Antiplasmodial and Antioxidant Isoquinoline Alkaloids from Dehaasia longipedicellata

Abstract

The crude extract of the bark of Dehaasia longipedicellata exhibited antiplasmodial activity against the growth of Plasmodium falciparum K1 isolate (resistant strain). Phytochemical studies of the extract led to the isolation of six alkaloids: two morphinanidiones, (+)-sebiferine (1) and (-)-milonine (2); two aporphines, (-)-boldine (3) and (-)-norboldine (4); one benzylisoquinoline, (-)-reticuline (5); and one bisbenzylisoquinoline, (-)-O-O-dimethylgrisamine (6). Their structures were determined on the basis of 1D and 2D NMR, IR, UV, and LCMS spectroscopic techniques and upon comparison with literature values. Antiplasmodial activity was determined for all of the isolated compounds. They showed potent to moderate activity with IC50 values ranging from 0.031 to 30.40 µM. (-)-O-O-dimethylgrisamine (6) and (-)-milonine (2) were the two most potent compounds, with IC50 values of 0.031 and 0.097 µM, respectively, that were comparable to the standard, chloroquine (0.090 µM). The compounds were also assessed for their antioxidant activities with di[phenyl]-(2,4,6-trinitrophenyl)iminoazanium and metal chelating activities had IC50 values of 18.38 and 64.30 µg/mL, respectively. Thus it may be considered as a good reductant with the ability to chelate metal and prevent pro-oxidant activity. In addition to the antiplasmodial and antioxidant activities, the isolated compounds were also tested for their cytotoxicity against a few cancer and normal cell lines. (-)-Norboldine (4) exhibited potent cytotoxicity towards pancreatic cancer cell line BxPC-3 with an IC50 value of 27.060 ± 1.037 µM, and all alkaloids showed no toxicity towards the normal pancreatic cell line (hTERT-HPNE).

Abbreviations

- BHA: butylated hydroxyanisole
- DCE: dichloromethane crude extract
- DPPH: di[phenyl]-(2,4,6-trinitrophenyl)iminoazanium
- EDTA: ethylenediaminetetraacetic acid
- FRAP: ferric reducing power assay
- ROS: reactive oxygen species
- RF: retention time
- SOD: superoxide dismutase
- TLC: thin-layer chromatography

Introduction

Malaria is an important parasitic disease transmitted to humans by the bite of an infected female mosquito. Approximately 219 million people worldwide are affected by malaria and 600,000 deaths in 2010 have been reported in the World Malaria Report [1]. Malaria remains one of the most important infectious diseases in the developing world. In Malaysia, the parasites Plasmodium falciparum, P. knowlesi, and P. vivax are recognized as the common cause of malaria. Symptoms of malaria include fever, shivering, joint pain, vomiting, and retinal damage [2]. Global warming could increase malaria by expanding the area in which the ambient temperature and climate conditions are suitable. This could lead to the resistance of common antimalarial drugs such as artemisinin-based monotherapies. Parasite resistance to artemisinin has now been detected in 4 countries: Cambodia, Myanmar, Thailand, and Vietnam [1]. Thus, there...
is an urgency for research to be done on new antimalarial drugs from natural resources [3].

Antioxidants are widely used as food additives to provide protection against oxidative degradation of food by free radicals [3]. Insufficient levels of antioxidants or inhibition of the antioxidant enzymes can cause oxidative stress. The antioxidant activity is important in the treatment of malaria since oxidative stress is normally synchronized with a malaria infection [4, 5]. Antioxidant activity is believed to act in a synergistic way, protecting the body against oxidative stress [6]. In our continuing interest in searching for new and biologically active compounds [7–11], under the framework of the Malaysian-French scientific collaboration, a survey of several crude extracts from Malaysian plants has shown that Dehaasia longipedicellata (Ridl.) Kosterm. exhibited promising antiplasmodial activity (IC50 = 1.30 µg/mL). Therefore, we have embarked on the investigation of the antiplasmodial and antioxidant activities of compounds isolated from D. longipedicellata. Hence, in this study, we communicate the isolation of alkaloids from the active extract and their bioactivities, antiplasmodial and antioxidant.

Dehaasia is a member of the Lauraceae family. It is an evergreen tree of moderate size, with large leaves, found growing in the western parts of Malaysia, China, and the Philippines [12]. About 35 species of Dehaasia are spread out worldwide and 9 species are found in Malaysia [13]. Dehaasia is locally known as “gajus hutan” or “pekan”, and the timber is durable and used for building houses. D. longipedicellata is a small tree with leaves that are apex pointed, blades that are soft and hairy on the undersurface, and are broadly elliptic to obvate. According to the Sakai of the Tapah Hills, the fruit is very poisonous. This is the first report on the antimalarial and antioxidant activities of isoquinoline alkaloids from Dehaasia.

Results and Discussion

In spite of the large diversity of the compounds which have been studied phytochemically from the Dehaasia genus [14–17], this report communicates for the first time the occurrence of (+)-sebiferine (1), (-)-boldine (3), (-)-norboldine (4), (-)-reticuline (5), and (-)-O-O-dimethylgrisabine (6) in D. longipedicellata (Fig. 1). Structural elucidation was performed with the aid of spectroscopic methods, notably UV, IR, LCMS, and 1D and 2D NMR (COSY, HMBC, HMQC, and NOESY).

Based on the potent screening result of the DCE (IC50 = 1.30 µg/mL), the compounds isolated from the bark of D. longipedicellata were then subjected to in vitro antimalarial evaluation against a chloroquine-resistant strain of P. falciparum, K1 (Table 1). Among the six compounds evaluated for their antimalarial activity, (-)-O-O-dimethylgrisabine (6) clearly showed the most potent in vitro antimalarial activity with an IC50 value of 0.031 µM, which was slightly better than the positive control chloroquine. In addition, (-)-milonine (2) also displayed a strong inhibition capacity with an IC50 value of 0.097 µM, followed by (-)-boldine (3), (-)-norboldine (4), (+)-sebiferine (1), and (-)-reticuline (5).

(-)-O-O-dimethylgrisabine (6), a bisbenzylisoquinoline, also showed a high scavenging activity of free radical DPPH with an

![Fig. 1 Structures of isolated compounds from Dehaasia longipedicellata.](image-url)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC50 µg/mL</th>
<th>K1</th>
<th>A549</th>
<th>A375</th>
<th>BxPC-3</th>
<th>hTERT- HPNE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-Sebiferine (1)</td>
<td>0.097</td>
<td>22.460</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>93.390 ± 5.564</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>(-)-Milonine (2)</td>
<td>0.097</td>
<td>2.602</td>
<td>117.570 ± 0.067</td>
<td>112.530 ± 3.484</td>
<td>45.500 ± 2.949</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>(-)-Boldine (3)</td>
<td>0.097</td>
<td>2.602</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>82.850 ± 8.735</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>(-)-Norboldine (4)</td>
<td>0.097</td>
<td>2.602</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>82.850 ± 8.735</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>(-)-Reticuline (5)</td>
<td>0.097</td>
<td>2.602</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>82.850 ± 8.735</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>(-)-O-O-dimethylgrisabine (6)</td>
<td>0.097</td>
<td>2.602</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>82.850 ± 8.735</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>0.097</td>
<td>2.602</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>82.850 ± 8.735</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>Artemisinin</td>
<td>0.097</td>
<td>2.602</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>82.850 ± 8.735</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>0.097</td>
<td>2.602</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>82.850 ± 8.735</td>
<td>&gt; 200</td>
</tr>
</tbody>
</table>

* Results expressed as mean ± SE; † results expressed in µg/mL for extracts and µM for pure compounds or standards. Bold numbers indicate most active compounds comparable to the standard.

All of the isolated compounds showed no potency against lung iron binding and antiplasmodial activity, and the latter is an oxidant compound, which could lead to the killing of the parasites from malaria infection are synergistic to Plasmodium (82.850 ± 8.735 µM). However, for pancreatic cancer cells (BxPC-3), a great potency was shown by (−)-norbornele (4) with an IC$_{50}$ of 27.060 ± 1.037 µM. The same compounds were tested against normal pancreatic cells (hTERT-HPNE) and no cytotoxicity was observed. Therefore, (−)-norbornele (4) should be studied further as a potential lead for drug discovery in cancer studies (Table 1).

### Materials and Methods

#### General experimental procedures

Spectra were recorded on the following instruments: UV, Shimadzu UV-250, UV-visible spectrometer; IR, Perkin Elmer 1600; NMR, AVN BRUKER with CDCl$_3$ as the solvent. These were used to obtain the 400 MHz for the proton spectrum and 100 MHz for the carbon spectrum. Mass spectra were obtained using Agilent Technologies 6530 accurate-mass Q-TOF LC/MS, with a ZORBAX Eclipse XDB-C18 rapid resolution HT 4.6 mm i.d. × 50 mm × 1.8 µm column. All solvents, except those used for bulk extraction, were AR grade. Column chromatography separations were conducted using Merck silica gel 60 (230–400 mesh) and silica gel 60 F$_{254}$ for TLC monitoring. Glass and aluminium supported silica gel 60 F$_{254}$ plates were used for TLC. TLC spots were visualized under UV light (254 and 365 nm) followed by spraying with Dragendorff’s reagent for alkaloid detection. HPLC was performed on a Waters system equipped with a binary gradient module (Waters 2545), system fluidics organizer (Waters SFO), photoiodide array detector (190–600 nm, Waters 2998), and a sample manager (Waters 2767). Chromatographic analysis and separations were performed on a Chromolith semiprep RP-18 endcapped HPLC column. The samples were eluted at a flow rate of 4 mL/min.

### Plant material

The bark of D. longipedicellata was collected on 29 February 2009 at Sungai Tekam Reserve Forest, Jerantut, Pahang by the phyto-chemical group of the Department of Chemistry, Faculty of Science, University of Malaya. The plant specimen was identified by Mr. Teo Leong Eng and Mr. Din Mat Nor. A voucher specimen (KL5634) has been deposited at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

#### Extraction and separation

Dried, ground bark of D. longipedicellata (2.0 kg) was first de-fatted with hexane twice for a 3-day period. The hexane extracts were combined and the solvent was evaporated. The plant material was dried up and then soaked with 25% NH$_4$OH for 2 hours. It was then macerated twice for a 3-day period with dichloromethane. DCE was finally concentrated to give a crude alkaloid extract of 18.7 g. DCE 10.5 g was subjected to column chromatography...
over silica gel (0.04–0.063 mm; 6 × 65 cm) eluting with a mixture of a dichloromethane: methanol solvent in proportions of 100:0, 99:1, 98:2, 97:3, 96:4, 95:5, 94:6, 92:8, 90:10, 80:20, 50:50, and 0:100, v/v, each 1000 mL, in gradient to obtain 7 main fractions (Fr. 1–Fr. 7) based on their TLC profiles. Further purification of Fr. 4 by preparative TLC (1.00 g using [CH2Cl2 : MeOH at a ratio of 97:3, v/v], saturated with NH4OH; Dragendorff reagent) led to the isolation of (+)-sebiferine (1) [20 mg, Rf: 0.58, ([α]27 +92.31, c = 0.00013, MeOH)], (−)-milonine (2) [17 mg, Rf: 0.42, ([α]27 − 109.09, c = 0.00011, MeOH]), and (−)-boldine (3) [10 mg, Rf: 0.33, ([α]27 + 66.67, c = 0.00003, MeOH)]. Preparative TLC of Fr. 5 (80 mg [CH2Cl2 : MeOH 96:4, v/v], saturated with NH4OH; Dragendorff reagent) yielded (−)-reticuline (5) [10 mg, Rf: 0.38, ([α]27 + 66.67, c = 0.00003, MeOH)] and (−)-norboldine (4) [10 mg, Rf: 0.42, ([α]27 − 100.00, c = 0.00010, MeOH)]. The Rf of (−)-norboldine (4) was 2.00 minutes. The degree of purity of the isolated compounds was determined by 1H NMR spectroscopy and was found to be 99% for compounds 1, 2, 3, and 4, and 98% for compounds 5 and 6.

Antiplasmodial assay

The DCE and the isolated compounds were evaluated for their in vitro antimalarial activity against P. falciparum strain K1, which is resistant to chloroquine. Chloroquine (purity 98.0%) and artemisinin (purity 98.0%) were purchased from Sigma Chemicals and used as positive controls. Isolated and standard compounds were maintained in a continuous culture as described by Trager and Jensen [31], with some modifications. The synchronization of the malaria culture to one stage follows the method of Lambros and Vanderberg [32]. Antiplasmodial activity was evaluated using a histidine-rich protein II (HRPII) assay from Noedl et al. [33]. The antimalarial activity of each compound was expressed as an IC50 value, defined as the concentration of the compound causing 50% inhibition of parasite growth relative to the untreated control.

Antioxidant assay

DPPH assay: The DPPH antioxidant assay was determined as described by Shimada et al. [34]. Briefly, DPPH (1 mL, 0.1 mM) dissolved in ethanol was added to an ethanol solution (3 mL) of the tested compounds at different concentrations (0, 50, 100, 150, and 200 µg/mL). An equal volume of ethanol was added in the control test. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, the absorbance at 517 nm was measured with a UV–VIS spectrophotometer. The percentage of scavenging of DPPH was calculated using the following equation:

\[
\text{DPPH scavenging effect (\%)} = \frac{A - A_1}{A} \times 100
\]

Where \(A\) is the absorbance of the control reaction and \(A_1\) is the absorbance in the presence of the sample. Ascorbic acid (purity 99.0%) was purchased from Sigma and used as the standard reference.

A Queous One Solution, Promega) according to the manufacturer’s protocol. Microplates were returned to the incubator for 1–2 h and absorbance of the formazan product was read on a microplate reader at 490 nm with 690 nm as the background wavelength (Infinite 200, Teco). The IC50 values of the samples and drug standards were determined using dose-response curves, and statistical analysis using the Student’s t-test (p < 0.05) was performed in Prism 5.02 software (GraphPad Software, Inc.).

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Conflict of Interest

The authors have declared that there is no conflict of interest.

References

16 Lee SS, Chen CK, Chen CH. Chemical constituents from Dehaasia triandra. II. Five new alkaloids, secoxanthophane, dehatriphilone, 11β,8β-dihydroxyisocorydione, 11β,8β-dihydroisocorydione, 11β,8β-dihydroisocorydione, and 8β,11β-dihydroisocorydione, isolated from the leaves. Tetrahedron 1996; 52: 6561–6568
27 Weinberg ED. Iron availability and infection. Biochim Biophys Acta 1999; 1790: 600–605