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

Sundaram Ravikumar, Ganesan Ramanathan, Murugesan Gnanadesigan

School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi – 623 409, Ramanathapuram District, Tamil Nadu, India

Department of Microbiology, V. H. S. N. College, Virudhunagar District, Tamil Nadu, India

Department of Microbiology, Selvamm Art and Science College, Namakkal–637003, Tamil Nadu, India

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 far. In this connection, the present study was initiated to investigate the in vitro antimalarial 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 11°27’N; Lan 79°47’E), South East coast of India, Tamilnadu, India. The plant names, families and voucher specimen numbers...
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 with methanol solvent under dark for 7 d. The extract was filtered and concentrated in rotary evaporator in vacuo at 54 °C 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 residues. Further, the most potent plant of *A. corniculatum* was subjected for the sequential fractionation with 500 g of residues.

### Table 1

| 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 annamalayana*, Kathir.| AUOCAS017   | 654                           | 54.30 8.30                 |
|                      | *Rhizophora apiculata*, Blume     | AUOCAS018   | 432                           | 38.76 8.97                 |
| Avicenniaceae        | *Avicennia cylindrica* (L.) Bl.   | AUOCAS020   | 329                           | 27.98 8.50                 |
|                      | *Avicennia officinalis* (L.)      | AUOCAS021   | 267                           | 19.54 7.32                 |
|                      | *Avicennia marina* (Forsk.) Vierh.| AUOCAS022   | 560                           | 49.65 8.86                 |
| Acanthaceae          | *Acanthus ilicifolius* (L.)       | AUOCAS023   | 437                           | 38.21 8.74                 |
| Myrsinaceae          | *Aegiceras corniculatum* (L.)     | AUOCAS024   | 421                           | 27.65 6.56                 |
| Euphorbiaceae        | *Exoecaria agallocha* (L.)        | AUOCAS025   | 478                           | 41.65 8.71                 |
| Combretaceae         | *Lumnitzera racemosa* (Wildl.)    | AUOCAS026   | 620                           | 58.98 9.51                 |

The antiplasmodial activity of mangrove leaves extracts (including chromatography fraction) were expressed by the inhibitory concentrations 50 (IC50), representing the concentration of drug that induced a 50% parasitaemia decrease compared to the positive control culture referred as 100% parasitaemia.

#### 2.4. Antiplasmodial activity calculation and analysis

The antiplasmodial activity of *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]  

#### 2.5. Chemical injury to erythrocytes

To assess the chemical injury of *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 °C, acceleration and reflector temperature 10 °C/min, initial temperature 70 °C).
The maximum percentages of compounds obtained from the extracts were identified by chemical library search (TUTOR.LIB, WILEY139.LIB).

2.7. Statistical analysis

The IC50 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 antimalarial 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 IC50 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).

Table 2

| 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

| Solvents used | 3.125 μg/mL(%) | 6.25 μg/mL(%) | 12.5 μg/mL(%) | 25 μg/mL(%) | 50 μg/mL(%) | 100 μg/mL(%) | IC50 (μg/mL) |
|---------------|----------------|---------------|---------------|-------------|-------------|--------------|--------------|
| Acetone       | 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 phytochemical 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 cytotoxic 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 in 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.

References

[1] Ravikumar S, Jacob Inbanesos S, Suganthi P, Gnanadesigan M. In vitro antiplasmodial activity of ethanolic extracts of mangrove plants from South East coast of India against chloroquine-sensitive Plasmodium falciparum. Parasitol Res 2011; 108: 873–878.

[2] WHO. World malaria report. [Online] Available from http://www.who.int/wmr [Accessed on Sep 18, 2008].

[3] Wu J, Xiao Q, Xu J, Li MY, Pana JY, Yang M. Natural products from the mangrove flora: source and bioactivities. Nat Prod Rep 2008; 25: 955–981.

[4] Ravikumar S, Gnanadesigan M, Jacob Inbanesos S, Kalaiaarasi A. Heparotrophic and antioxidant properties of Suaeda maritime (L.) Dumrot ethanol extract on concanavalin-A induced heaptopotoxicity in rats. Indian J Exp Biol 2011; 49: 455–460.

[5] Ravikumar S, Ramanathan G, Jacob Inbanesos S, Ramu A. Antiplasmodial activity of two marine polyherbal preparations from Chaetomorpha antennina and Aegiceras corniculatum against Plasmodium falciparum. Parasitol Res 2011; 108: 107–113.

[6] Ravikumar S, Gnanadesigan M, Suganthi P, Ramalakshmi A. Antibacterial potential of chosen mangrove plants against isolated urinary tract infectious bacterial pathogens. Int J Med Med Sci 2010; 2(3): 94–99.

[7] Ravikumar S, Jacob Inbanesos S, Suganthi P, Venkatesan M, Ramu A. Mangrove plants as a source of lead compounds for the development of new antiplasmodial drugs from South East coast of India. Parasitol Res 2011; 108: 1405–1410.

[8] Ravikumar S, Ramanathan G, Subhakaran M, Jacob Inbanesos S. Antimicrobial compounds from marine halophytes for silkworm disease treatment. Int J Med Med Sci 2009; 1(5): 184–191.

[9] Arwa PS, Onyango JC, Nyunja RO. Phytochemical compounds and antimicrobial activity of Rhoicissus plant (Rhoicissus revelli) (Planch). Plant Sci Res 2008; 1(3): 68–73.

[10] Margret JR, Kumaresan S, Ravikumar S. A preliminary study on the anti-inflammatory activity of methanol extract of Ulva lactuca in rat. J Envi Biol 2009; 30(5): 899–902.

[11] Kraft C, Jenett-Siems K, Köhler I, Siems K, Abild D, Bienze U, et al. Andirol A and B, two unique 6 hydroxymethylpterocarpenes from Andira inermis. Z Naturforsch 2002; 57: 785–790.

[12] Inbanesos SJ, Ravikumar S. In vitro antiplasmodial activity of marine sponge Hyattella intestinalis associated bacteria against Plasmodium falciparum. Asian Pac J Trop Biomed 2011; 1 (Suppl 1): S100–S104.

[13] Okokon JE, Etebong EO, Udohang JA, Obot J. Antiplasmodial and antinociceptive activities of Melanthera scadens. Asian Pac J Trop Biomed 2012; 2 (1): 16–20.

[14] Inbanesos SJ, Ravikumar S, Suganthi P. In vitro antiplasmodial effect of ethanolic extracts of coastal medicinal plants along Palk Strait against Plasmodium falciparum. Asian Pac J Trop Biomed 2012; 2(5): 364–367.

[15] Kumar S, Diwan SK, Mahajan SN, Rawankule S, Mahure C. Case report of Plasmodium falciparum malaria presenting as wide complex tachycardia. Asian Pac J Trop Biomed 2011; 1 (Suppl 2): S305–S306.

[16] Akpan EJ, Okokon JE, Enuk IC. Antiplasmodial and antipyretic studies on root extracts of Anthocleista djalonensis against Plasmodium berghei. Asian Pac J Trop Dis 2012; 2(1): 36–42.

[17] Chandrasekaran M, Kannathasan K, Venkatesalu V, Prabhakar K. Antibacterial activity of some salt marsh halophytes and mangrove plants against methicillin resistant. World J Microbiol Biotechnol 2009; 25(1): 155–160.

[18] Aparicio RM, Garcia-Celma MJ, Vinardell MP, Mitjans M. In vitro studies of the hemolytic activity of microemulsions in human erythrocytes. J Pharm Biomed Anal 2005; 39: 1063–1067.

[19] Lexis LA, Fassett RG, Coombes JS. α—tocopherol and α—lipoic acid enhance the erythrocyte antioxidant defense in cyclosporine A—treated rats. Basic Clin Pharmacol Toxicol 2006; 98: 68–73.