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Antiplasmodial ativity of akaloids from *Garcinia Parvifolia* Miq. Stem Bark

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Abstract

Garciniavalline (**1**), a novel aporphinoid alkaloid, in addition to four known alkaloids, cleistopholine (**2**), O-methylmoschatoline (**3**), (-)-oliveroline (**4**) and (-)-oliveridine (**5**), were isolated and characterized from *Garcinia Parvifolia* Miq. **s**tem bark. Structural elucidation of these compounds was established by spectroscopic methods. Among them, alkaloids (**2**) and (**4**) exhibited antiplasmodial activity against *Plasmodium falciparum*

Keywords: Isoquinoline alkaloids; *Garcinia Parvifolia* Miq.; Antiplasmodial.

1. Introduction

The genus *Garcinia* (Guttiferae) is composed of about 250 different species confined to the warm humid tropics of the world [1]. Extensive chemical investigation of this genus has resulted in the isolation of a wide variety of natural products, including xanthones, coumarins, flavonoids, chalcones, benzofurans and triterpenes [2-4]. Some of these species are frequently employed in folk medicine to treat several injuries [5]. Several experimental studies have reported that extracts and/or the oil of several species of this genus are potential antimalarial [6-9] and antifungal sources [2]. *Garcinia Parvifolia* Miq., an endemic plant found in Indonesia, has not previously been the subject of phytochemical analyses. In this paper we discuss the isolation and structure elucidation of a new oxoaporphine, garciniavalline (**1**), along with four known compounds, cleistopholine (**2**) [10], O-methylmoschatoline (**3**) [11], (-)-oliveroline (**4**) and (-)-oliveridine (**5**) [12], from the alkaloidal extract of the *Garcinia Parvifolia* Miq. stem bark. The compounds were evaluated for their antiplasmodial activity against *Plasmodium falciparum*.

2. Experimantal

2.1. Plant material

Garcinia Parvifolia Miq. was collected in july 2008 in the West Province of Indonesia. A specimen was identified and maintained at the Herbarium Bogoriense, Bogor, Indonesia.

2.2. General procedures

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The UV spectra were obtained in MeOH, using Shimadzu UV 1201 spectrophotometer, IR spectra were recorded on a Perkin-Elmer 241 MC (FT-IR). ^{1}H NMR (300 and 400 MHz) and ^{13}C NMR (75 MHz) spectra (all in $CDCl_3$) were recorded with a Bruker AMX 300 and Bruker AM 400, using TMS as internal standard. The mixing time for the HMBC spectra was 0.8 s, and the delay, in NOESY experiments, 2 s. CIMS were obtained with a Nermag-Sidar R10-10C mass spectrometer. Si gel 60 (Merck 0.063-0.200 mesh) was used for column chromatography, precoated Si gel plates (Merck 60 F_{254} 0.2 mm) were used for TLC. Plates were visualized by spraying with Dragendorff's reagent or with 50% H_2SO_4 and then heating.

2.3. Extraction and isolation

The air-dried stem bark of *Garcinia Parvifolia* Miq. (1,5 kg) were defatted by percolation with petroleum ether; the solid residue was then basified with 5% aq. NH_4OH solution and extracted with CH_2Cl_2. The combined organic extracts were then evaporated under reduced pressure. The bases were extracted with 3% aq. HCl from the CH_2Cl_2 solution. The HCl solution was basified with NH_4OH (pH 8-9) and extracted with CH_2Cl_2. The CH_2Cl_2 solution was dried over anhydrous Na_2SO_4, filtered and then evaporated to leave a brownish solid residue (17 g, 1.1%). The residue was flash chromatographed on Si gel (500 g), eluted with increasing polarities of CH_2Cl_2/MeOH mixtures. Ninety fractions of 100 mL each were collected. Fractions of similar composition (as indicated by TLC) were combined. From fractions 30-36 and fractions 37-40, cleistopholine (**2**, 200 mg) and O-methylmoschatoline (**3**, 500 mg) were obtained respectively. Fractions 41-44 (600 mg) was subjected to Si gel CC (100 g), eluting with EtOAc/MeOH (99:1). Thirty fractions were collected; fractions 5-12 furnished garciniavalline (**1**, 50 mg). Fractions 52-56 (1200 mg), were subjected to Si gel CC (200 g), eluting with EtOAc/MeOH (96:4). Fifty fractions were thus collected. Fractions 10-25 were, subjected to preparative TLC, using hexane:EtOAc:diethylamine (8:1:1) as eluent, by which (-)-oliveroline (**4**, 150 mg) and (-)-oliveridine (**5**, 200 mg) were obtained.

2.4. Antiplasmodial Assay

Antiplasmodial activity of the compounds was assessed by an *in vitro* radioisotope incorporation test using [^{3}H] hypoxanthine [13]. Each compound, plus chloroquine as a control, was assayed in triplicate at 4 different concentrations. Concentrations of both compounds tested, and positive controls, which inhibited parasitespecific incorporation of [^{3}H] hypoxanthine by 50% (IC_{50}), were determined by non-linear regression analysis. Zero-drug controls were defined as 100% incorporation.

3. Results and discussion

Garciniavalline (**1**): orange needles (CH_3Cl); m.p. 198-200 ºC; UV (MeOH) λ_{max} 205 (3.01), 214 (3.09), 223 (3.73), 289 (3.04), and 385 (2.11) nm; IR (KBr) ν_{max} 2936, 1638, 1596, 1446, 1337, 1202 and 963 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table **1**. LRMS *m/z* 366 $[M+H]^+$ (48), 351 (100), 334 (56), 223 (8), 308 (4) and 272 (2).

Garciniavalline (**1**) was obtained as orange needles from $CHCl_3$, m.p. 198-200 ºC. It displayed a green spot on spraying with Dragendorff's reagent. The CIMS data showed the $[M+H]^+$ at *m/z* 366 corresponding to the molecular formula $C_{20}H_{15}O_6N$. An IR band at ν1638 cm^{-1} and a signal at δ 183.3 ppm in the ^{13}C NMR spectrum indicated that a carbonyl group was present. Its UV absorption maxima at λ 205, 214, 223, 289, and 385 nm were characteristic of an oxoaporphine skeleton [10]. The ^{1}H NMR spectrum of (**1**) (Table **1**) showed the presence of a methylenedioxy singlet at δ 6.25 assigned to positions 1 and 2, whereas three methoxy groups

were identified, respectively at δ 4.24 characteristic of position 3 [11] and δ 3.98 and 3.96 located in ring D.

Table 1
Correlated ^{13}C-NMR, ^{1}H-NMR, HMBC and COSY for Compound (1) in $CDCl_3$.

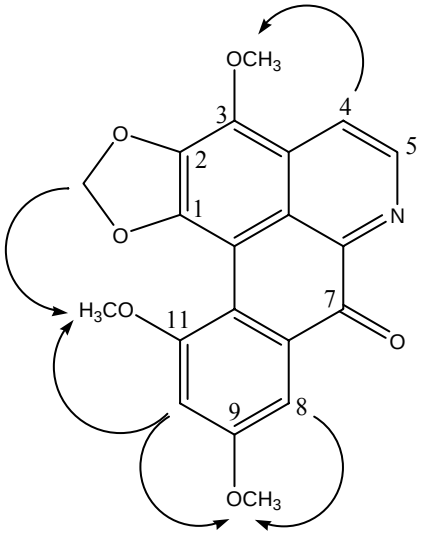
Position	δ_C	δ_H	HMBC	^{1}H -^{1}H COSY
1	136.6		6.25	
1a	107.5			
1b	111.4			
2	135.2		4.25	
3	152.0		4.25	
3a	121.6	8.12 d 5.2		
4	118.0	8.86 d 5.2	8.86	8.86
5	144.4		8.12	8.12
6a	148.3			
7	183.3		7.65	
7a	133.8		7.65	
8	102.7	7.65 d 2.5	6.83	6.83
9	161.6		6.83, 3.98	
10	107.9	6.83 d 2.5	7.65	7.65
11	154.8		3.96, 6.83	
11a	125.0			
OCH_2O	101.3	6.25		
MeO-3	61.8	4.25		
MeO-9	55.6	3.98		
MeO-11	55.2	3.96		

The aromatic region of the spectrum revealed the presence of one pair of doublets at δ 8.86 and 8.12 (J = 5.2 Hz), assigned to H-5 and H-4 [12], and another pair at δ 7.65 and 6.83 (J = 2.5 Hz), which was in accord with a *meta*-substitution in ring D. The absence of the characteristic deshielded H-11 signal indicates the MeO-9/MeO-11 substitution. The unambiguous assignment of the *meta* substitution was achieved by NOESY experiments (Scheme 1). Observation of the NOESY correlations between MeO-11 and both 1,2-methylenedioxy and H-10, between MeO-9 and both H-8 and H-10, and between H-4 and both MeO-3 and H-5 protons corroborated the MeO-9/MeO-11 substitution. Further HMQC, HMBC, COSY data (Table 1) provided confirmation of structure (**1**).

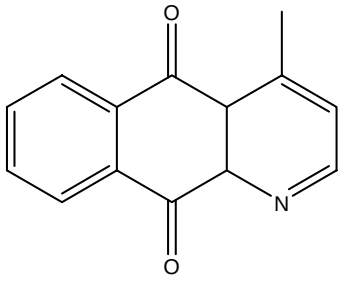
The *meta*-substitution pattern in the D ring of aporphines has a taxonomic significance in the Annonaceae family. Although some aporphines with *meta*-substitution in the D ring have been reported in other families [12], these alkaloids are mainly present in the *Duguetia* and *Guatteria* genera [13]. Thus, only one oxoaporphine with a 9,11 *meta*-substitution pattern was reported from *Guatteria discolor* [14].

Chromatographic separation also yielded cleistopholine (**2**), O-methylmoschatoline (**3**), (-)-oliveroline (**4**), and (-)-oliveridine (**5**) from the total alkaloidal extract; their structures were established by comparison of their physical and spectral data (UV, IR, ^{1}H NMR, ^{13}C NMR, LRMS) with those published in the literature. The structures of (**4**) and (**5**) were further supported by NOESY data.

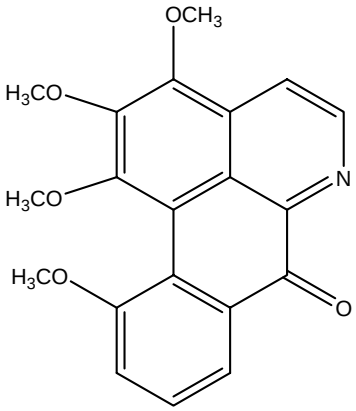
The antiplasmodial activity of the isolated compounds was assayed against *Plasmodium falciparum* sensitive strain ITG2 (Table 2). In this study, the most active compounds were cleistopholine (**2**), and (-)-oliveroline (**4**), which showed IC_{50} = 17.8 and 14.9 μM, respectively.



(1)



(2)



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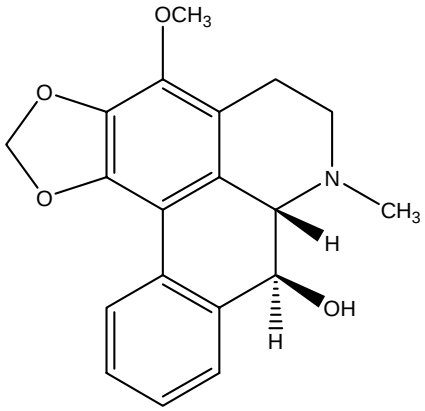

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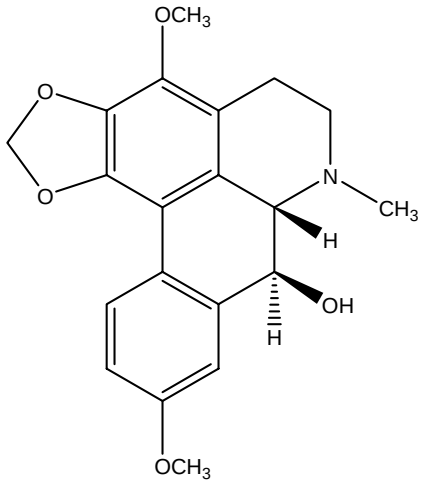

(5)

Scheme 1

Table 2
The *in Vitro* Antiplasmodial Activity of Alkaloids 1-5 Against *Plasmodium Falciparum.*

Compound	IC_{50} (μmol mL^{-1})
1	75.9
2	17.8
3	32.3
4	14.9
5	55.7
Chloroquine	0.06

4. Conclusion

In conclusion, these studies demonstrated the good *in vitro* antiplasmodial activity of the compounds isolated from *Garcinia Parvifolia* Miq. stem bark. Efforts will be undertaken to continue the bioassay guided fractionation in order to isolate and identify the active compounds, as well as to understand the mechanism of action.

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