Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

In vitro antiplasmodial activity of spiro benzofuran compound from mangrove plant of Southern India

Sundaram Ravikumar[1*], Ganesan Ramanathan[2], Murugesan Gnanadesigan[3]

[1]*School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi - 623 409, Ramanathapuram District, Tamil Nadu, India*
[2]*Department of Microbiology, V. H. S. N. College, Virudhunagar District, Tamil Nadu, India*
[3]*Department of Microbiology, Selvamm Art and Science College, Namakkal-637003, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 10 November 2011
Received in revised form 15 January 2012
Accepted 15 March 2012
Available online 20 May 2012

Keywords:
Antiplasmodial
Benzofuran compounds
Mangrove plants

ABSTRACT

Objective: To find out the *in vitro* antipalsmodial activities of mangrove leaf extracts. **Methods:** *In vitro* antiplasmodial assay was carried out with 13 different mangrove plants. Column chromatography was performed with the most potent *Agecerious corniculatum (A. corniculatum)* by using various solvent extractions. GC-MS was also preformed with the most potent ethanolic fraction of the *A. corniculatum* extract. **Results:** Of the 13 mangroves plants, *A. corniculatum* showed maximum percentage of parasitemia suppression (94.98 ± 1.16)%. Column chromatography was performed with *A. corniculatum* with different solvents and the methanolic extract showed maximum percentage (99.73±1.63)% of parasitemia inhibition at 150 μg/mL concentration with the IC_{50} value of (29.28±3.23) μg/mL concentration. The results of the GC-MS analysis observed that, the most potent methanolic extract showed maximum retention time (30.687 RT) and the chemical class was identified as Spiro [benzofuran-2(3 H), 1′-(3 cyclohexane)-2′,3-dione, 7-chloro-4′,6] which was responsible for the antiplasmodial activity. **Conclusions:** It is concluded from the present study that, the chemical constituents of *A. corniculatum* collected from Pichavaram mangrove forest can be used as a putative antiplasmodial drugs in future.

1. Introduction

Malaria is one of the most important parasitic diseases in tropical and subtropical countries. More than 250 million clinical cases resulting at least 1-2 million deaths per year with fatality rate being extremely high among young children below 5 years old and pregnant women[1,2].

In addition, resistance of *Plasmodium falciparum (P. falciparum)* to currently used antimalarial such as chloroquine is spreading rapidly. Therefore it is important to develop new and effective antimalarial drugs are highly warranted. In this connection, marine halophytes such as mangroves and mangrove halophytes are well known to produce natural metabolites with various biological activities such as antibacterial, antiviral, antidiarrhoeal, antifeedant, insecticidal, hepatoprotective, cytotoxicity and anticancer[3-5]. Chemical classes of mangrove and associates contains steroids, triterpenes, saponins, flavonoids, alkaloids, tannins and phenols[6,7] which have wide range of therapeutic uses. However, active chemical classes responsible for antiplasmodial property is not attempted so for. In this connection, the present study was initiated to investigate the *in vitro* antiplasmodial activities of mangrove halophytes.

2. Materials and methods

2.1. Collection and extraction of mangrove plants

Fresh elder leaves from mangrove plants were collected from the Pichavaram mangrove forest (Lat 110°27′N; Lan 79°47′E), South East coast of India, Tamilnadu, India. The plant names, families and voucher specimen numbers

*Corresponding author: Sundaram Ravikumar, School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi - 623 409, Ramanathapuram District, Tamil Nadu, India.
Tel: +914561-243470
E-mail: ravibiotech201321@gmail.com

and the percentage yield of extraction are given in Table 1. Specimens of mangrove halophytes were authenticated by Prof. Dr. K. Kathiresan, CAS, Annamalai University, Parangipettai, Tamil Nadu, India. Voucher specimens of all the samples were deposited in the herbarium cabinet facilities (sponsored by ICMR, New Delhi) at the department of Oceanography and Coastal Area Studies, Alagappa University, Thondi campus, Thondi, Tamil Nadu, India. All the samples were washed thrice with sterile distilled water to remove adherent soil and salt contaminants. Washed samples were chopped into small pieces and shade dried. The dried samples were further subjected to percolation

Table 1

Name of the mangrove plants chosen for antiplasmodial screening.

Family	Name of the plant species	Specimen no	Weight of the plant part (g)	Yield of the extract	
				(g)	(%)
Rhizophoraceae	*Rhizophora mucronata*. Poir	AUOCAS015	495	39.87	8.05
	Ceriops decandra. Griff Din Hou	AUOCAS016	346	25.98	7.51
	Rhizophora annamalayan. Kathir.	AUOCAS017	654	54.30	8.30
	Rhizophora apiculata. Blume	AUOCAS018	432	38.76	8.97
	Bruguiera cylindrica (L.) Bl.	AUOCAS020	329	27.98	8.50
Avicenniaceae	*Avicennia officinalis* (L.)	AUOCAS021	267	19.54	7.32
	Avicennia marina (Frosk.) Vierh	AUOCAS022	560	49.65	8.86
Acanthaceae	*Acanthus ilicifolius* (L.)	AUOCAS023	437	38.21	8.74
Myrsinaceae	*Aegiceras corniculatum* (L) Blanco	AUOCAS024	421	27.65	6.56
Euphorbiaceae	*Exoecaria agallocha* (L.)	AUOCAS025	478	41.65	8.71
Combretaceae	*Lumnitzera racemosa* (Willd)	AUOCAS026	620	58.98	9.51

with methanol solvent under dark for 7 d. The extract was filtered and concentrated in rotary evaporator in vacuo at 54 ℃ and further lyophilized to remove excess organic residues. Further, the most potent plant of *A. corniculatum* was subjected for the sequential fractionation with 500 g of silica gel (230–400 mesh, 2 cm×50 cm column, MERCK) by using 30 mL of acetone, chloroform, methanol and ethanol solvents.

2.2. Parasite cultivation

The antiplasmodial activity of plant extracts was assessed against *P. falciparum* obtained from the Jawaharlal Nehru Centre for Advanced Scientific Research, Indian Institute of Science, Bangalore, India. *P. falciparum* are cultivated in human O Rh^+ red blood cells using RPMI 1640 medium (HiMedia Laboratories Private Limited, Mumbai, India) supplemented with O Rh^+ serum (10%), 5% sodium bicarbonate (HiMedia Laboratories Private Limited, Mumbai, India) and 40 μg/mL of gentamycin sulphate (HiMedia Laboratories Private Limited, Mumbai, India). Hematocrits were adjusted at 5% and parasite cultures were used when they exhibited 2% parasitaemia.

2.3. In vitro antiplasmodial assay

Filter sterilized leaves extracts (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% hematocrit. Negative control was maintained with fresh red blood cells and 2% parasitized *P. falciparum* diluted to 2% hematocrit, positive control was maintained with parasitized blood cells culture treated with chloroquine and artemether. Parasitaemia was evaluated after 48 h by giemsa stain and the average percentage suppression of parasitaemia was calculated by the following formula: Average % suppression of parasitaemia = Average % parasitaemia in control−Average % parasitaemia in test/ Average % parasitaemia in control ×100.

2.4. Antiplasmodial activity calculation and analysis

The antiplasmodial activity of mangrove leaves extracts (including chromatography fraction) were expressed by the inhibitory concentrations 50 (IC_{50}), representing the concentration of drug that induced a 50% parasitaemia decrease compared to the positive control culture referred as 100% parasitaemia.

2.5. Chemical injury to erythrocytes

To assess the chemical injury of the *Avicennia corniculatum (A. corniculatum)* leaf extract on erythrocytes, 200 μL of erythrocytes were incubated with 200 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 microscopy. The morphological findings were compared with those in erythrocytes that were uninfected and not exposed to extract[7].

2.6. GC–MS analysis of A. corniculatum

About 10 mg of the most potent *A. corniculatum* methanolic leaf extract was dissolved in 1 mL of methanol. From that, 0.1 μL was injected in to GC-MS (GC 17A, Japan) with standard specification (column size 0.25 mm × 25 m, Carrier gas-Helium, Column 5% phenyl polysiloxane, flow rate 0.4 m/min, sample injection temperature 25 ℃, acceleration and reflector temperature 10 ℃/min, initial temperature 70 ℃).

The maximum percentages of compounds obtained from the extracts were identified by chemical library search (TUTOR. LIB, WILEY139.LIB).

2.7. Statistical analysis

The IC_{50} values were calculated (concentration of extract in X axis and percentage of inhibition in Y axis) using Office XP (SDAS add-ins programme) software with linear regression equation.

3. Results

The *in vitro* antiplasmodial activity of mangrove halophytes were reported in Table 2 and the results revealed that, all the plant extracts showed dose dependent parasitemia suppression. Of the selected mangrove plants, *A. corniculatum* showed maximum percentage (94.98±1.16)% of parasitemia suppression followed by, *Rhizophora mucronata* (75.00± 0.408)%, *Rhizophora apiculata* (41.50±0.408)%, *Bruguiera cylindrica* (40.80±0.653)% and *Avicennia marina* (40.20± 0.163)%. The maximum percentage of parasitemia inhibition by the mangrove plant *A. corniculatum* was further fractioned with different solvents. The methanolic extract showed maximum percentage (91.98±2.40)% of parasitemia inhibition at concentration of 100 μg/mL and minimum percentage(2.90±0.09)% of parasitemia inhibition was found with the 25 μg/mL concentration of acetone extract with the minimum IC_{50} value (29.28±3.23) μg/mL (Table 3). The GC-MS results of the most potent methanolic extract of *A. corniculatum* showed 52 numbers of peak values with different time intervals of that, the maximum retention time value was identified at 30.687 RT and the chemical class was identified as Spiro [benzofuran-2(3 H), 1′- (3 cyclohexane)- 2′,3-dione, 7- chloro-4′,6] (Figure 1).

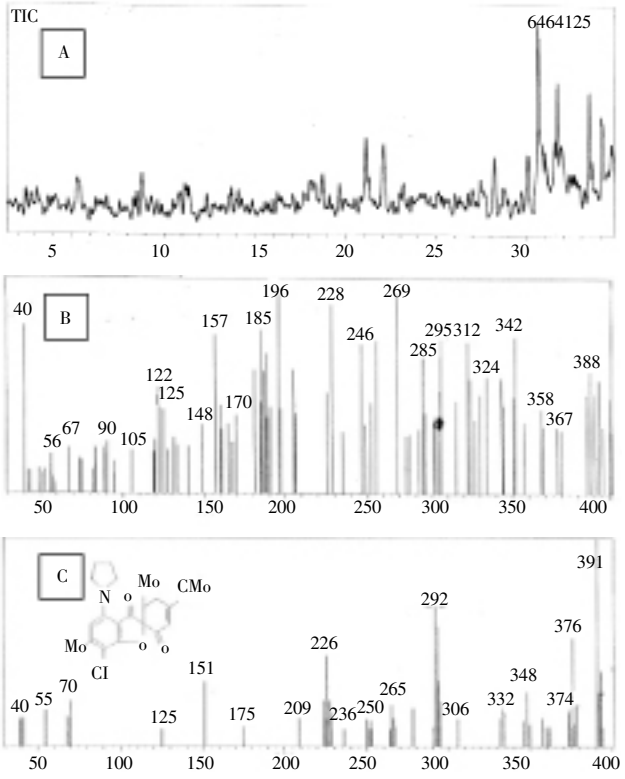

Figure 1. GC-MS analysis of the methanolic extract of *A. corniculatum*. A) Detection of various bioactive metabolite peaks; B) Peak separation at the retention time of 30.687; C) Identified chemical constituent [Spiro (benzofuran-2(3 H), 1′- (3 cyclohexane)- 2′,3-dione, 7-chloro-4′,6].

Table 2
Preliminary *in vitro* antiplasmodial activity of marine halophytes against *P. falciparum*.

Plant species	Average % suppression of parasitaemia		
	50 μg/mL	100 μg/mL	150 μg/mL
Rhizophora mucronata	17.30 ± 1.24	43.10 ± 2.08	75.00 ± 1.40
Rhizophora apiculata	26.50 ± 3.40	29.30 ± 1.24	41.50 ± 3.59
Ceriops decandra	8.90 ± 1.73	12.80 ± 3.65	21.90 ± 2.73
Bruguiera cylindrica	22.30 ± 2.25	34.60 ± 2.49	40.80 ± 1.65
Lumnitzera racemosa	20.70 ± 1.57	29.60 ± 1.49	29.90 ± 3.73
Avicennia marina	25.40 ± 3.32	31.00 ± 3.48	40.20 ± 2.16
Avicennia officinalis	18.40 ± 1.38	19.30 ± 2.24	21.50 ± 1.40
Exoecaria agallocha	19.80 ± 2.65	22.60 ± 1.49	28.50 ± 3.46
Acanthus ilicifolius	18.40 ± 1.32	28.50 ± 3.40	32.40 ± 2.32
Aegiceras corniculatum	59.70 ± 3.57	74.50 ± 1.40	94.98 ±1.16
Rhizophora annamalayana	10.10 ± 2.08	20.10 ± 2.08	39.10 ± 3.08

Table 3
In vitro antiplasmodial activity of various solvent extracts of *A. corniculatum*.

Solvents used	3.125 μg/mL(%)	6.25 μg/mL(%)	12.5 μg/mL(%)	25 μ/mL(%)	50 μg/mL(%)	100 μg/mL(%)	IC_{50} (μg/mL)
Acetone	0.00	0.00	0.00	2.90±0.09*	12.98±0.97*	27.98±1.09*	>100
Chloroform	0.00	3.59±0.84*	7.87±2.98*	23.98±2.93*	36.98±7.87*	56.84±2.98*	83.04±12.09
Methanol	18.52±3.54*	29.47±2.83*	43.98±1.65*	58.09±2.85*	74.70±1.87*	91.98±2.40*	29.28±3.23
Ethanol	0.00	11.77±1.76*	14.98±5.29*	17.93±2.98*	36.98±4.84*	54.87±4.87*	85.46±12.76

Values are the average of the three replicates and found significant *($P<0.01$) between concentration and plants.

4. Discussion

Nature has good source of medicinal agents for thousands of years and an immersive number of modern drugs have been isolated from natural sources based on the traditional information. In that, marine plants are proved to have traditional and medicinal uses[8,9]. In view of this, the present study was investigated to find out the *in vitro* antiplasmodial activities of mangrove plants and the results showed that, all the mangrove plants showed suppression of parasitemia with different concentrations of the extracts and this might be due to the presence of unique phytochemcial constituents[10]. Moreover, single or heterogeneous mixture of active biochemical constituents showed a broad spectrum of biological and pharmacological activity[6]. But, the *A. corniculatum* showed minimum level of IC_{50} value with the methanolic fractionation and this might be due to the presence of high content of flavonoid based benzofuran compounds [spiro (benzofuran-2(3 H), 1′- (3 cyclohexane)- 2′,3-dione, 7- chloro-4′,6.][11-16]. Previously, *A. corniculatum* was also proved to have antibacterial and antiviral properties[17]. In addition, none of the plant extracts were not showed any of the chemical injury to the erythrocyte membrane throughout the experiment. Hence, the erythrocytic membrane is a delicate structure that can be significantly altered by drug interactions[18,19]. The mechanical stability of the erythrocyte membrane is a good indicator of *in vitro* studies for cytotoxicity screening, since its structural dynamics favor interactions with drugs and this indicates that, the possible utilization of mangrove extracts as antiplasmodial drug in future. According to Ravikumar *et al*[7] the plant extracts which showing i*n vitro* antiplasmodial activity more than 100 μg/mL concentration is inactive, from the present studies, the IC_{50} value of methanolic extract from *A. corniculatum* (29.28±3.23) μg/mL was showed less than 50 μg/mL hence this could be used as a potential antiplasmodial drug. It is concluded from the present study that, the mangrove leaf extract of *A. corniculatum* collected from the Pichavaram mangrove forest, Tamilnadu, India were showed potential *in vitro* antiplasmodial activity against *P. falciparum*.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are thankful to the authorities of Alagappa University for providing required facilities and also to Indian Council of Medical Research, New Delhi for financial assistance.

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