Antimalarial efficacy of nine medicinal plants traditionally used by the Karens of Andaman and Nicobar Islands, India
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M. Punnam Chander¹, C. R. Pillai² and P. Vijayachari¹

¹Regional Medical Research Centre (Indian Council of Medical Research), WHO Collaborating Centre for Diagnosis, Reference, Research and Training in Leptospirosis, Port Blair 744 101, Andaman and Nicobar Islands, India; ²National Institute of Malaria Research (Indian Council of Medical Research), New Delhi, India.

Introduction
Andaman and Nicobar Islands, a union territory of India, has been historically known for high malaria transmission. The tropical climate prevailing throughout the year provides an ideal environment for mosquito proliferation and breeding in Nicobar group of islands (Manimunda et al., 2011). Entire land mass in the island has a network of creeks which results in ideal brackish water habitats for the breeding of Anopheles sundaicus, the predominant vector of malaria in Andaman and Nicobar Islands (Krishnamoorthy et al., 2005).

Malaria prevalence has increased alarmingly in past few years due to the development of drug resistance by Plasmodium falciparum (Parzy et al., 1997). Therefore, new and more effective therapeutic agents are urgently required to overcome the problem of malaria.

The Karen tribe is a lesser-known community in these Islands, settled in Mayabunder, North and Middle Andaman District. Karen derives from the word ‘Kawin’ as British called them, while they called themselves as Pwakanyaw, the quiet and easy going people, hailing from Henzada, Pathe and Nachaon areas of Pegu district in Western Myanmar (Oberai, 2000). Presently they inhabit three villages depending on agriculture and fishing for livelihood. Most of them depend on traditional medicine, though modern medicine had increased rapidly during recent times (Chander et al., 2015). As a part of the ongoing studies documented their traditional treatment practices and collected plants which were reported to be efficacious in the treatment of traditional medicine system.

In the present study, nine of these plants were subjected to in vitro antimalarial activity against Plasmodium falciparum chloroquine-sensitive MRC-2 isolate.

Materials and Methods

Plant materials
Nine plants were selected for evaluation on the basis of their ethnomedicinal histories. The names of these plants are: Z. spectabilis, S. wallichiana, C. pulcherrima, Amomum sp., Sassafras albidum, A. coriaceum, A. fortunei, A. indica, and A. naturalis.
plants in alphabetical order, with their respective scientific name, voucher numbers, family and vernacular name (Karen language) are presented in the Table I. Plant materials were collected in the three villages of Mayabunder, North and Middle Andaman District along with Traditional Knowledge Providers (TKPs) and authenticated by the plant taxonomist at the Botanical Survey of India, Andaman and Nicobar Islands, where voucher specimens were deposited in the Medicinal plant laboratory at the Regional Medical Research Centre (ICMR), Port Blair.

Extraction preparation
The plants were washed, shade dried at room temperature and ground into a fine powder. One hundred gram of each plant powder was subjected to percolation by soaking in 95% methanol. After 7 days of dark incubation, the filtrate was concentrated separately by rotary vacuum evaporation (45°C). Stock solutions of crude extracts were made in dimethyl sulfoxide (DMSO).

Parasite cultivation
The antimalarial activity of plant extracts was assessed against chloroquine-sensitive P. falciparum (MRC-2) isolate obtained from the National Institute of Malaria Research (ICMR), New Delhi, India. P. falciparum is cultivated in human A Rh+ red blood cells using RPMI 1640 medium (Sigma, India) supplemented with AB Rh+ serum (10%), 5% sodium bicarbonate (Sigma, India) and 40 μg/mL of gentamicin sulfate (Sigma, India) (Trager and Jensen, 1976).

In vitro test for antimalarial activity
The in vitro activity of P. falciparum intraerythrocytic stage on crude plant extracts was evaluated by Schizont maturation Inhibition M-III method (WHO, 2001). Briefly, crude extracts were dissolved in DMSO and serially diluted with RPMI 1640 medium to reach 1 mg/mL before use. Serial double dilutions were made in 96-well microtiter plates (in triplicate) with a concentration range of 1.56-100 μg/mL. The cultures, before testing, were synchronized by treatment with 5% D-sorbitol with a parasitemia of 0.6-0.8%. Each well received 10 μL of parasite-infected erythrocytes, 5% hematocrit and 90 μL of different drug dilutions. Chloroquine and solvent controls contained similar concentrations of solvent, as that of test wells. The plates were incubated at 37°C for 24 hours. After confirmation of the presence of 10% mature schizonts in control wells (without drug), the blood from each well was harvested, and a thick film was prepared on a glass slide. The blood films were stained for 40 min with Giemsa stain at a dilution of 10% in double distilled water. Three independent optical-microscopy readings of the number of schizonts with three of more nuclei were carried out in 200 parasitized red blood cells for each dilution and duplicate. Growth inhibition was expressed as the percentage of schizonts in each concentration, compared with controls.

Antimalarial activity calculation and analysis
The number of schizonts observed per well was directly entered into the nonlinear regression software, HN-

<table>
<thead>
<tr>
<th>Scientific name (Voucher No.)</th>
<th>Family</th>
<th>Local Name</th>
<th>Antimalarial activity (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amomum spp.</em> (AN-509)</td>
<td>Zingiberaceae</td>
<td>Thajokha</td>
<td>37.3 ± 2.5</td>
</tr>
<tr>
<td><em>Boesenbergia rotunda</em> (L.) Mansf. (AN-505)</td>
<td>Zingiberaceae</td>
<td>Sue</td>
<td>&gt;100.0</td>
</tr>
<tr>
<td><em>Caesalpinia pulcherrima</em> (L.) Sw. (AN-521)</td>
<td>Caesalpiniaceae</td>
<td>Posibo</td>
<td>14.6 ± 1.3</td>
</tr>
<tr>
<td><em>Cinnamomum tamala</em> (Buch.-Ham.) T. Nees &amp; Eberm. (AN-524)</td>
<td>Lauraceae</td>
<td>Thechipogow</td>
<td>&gt;100.0</td>
</tr>
<tr>
<td><em>Moringa oleifera</em> Lam. (AN-512)</td>
<td>Moringaceae</td>
<td>Dandloadaw</td>
<td>&gt;100.0</td>
</tr>
<tr>
<td><em>Senna alata</em> (L.) Roxb. (AN-502)</td>
<td>Caesalpiniaceae</td>
<td>Katheepaw</td>
<td>&gt;100.0</td>
</tr>
<tr>
<td><em>Solanum torvum</em> Sw. (AN-522)</td>
<td>Solanaceae</td>
<td>Takotako</td>
<td>&gt;100.0</td>
</tr>
<tr>
<td><em>Strychnos wallichiana</em> Steud. ex DC. (AN-511)</td>
<td>Loganiaceae</td>
<td>Niveka</td>
<td>12.0 ± 2.5</td>
</tr>
<tr>
<td><em>Zingiber spectabilis</em> Griffith (AN-503)</td>
<td>Zingiberaceae</td>
<td>Methalow</td>
<td>5.5 ± 0.7</td>
</tr>
<tr>
<td>Choloroquine</td>
<td></td>
<td></td>
<td>1.3 ± 0.1</td>
</tr>
</tbody>
</table>

IC₅₀ values are expressed as a mean of three independent values and were recorded in μg/mL.
NonLin V 1.1, which was specific for the analysis of in vitro drug sensitivity assay for malaria. Individual dose response curves were generated and their IC₅₀ values were determined.

Chemical injury to erythrocytes

In order to determine the possibility of chemical injury to the erythrocytes due to the extract, 100 μL of 5% erythrocytes were incubated with 100 μL/mL of the extract at a dose equal to the highest used in the antimalarial assay. During the experiment, the conditions were maintained as in the case of antimalarial assay. After 24 hours of incubation, thin blood smears were stained with Giemsa and observed microscopically for any morphological changes. The morphological findings were compared with those of erythrocytes which are not exposed to the extract.

Results

The leaf extract of Z. spectabilis (IC₅₀ 5.5 ± 0.7 μg/mL) showed excellent antimalarial activity followed by extracts of S. wallichiana (IC₅₀ 12.0 ± 2.5 μg/mL), C. pulcherrima (IC₅₀ 14.6 ± 1.3 μg/mL) and Amomum sp. (IC₅₀ 37.3 ± 2.5 μg/mL). Moreover, the leaf extract of C. tamala, S. torvum, M. oleifera, S. alata and B. rotunda were relatively inactive with IC₅₀ values higher than 100 μg/mL (Table I).

The microscopic observation of uninfected erythrocytes incubated with the methanol extracts and uninfected erythrocytes from the blank column of the 96-well plate showed no morphological differences after 48 hours of incubation.

Discussion

In the early studies, we reported the list of plants used by the Karens for various illnesses (Chander et al., 2015) and we selected nine plants routinely used by the karens. Methanol extracts of Z. spectabilis, S. wallichiana and C. pulcherrima showed the antimalarial activity and no previous reports available on the antimalarial properties of these medicinal plants in the literature. A previous phytochemical investigation on non-volatile constituents of the rhizomes of Z. spectabilis afforded several flavonoids, terpenoids and also demonstrated the antibacterial activity (Sivasothyu et al., 2012). In the present study, the leaf extract of Z. spectabilis showed high antimalarial activity in crude form.

According to Rasaonaivo et al. (1992), an extract is very active if IC₅₀ < 5 μg/mL, active 5 μg/mL < IC₅₀ < 50 μg/mL, weakly active 50 μg/mL < IC₅₀ <100 μg/mL and inactive IC₅₀ > 100 μg/mL.

S. wallichiana is specifically used for malaria fever in Karen community and it showed good antimalarial activity. In previous reports, the presence of various phytochemical and antimicrobial activities of S. wallichiana was demonstrated (Mallikharjuna et al., 2010). Vivek et al. (2013) reported the antimicrobial and anti-oxidant properties of leaf and flower extracts of C. pulcherrima. In the present study, leaf extract showed the active antimalarial property.

The in vitro antimalarial activity might be due to the presence of alkaloids, flavonoids, triterpenes, sterols, tannins and saponins in the methanol extracts of tested plants. The mechanism of action might be due to the inhibition of hemozoin biocrystallization, inhibition of protein synthesis, decreased mitochondrial membrane potential and DNA fragmentation (Dubar et al., 2011; Lopez et al., 2010).

Conclusion

The methanol leaf extracts of Z. spectabilis, S. wallichiana and C. pulcherrima possess significant suppressive effects on in vitro cultures of chloroquine-sensitive P. falciparum. These plants could serve as useful sources for new antimicrobial agents.

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Conflict of Interest

Authors declare no conflict of interest

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