield of extract (%)	Suppression of parasitaemia (%) at 48 h						
		100	50	25	12.5	6.25	3.125	IC_{50}
C. vulpina	4.8%	78.12	71.87	62.50	53.12	43.75	34.37	14.75
Chloroquine	–	81.25	75.00	65.62	56.25	46.87	37.50	7.00

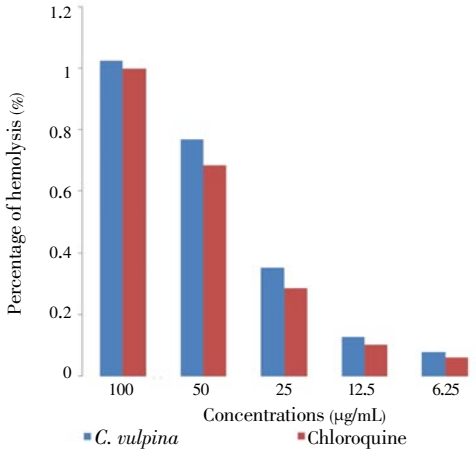

Figure 1. Hemolytic activity of crude extract of marine sponge *C. vulpina*.

4. Discussion

The rich diversity of bioactive compounds from sponges have provided molecules that interfere with the pathogenesis of diseases at many different points, which increase the chance of developing selective drugs against specific targets. Marine sponges have provided many examples of novel secondary metabolites that possess varied chemical status and potent antimalarial activity[5]. Marine sponges belonging to the genus *Ircinia* are known to be a very rich source of terpenoids, several of which have shown a wide variety of biological activities. The variabilins from terpenoids which were polyprenyl - hydroquinones, had analgesic and anti-inflammatory properties[13]. Among the halogenated alkaloids, bromoalkaloids form the most widely distributed group of natural compounds, which are predominantly found in marine eukaryotes like sponges[14]. Polyacetylenenic alcohols, including (35,145)- petrocortyre A, purified from the marine sponge *Petrosia* sp., which possess the cytotoxic activity against a small panel of human solid tumor cell liner by inhibiting DNA replication[15]. The sponge *Ircinia vulpin* has also shown to possess antiviral, central nervous system stimulatory and antialgal properties[16]. *Aplysina cavernicola*, a much studied sponge which produces aeroplysinin and aerthionin and other dibromo and dichlorotyrosine derivatives, found to have antibiotic activity against *Bacillus subtilis* and *Proteus vulgaris*[13]. An ethanolic extract

of *Haliclona virdis* showed a significant hypoglycemic effect lasting for more than 8 h after single oral doses of 200 or 500 mg/kg to normal mice[17]. Considering these significances, present investigation has been made an attempt to evaluate the antiplasmodial activity of marine sponge *C. vulpina* against chloroquine-sensitive *P. falciparum*. The extract of the *C. vulpina* exhibited excellent antiplasmodial activity with the IC_{50} value of 14.75 μg/mL. And the extract showed a slight haemolytic actvity of 1.0% at the concentration of 100 μg/mL and decreased significantly in lower concentrations. Song *et al.* reported that the saponins of *Panax notiginseng* exhibited the haemolytic activity of 11.6% and 3.6% at 500 mg/L and 250 mg/Lconcentration respectively[18]. The IC_{50} value of *C. vulpina* extract showed below 50 μg/mL concentration in the present investigation. According to Rasoanaivo *et al*., the extract which shows the *in vitro* antiplasmodial activity by <50 μg/mL is active[10]. According to the above said point, the extract of *C. vulpina* is active and can be used as a potential active antiplasmodial drug in future. The mechanism of action might be due to the inhibition of *P. falciparum* merozoites invasion into the erythrocytes or disruption of *P. falciparum* rosettes[19-21]. Ravikumar *et al.* reported that the bark extract from the mangrove plant of *Avicennia marina* exhibited minimum concentration of inhibitory activity of IC_{50} 49.63 μg/mL[22]. When compared with the above report, *C. vulpina* extract is more potent against the chloroquine sensitive *P. falciparum*. The antiplasmodial activities of sea weeds[23], sponge associated bacterium[24], terrestrial medicinal plants[25], coastal medicinal plants[8], traditional medicinal plants[26] have been reported and found to have good antiplasmodial activity against *P. falciparum*. It is reported that the endophytic fungi from Thai medicinal plants collected from forest region of Thailand, found to have excellent antiplasmodial activity with the IC_{50} value of 1.6-8.0 μg/mL[9]. Fattorusso *et al.* reported that the cycloperoxide compounds obtained from the sponge *Plakortis simplex* showed a good antiplasmodial activity against chloroquine sensitive *P. falciparum* with IC_{50} 26.81 to 1 263.52 nmol/L[5]. It is also reported that the marine sponge *C. vulpina* associated bacteria have a potent antiplasmodial activity of IC_{50} 20.73 μg/mL[27]. Ravikumar *et al.* reported that the South Indian medicinal plant *Azadirachta indica* (bark extract) showed a good antiplasmodial activity (IC_{50} 29.77 μg/mL)[28]. These findings could encourage the development of new antiplasmodial drugs from the marine natural products. It is concluded from the present findings that the sponge *C. vulpina* collected from the Thondi coast, Palk Strait region, Tamil Nadu, possesses a significant suppressive effect on *in vitro* cultures of chloroquine sensitive *P. falciparum*, which could be used as a potential antiplasmodial drug after completing successful clinical trials.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to the authorities of Alagappa University for providing required facilities. This work was finiancially supported by Indian Council of Medical Research, New Delhi.

Comments

Background

Malaria is an infectious disease caused by the genus *Plasmodium*. Among them, *P. falciparum* is the parasite responsible for most severe diseases and fatal cases, which may kill over one millions of people per annum. Authors have highlighted the literature report about the incredible potential of marine organisms (mostly invertebrates, such as sponges, tunicates, and soft corals) known to produce a large array of secondary metabolites. Authors selected sponge as a source material for screening antiplasmodial activity. Most of the bioactive compounds from sponges were used for various bioassay such as anti-inflammatory, antitumor, immunosuppressive (or) neurosuppressive, antiviral, antibiotics, antifouling and antimalarial properties.

Research frontiers

Studies are being performed in order to screen the antiplasmodial activity from the crude extract of sponge *C. vulpine*. Marine sponges have provided many examples of novel secondary metabolites that possess varied chemical status and potent antimalarial activity.

Related reports

Authors have mentioned many related article to support this research. Compared with other extract of various sources, the extract of *C. vulpina* exhibited excellent antiplasmodial activity with less IC_{50} value of 14.75 μg/mL.

Innovations & breakthroughs

When compared with the mentioned report in this research, *C. vulpina* extract is more potent against the chloroquine sensitive *P. falciparum* with less IC_{50} value.

Applications

The study has showed *C. vulpina* extract is more potent against the chloroquine sensitive *P. falciparum*. The IC_{50} value of *C. vulpina* extract showed below 50 μg/mL concentration in the present investigation, so *C. vulpina* extract can be used as a potential active antiplasmodial drug in future.

Peer review

This is a good study in which the authors mentioned about the sponge *C. vulpina* possesses a significant suppressive effect on *in vitro* cultures of chloroquine sensitive *P. falciparum*. The results are interesting that *P. falciparum* could be used as a potential antiplasmodial drug.

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