SHORT COMMUNICATION

Screening of Thai Medicinal Plant Extracts and Their Active Constituents for In Vitro Antimalarial Activity

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To discover antimalarial substances from plants cultivated in Thailand 80%-EtOH extracts from selected plants were screened for in vitro antimalarial activity against the drug resistant K1 strain of Plasmodium falciparum. In total, 86 Thai medicinal plant samples representing 48 species from 35 genera in 16 families were screened and two species (Polyalthia viridis and Goniothalamus marcanii) were found to show notable antimalarial activity (IC50: 10.0 and 6.3 µg/mL). Marcanine A and 16-hydroxycleroda-3,13(14)Z-dien-15,16-olide were identified as the respective major active constituents in P. viridis and G. marcanii, respectively.

Keywords: antimalarial activity; Polyalthia viridis; Goniothalamus marcanii; marcanine A; 16-hydroxycleroda-3,13(14)Z-dien-15,16-olide.

INTRODUCTION

Malaria is the major parasitic infectious disease in many tropical and subtropical regions, leading to more than 1 million deaths (principally among young African children) out of 400 million cases each year (Greenwood and Mutabingwa, 2002). Globally, the incidence of malaria is increasing, a situation caused by behavioral changes of the mosquitoes as a result of increased resistance to insecticides. The present available drugs are losing effectiveness due to the occurrence of drug-resistant strains of Plasmodium falciparum against the existing antimalarial drugs, and many efforts are being made on a global scale to discover new, more effective antimalarial drugs.

Thailand is a tropical country which has an abundance of diverse plant resources and most of them are reported to have medicinal values. Because Thai medicinal plants are still widely and legally used in traditional Thai medicine, these have the possibility of containing active substances for the search of new drugs, especially to treat tropical infectious diseases, cancer, AIDS and so on. In the course of our screening program to discover new antimalarial substances, 86 Thai plant extracts were investigated. The present paper describes the results from screening the plant extracts and the identification of two kinds of antimalarial constituents.

MATERIALS AND METHOD

Plant materials. Plant materials were collected from various locations in Thailand. The plants were identified by Associate Professor Wongsatit Chuakul, Department of Pharmaceutical Botany, School of Pharmacy, Mahidol University. Voucher specimens are kept at the Herbarium of Faculty of Pharmacy, Mahidol University.

Extraction procedure. Various parts of the plants were dried and ground into powder. Ten grams of each sample was macerated overnight in 200 mL of 80% ethanol and filtered through filter paper (Whatman no. 1). The filtrates were evaporated under reduced pressure to give the ethanol extracts.

Isolation of the active substances. 16-Hydroxycleroda-3,13(14)Z-dien-15,16-olide (1) was isolated by activity guided purification as follows. The 80% EtOH extract of P. viridis was evaporated and dissolved in MeOH. The MeOH-soluble part was passed through a Sep-Pak C18 cartridge equilibrated with MeOH, and the resulting unabsorbed fraction was chromatographed by HPLC. HPLC was performed on a column of PEGASIL.
Table 1. Antimalarial activity of selected plant ethanol extracts of G. marcanii and P. viridis, and active constituents

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Extract/compound</th>
<th>IC$_{50}$ (µg/mL) for K1</th>
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<tbody>
<tr>
<td><em>Polyalthia viridis</em></td>
<td>80% EtOH ext.</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>16-Hydroxycleroda-3,13(14)Z-dien-15,16-olide</td>
<td>3.6</td>
</tr>
<tr>
<td><em>Goniothalamus marcanii</em></td>
<td>80% EtOH ext.</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>Marcanine A</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Artemisinin*</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Chloroquine*</td>
<td>0.11</td>
</tr>
</tbody>
</table>

* Positive control.

RESULTS AND DISCUSSION

In the present study, 86 Thai medicinal plant samples were collected, and their 80% EtOH extracts were tested for antimalarial activity against the drug resistant *P. falciparum* (K1) strain. All the samples were tested for activity at two doses (1.56 and 12.5 µg/mL), and only two samples (stem bark of *Polyalthia viridis* and leaves of *Goniothalamus marcanii*) were found to show antimalarial activity at a dose of less than 12.5 µg/mL (Table 1). The extract of the leaves of *G. marcanii* was more active than that of *P. viridis*. It has not been reported that these two plants contain antimalarial constituents.

Antimalarial constituents were purified from the extracts of *P. viridis* and *G. marcanii*. Fractionation of the MeOH soluble part in the extract from *P. viridis* gave seven fractions by reverse phase HPLC and only one fraction including a major peak showed antimalarial activity against the drug resistant *P. falciparum* (K1) strain (IC$_{50}$ 3.6 µg/mL). The active ingredient was identified to be 16-hydroxycleroda-3,13(14)Z-dien-15,16-olide by spectroscopic analysis on HR- and LR-MS, and NMR (Fig. 1 and Table 1). This compound has been isolated as the cytotoxic and antibacterial active ingredient (Ma et al., 1994; Rashid et al., 1996; Hagiwara et al., 1995). However, it has not been reported to have antimalarial activity. HPLC analysis of the extract of *G. marcanii* also indicated the presence of one major peak, which was identified to be marcanine A (Fig. 1). The compound has been isolated as the cytotoxic constituent from *G. marcanii* (Soonthornchareonnon et al., 1999). Therefore this compound was tested to evaluate the antimalarial activity, and marcanine A showed antimalarial activity against *P. falciparum* (K1) strain (IC$_{50}$ 2.5 µg/mL) (Table 1). The present study is the first report that marcanine A has antimalarial activity. The present results indicate further possibility for discovering useful active natural products for antimalarial activity from plant sources grown in Thailand.

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REFERENCES
