Antiplasmodial Compounds from Indonesian Marine Sponge, *Xestospongia* sp, against *Plasmodium falciparum* 3D7

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**ABSTRACT**

A part of our continuing search for antiplasmodial compounds from Indonesian marine sponge, two steroidal alkaloids, epoxysarcovagenine-D (1) and epoxyepapakistamine-A (2), have been isolated from the ethyl acetate extract of the Indonesian marineponge, *Xestospongia* sp. Their structures were identified on the basis of spectroscopic data analysis and by comparison with published spectroscopic and physicochemical properties data. Compounds 1 and 2 were isolated first time from marine sponge, *Xestospongia* sp., and showed strongest plasmodial activity against *Plasmodium falciparum* 3D7 strain with IC$_{50}$ values of 0.013 and 0.158 µM, respectively.
Keywords: Steroidal alkaloid, Xestospongia sp, Petrosiidae, Antiplasmodial activity

INTRODUCTION

The genus Xestospongia is the marine sponge belong to Petrosiidae family, comprises approximately 40 species that are mainly distributed in north-western Australia, Papua New Guinea, Solomon Island, Thailand and Indo-Malaysia Peninsula (Fromont, 1991; Calcul, 2003; Laurent et al., 2006; Aguinaga et al., 2010). Xestospongia is known to settle and grow on a variety of substrates, such as sand, rock beds, dead coral rubble and coral heads (Kerr and Borges, 1994; Williams et al., 1998; Krisanapuntu et al., 2001; Bell and Smith, 2004; Armstrong et al., 2006). Chemical investigations on the species of this genus have led to the isolation of a large array of structurally diverse secondary metabolites with significant biological activities including antimalarial alkaloids (Girard et al., 2004; Darumas et al., 2007; Ashok et al., 2014), antifungal alkaloids (Moon et al., 2012), cytotoxic and inhibition of the aspartic protease of the quinones and hydroquinones (Aguinaga et al., 2010; Dai et al., 2010), antimalarial quinones (Laurent et al., 2006), antiplasmodial benzaldehyde (Murtihapsari et al., 2019) and antiplasmodial sterol (Renga et al., 2012).

During the course of our continuing search for antiplasmodial compounds from Indonesian marine sponge, the methanolic extract of Xestospongia sp exhibited a significant antiplasmodial activity against Plasmodium falciparum 3D7 strain. Xestospongia sp is distributed in the eastern part of Indonesia, and previous investigation have led to the isolation of several steroid with antiplasmodial activity (Muptihapsari et al., 2019). Owing to our interest in antiplasmodial compounds from this species, we investigated the ethyl acetate extract of the Xestospongia sp and obtained two antiplasmodial steroidal alkaloid. Here, we describe the structural identification of the isolates and their antiplasmodial activity.

MATERIALS AND METHODS

General
Melting points were obtained on an electrothermal melting point apparatus. Optical rotations were measured on an ATAGO AP-300 automatic polarimeter. IR spectra were obtained with a Perkin Elmer spectrum-100 spectrophotometer using KBr pellets. Mass spectra were recorded on Water Qtof HR-MS XEVotm mass spectrometers. 1D and 2D-NMR spectra were run on a JEOL ECZ A-600 spectrometer with tetramethyl silane as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Column chromatography was performed on silica gel (70-230 and 200-400 mesh, Merck,
Germany) and RP-18 gel (20−45 μm, Fuji Silysia Chemical Ltd., Japan). Fractions and spots were monitored by TLC (GF<sub>254</sub>, Merck, Germany), and spots were visualized by heating silica gel plates sprayed with 10% H<sub>2</sub>SO<sub>4</sub> in EtOH followed by heating.

**Sponge materials**

Material of porifera *Xestospongia* sp, was obtained by scuba in about 10 m depth in the south west of Kaimana, West Papua, Indonesia (GPS: 4°20.341’ S-133°30.265’E). It has been identified taxonomically as *Xestospongia* sp. (Darumas et al., 2007), the species belongs to the *Xestospongia*. Identification taxonomic and nomenclature were provided by the Laboratory Biology and Conservation, Jakarta Fisheries University, Ministry of Marine Affairs and Fisheries (LIN BIOVASI, No: 041/STP-V/2016, Catalogue: MS041.1-5).

**Parasite cultivation**

*Plasmodium falciparum* strain 3D7, which is resistant to chloroquine (Singh and Rosenthal, 2001), was cultured in sealed flask at 37 °C, in a 3% O<sub>2</sub>, 5% CO<sub>2</sub> and 91% N<sub>2</sub> atmosphere in RPMI 1640, 25 μM HEPES, pH 7.4, supplemented with heat inactivated 10% human serum and human erythrocytes to achieve a 2% haematocrit. Parasites were synchronized in the ring stage by serial treatment with 5% sorbitol and studied at 1% parasitemia (Lambros et al., 1979).

**In vitro antiplasmodial**

Extracts and isolated compounds were prepared as 20 μg/mL stock solutions in DMSO (dimethyl sulfoxide), diluted as needed for individual experiment and tested in triplicate. The stock solutions were diluted in supplemented RPMI (*Rosewell Parla Memorial Institute* medium product) 1640 medium so as to have, at most, 0.2% DMSO in the final reaction medium. An equal volume of 1% parasitemia, 4% haematocrit culture was there after added and gently mixed thoroughly. Negative controls contained equal concentration of DMSO. Positive control contained an artemisinin. Cultures were incubated at 37 °C for 48 hours (one parasite erythrocytic life cycle). Parasites at ring stage were thereafter fixed by replacing the serum medium by an equal volume of 1% formaldehyde in PBS. (Phosphate Buffered Saline) aliquots (50 μL) of each culture were then added to 5 mL round-bottom polystyrenes tubes containing 0.5 mL 0.1% Triton X-100 and 1 nM YOYO nuclear dye (barand YOYO®-1) in PBS. Parasitemias of treated and control cultures were compared using a Becton-Dickinson FAC (brand BD-FAC™) Sort flow cytometer to count nucleated (parasitized) erythrocytes. An antiplasmodial examination was used 96 wells, each well filled by parasitemia culture 1%. About 50 μL of the compounds filled into the well with the following concentration 10<sup>-9</sup> to 10<sup>-2</sup> μg/mL. These data were normalized to percent control activity and 50% inhibitory concentration (IC<sub>50</sub>) calculated using Table probit.
Different concentration of the extract and isolated compounds were incubated at 37 °C with cultured 3D7 strain of *P. falciparum* parasites for 48 hours. Parasites were thereafter fixed and strained, and parasitemias of treated and control cultures were determined. Results are means, compared to untreated controls from 3 experiments. Error bars represent standard deviations of results (Bagavan et al., 2011).

**RESULTS**

**Extraction and isolation**

The milled fresh of marine sponge, *Xestospongia* sp (38 kg), were extracted with ethanol (30 L) at room temperature for 3 days. The ethanol extract was evaporated in vacuum to give a semisolid residue (376 g). After being suspended in water (2.0 L), the solution was successively fractionated with *n*-hexane, EtOAc and *n*-BuOH. Evaporation in vacuum resulted in the crude extracts of *n*-hexane (10.3 g), EtOAc (11.4 g), and *n*-BuOH (19.6 g), respectively. All extracts were evaluated for their antiplasmodial activity against *P. falciparum* 3D7 and showed antiplasmodial activity with IC\textsubscript{50} values of 1.95, 0.41, and 1.68 µg/mL, respectively.

The EtOAc extract (11.4 g) was applied to silica gel column chromatography, eluted with *n*-hexane-EtOAc (100:1 to 1:100, v/v) to produce eight fractions (F.1-8). Fraction F.4 (420 mg) was subjected to silica gel column chromatography eluted with *n*-hexane-acetone (100:1 to 1:100, v/v) to produce six subfractions, 4A-4F. Subfraction 4C (130 mg) was applied to silica gel column chromatography using CHCl\textsubscript{3}-MeOH (9.5:0.5, v/v), then octa desyl silane (ODS) eluting with MeOH-H\textsubscript{2}O (2:3, v/v) to afford 1 (12.3 mg). F.6 (370 mg) was subjected to silica gel column chromatography eluted with *n*-hexane-acetone (100:1 to 1:100, v/v) to produce seven subfractions, 6A-4G. Subfraction 6E (130 mg) was applied to silica gel column chromatography using *n*-hexane-acetone (8:2, v/v) to afford 2 (14.6 mg).

**Epoxysarcovagenine-D** (1). Yellowish solid, m.p. 113-115 °C; [α]\textsuperscript{20}\textsubscript{D} +16.5° (c = 0.1, CHCl\textsubscript{3}); UV λ\textsubscript{max} (MeOH) nm (log ε) 280 (5.2); IR (KBr) ν\textsubscript{max} cm\textsuperscript{-1}: 3440, 3240, 2920, 2880, 1670, 1645, 1146, 1056; \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 600 MHz): Table 1; \textsuperscript{13}C-NMR (CDCl\textsubscript{3}, 125 MHz): Table 1; HR-TOFMS m/z 441.3040 [M+H]\textsuperscript{+}, calcd. for C\textsubscript{27}H\textsubscript{40}N\textsubscript{2}O\textsubscript{3} m/z 440.3039.

**Epoxynepapakistamine-A** (2). White crystals; m.p 118-120 °C; IR (KBr) ν\textsubscript{max} cm\textsuperscript{-1}: 3446, 2945, 1728, 1660, 1150, 847; \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 600 MHz): Table 1; \textsuperscript{13}C-NMR (CDCl\textsubscript{3}, 125 MHz): Table 1; HR-TOFMS m/z 559.3670 [M+H]\textsuperscript{+}, calcd. for C\textsubscript{32}H\textsubscript{50}N\textsubscript{2}O\textsubscript{5} m/z 558.3669.
DISCUSSION

The ethanolic extract from the milled fresh of Xestospongia sp was concentrated and extracted successively with n-hexane, ethyl acetate and n-butanol. All of the extracts were evaluated for their antiplasmodial activity against P. falcifarum 3D7 in vitro and the ethyl acetate extract exhibited strongest antiplasmodial activity with an IC₅₀ value of 0.41 µg/mL. By using antiplasmodial assay to guide separations, the ethyl acetate fraction was separated by combination of column chromatography on silica gel and octadesylsilane to afford two antiplasmodial steroidal alkaloids, 1 and 2 (Figure 1).

![Figure 1](image1.png)

**Figure 1.** The chemical structures of 1 and 2 isolated from Xestospongia sp.

![Figure 2](image2.png)

**Figure 2.** Selected ¹H-¹H COSY and HMBC Correlations for Compounds 1 and 2.

Compound 1 was isolated as yellowish solid; m.p 113-115 °C; [α]²⁰D +16.5° (c = 0.1, CHCl₃). Its molecular formula was determined as C₂₇H₄₀N₂O₃ (nine indices of hydrogen deficiency) by HR-TOFMS m/z 441.3040 [M+H]+ and NMR spectral data (Table 1). The UV spectrum showed an absorption maximum at 280 nm (log ε 5.2), indicating the presence of an enone system (Supratman
et al., 2000). Infrared (IR) absorption bands due to amine, conjugated carbonyl, amide and ether groups were observed at 3440, 1670, 1645 and 1146 cm$^{-1}$, respectively.

The $^1$H-NMR spectrum of 1, displayed the presence of two tertiary methyl signals at $\delta_H$ 0.82 and 0.87 (each 3H, s, Me-18 and Me-19), a secondary methyl at [$\delta_H$ 1.12 (3H, d, J=6.5 Hz, Me-21), two methyl proton of tigloyl group at $\delta_H$ [2.40 (3H, d, J=6.9 Hz, Me-3’) and 2.53 (3H, d, J=1.2 Hz, Me-5’)] and a $N_b$-Me at $\delta_H$ 3.26 (3H, s). Proton signals of olefinic protons at $\delta_H$ [7.55 (1H, dd, J=6.9, 2.6 Hz, H-2) and 6.34 (1H, dd, J=6.9, 1.2 Hz, H-3’)], an oxygenated sp$^3$ methine at $\delta_H$ 3.10 (1H, dd, J=6.7, 2.4 Hz, H-16) and two N-H protons at $\delta_H$ [8.03 (1H, br.s, $N_a$-H) and 2.10 (1H, br.s, $N_b$-H) were also observed in the H-NMR spectrum. The $^{13}$C NMR spectrum showed 27 signals, which are clarified by their chemical shifts and the DEPT spectra, consisting of six methyls, six methylenes, eight methines (including two olefinic carbons at $\delta_C$ 125.9 and 131.2), three sp$^3$ quaternary carbons, two sp$^2$ quaternary carbons at $\delta_C$ 132.1 and 131.5, a carbonyl ketone at $\delta_C$ 196.2 and a carbonyl amide at $\delta_C$ 168.0. These functionalities accounted as four out of the total nine indices of hydrogen deficiency. The remaining five indices of hydrogen deficiency were consistent to the pregnane-type steroidal skeleton with a tigloyl amino group, $N$-methylaminoethane substituent and additional an epoxide ring (Kaulanai et al., 2002; Farabi et al., 2018; Wu et al., 2019). To clarify the position of functional group in 1, 2D NMR experiments were carried out and the results as shown in Figure 2. In the $^1$H-$^1$H COSY spectrum of 1 displayed correlations in C$_1$-C$_2$, C$_5$-C$_6$-C$_7$-C$_8$-C$_9$, C$_{11}$-C$_{12}$, C$_{14}$-C$_{15}$-C$_{16}$ and C$_{4’}$-C$_{3’}$ supporting the presence of pregnane-type steroidal structure with a tigloyl group and additional an epoxide ring. The HMBC correlations from tertiary methyl signals at $\delta_H$ 0.82 and 0.87 to C-10 ($\delta_C$ 38.0), C-1 ($\delta_C$ 38.9), C-5 ($\delta_C$ 54.2) and C-13 ($\delta_C$ 41.6), C-12 ($\delta_C$ 32.8), C-14 ($\delta_C$ 45.2) was enabled to assign two tertiary methyls were attached at C-10 and C-13, respectively. An olefinic signal at $\delta_H$ 7.55 was correlated to C-1 ($\delta_C$ 38.9), C-3 ($\delta_C$ 132.1) and C-4 ($\delta_C$ 196.2), whereas H-5 ($\delta_H$ 2.28) was correlated to C-4 ($\delta_C$ 196.2), suggest the position of $\alpha,\beta$-unsaturated ketone was located at C-2, C-3 and C-4. Another olefinic signal at $\delta_H$ 6.34 was correlated to Me-4’ ($\delta_C$ 12.20), Me-5’ ($\delta_C$ 13.9) and carbonyl amide ($\delta_C$ 168.0), suggested a tigloyl amino group was attached at C-3. An oxygenated proton at $\delta_H$ 3.10 was correlated to C-17 ($\delta_C$ 72.9) and C-15 ($\delta_C$ 27.0), whereas methine proton at $\delta_H$ 1.36 was correlated to C-16 ($\delta_C$ 59.4), indicated that epoxy ring was located at C-16 and C-17. A methine proton at $\delta_H$ 2.51 was mutually coupled to methyl at $\delta_H$ 1.12 and were correlated to oxygenated carbon at C-17 ($\delta_C$ 72.9), indicated $N$-methylaminoethane substituent was attached at C-17. The relative stereochemistry of 1 was determined on the basis of coupling constant in the $^1$H-NMR spectrum and a biogenetic point of view the occurrence of pregnane-type steroidal structure in natural (Rahman et al., 2002; Farabi et al., 2018). These
observations along with the similarity of spectral data and physicochemical properties between 1 and previously reported (20S)-20-(N-methylamino)-3β-(tigloyl-amino)-5α-pregnan-16α,17α-epoxy-4-one, isolated from the leaves Sarcococca coriacea (Kaulani et al., 2002; Wu et al., 2019), let us identify 1 as (20S)-20-(N-methylamino)-3β-(tigloyl-amino)-5α-pregnan-16α,17α-epoxy-4-one, which isolated from marine sponge Xestospongia sp for the first time.

Compound 2 was isolated as white crystal; m.p 113-115 °C; [α]$_D^{20}$ +20.5° ($c = 0.2$, CHCl$_3$). Its molecular formula was determined as C$_{32}$H$_{50}$N$_2$O$_6$ (nine indices of hydrogen deficiency) by HRTOFMS $m/z$ 559.3670 [M+H]$^+$ and NMR spectral data (Table 1). The UV spectrum showed an absorption maximum at 285 nm (log $\varepsilon$ 5.1) Shiono et al., 2016, indicating the presence of an enone system. Infrared (IR) absorption bands due to amine, conjugated carbonyl, amide and ether groups were observed at 3420, 1720, 1670 and 1146 cm$^{-1}$, respectively. NMR spectral of 2 was very similar to those of 1, except the absence of α,β-unsaturated ketone at C-2, C-3 and C-4 at [δ$^H$ 7.55 (1H, d, $J=6.9$, 2.6 Hz), δ$^C$ 125.9, 132.1 and 196.2] and appeared the oxygenated carbons at [δ$^H$ 4.53 (1H, d, $J=9.3$), δ$^C$ 71.7 and 4.52 (1H, dd, $J=8.7$, 2.4 Hz), δ$^C$ 74.1.)] and two acetyl groups [δ$^H$ 2.05 and 2.00 (each 3H, s), δ$^C$ 20.9, 21.3, δ$^C$ 170.1 and 170.4], suggesting that 2 was an acetyl derivative of 1. An oxygenated proton at δ$^H$ 4.53 was correlated to C-3 (δ$^C$ 50.0), C-1 (δ$^C$ 40.2) and carbonyl ester at C-1” (δ$^C$ 170.1), whereas another an oxygenated proton at δ$^H$ 4.52 was correlated to C-3 (δ$^C$ 50.0), C-5 (δ$^C$ 48.7) and carbonyl ester at C-1” (δ$^C$ 170.4), suggested two acetyl groups was located at C-2 and C-4, respectively.

These observations along with the similarity of spectral data and physicochemical properties between 1 and previously reported (20S)-20-(N-methylamino)-3β-(tigloyl-amino)-5α-pregnan-16α,17α-epoxy-2β,4β-di-O-acetate, isolated from the leaves Sarcococca coriacea (Kaulani et al., 2002; Wu et al., 2019), let us identify 2 as (20S)-20-(N-methylamino)-3β-(tigloyl-amino)-5α-pregnan-16α,17α-epoxy-2β,4β-di-O-acetate, which also isolated from marine sponge Xestospongia sp for the first time.

The antiplasmodial activity of the two isolated compounds 1 and 2 against P. falciparum was conducted according to the method described in previous paper (Fröhlich et al., 2016) and were used an artemisinin (5.20 × 10$^{-6}$ μM) as a positive control (Ravikumar et al., 2012; Fröhlich et al., 2016). Compounds 1 and 2 showed strong antiplasmodial activity with IC$_{50}$ values of 0.013 and 0.158 μM, respectively, suggesting the presence of acetyl groups can decrease antiplasmodial activity, in contras with the presence of an enone group can increase antiplasmodial activity. Both compounds, (20S)-20-(N-methylamino)-3β-(tigloyl-amino)-5α-pregnan-16α,17α-epoxy-4-one (1) and (20S)-20-(N-methylamino)-3β-(tigloyl-amino)-5α-pregnan-16α,17α-epoxy-2β,4β-di-O-acetate (2) have been isolated as a potent cholinesterase inhibiting compounds from
Sarcococca coriacea (Kaulani et al., 2002), but the antiplasmodial activity was shown in this study for the first time.

Table 1. NMR Data (600 MHz for $^1$H and 150 MHz for $^{13}$C, in CDCl$_3$) for 1 and 2.

| Position | $^{13}$C NMR $\delta_c$ (mult) | $^1$H NMR $\delta_H$ (Integral, mult, J=Hz) | $^{13}$C NMR $\delta_c$ (mult) | $^1$H NMR $\delta_H$ (Integral, mult, J=Hz) |
|----------|-------------------------------|---------------------------------|-------------------------------|---------------------------------|
| 1        | 38.9 (t)                      | 2.00 (1H, d, 6.9)               | 40.2 (t)                      | 1.73 (1H, m)                   |
|          |                               | 1.75 (1H, d, 2.6)               |                               | 1.48 (1H, d, 9.3)              |
| 2        | 125.9 (t)                     | 7.55 (1H, dd, 6.9, 2.6)        | 71.7 (d)                      | 4.53 (1H, d, 9.3)              |
| 3        | 132.1 (s)                     | -                              | 50.0 (d)                      | 4.72 (1H, d, 8.7)              |
| 4        | 196.2 (s)                     | -                              | 74.1 (d)                      | 4.52 (1H, dd, 8.7, 2.4)        |
| 5        | 54.8 (d)                      | 2.28 (1H, m)                   | 48.7 (d)                      | 2.00 (1H, m)                   |
| 6        | 20.4 (t)                      | 1.64 (1H, m)                   | 20.4 (t)                      | 1.64 (1H, m)                   |
|          |                               | 1.39 (1H, m)                   |                               | 1.39 (1H, m)                   |
| 7        | 20.5 (t)                      | 1.50 (1H, m)                   | 25.0 (t)                      | 1.52 (1H, m)                   |
|          |                               | 1.27 (1H, m)                   |                               | 1.27 (1H, m)                   |
| 8        | 33.1 (d)                      | 1.41 (1H, m)                   | 33.2 (d)                      | 1.41 (1H, m)                   |
| 9        | 54.9 (d)                      | 1.40 (1H, m)                   | 65.0 (d)                      | 1.40 (1H, m)                   |
| 10       | 38.0 (s)                      | -                              | 35.0 (s)                      | -                              |
| 11       | 30.2 (t)                      | 1.52 (1H, m)                   | 31.4 (t)                      | 1.52 (1H, m)                   |
| 12       | 32.8 (t)                      | 1.56 (1H, m)                   | 32.9 (t)                      | 1.56 (1H, m)                   |
|          |                               | 1.30 (1H, m)                   |                               | 1.38 (1H, m)                   |
| 13       | 41.6 (s)                      | -                              | 41.6 (s)                      | -                              |
| 14       | 45.2 (d)                      | 1.36 (1H, m)                   | 45.7 (d)                      | 1.40 (1H, m)                   |
| 15       | 27.0 (t)                      | 1.65 (1H, m)                   | 27.0 (t)                      | 1.65 (1H, m)                   |
|          |                               | 1.55 (1H, m)                   |                               | 1.55 (1H, m)                   |
| 16       | 59.4 (t)                      | 2.51 (1H, dd, 6.7, 2.4)        | 59.4 (d)                      | 2.51 (1H, m)                   |
| 17       | 72.9 (s)                      | -                              | 72.9 (s)                      | -                              |
| 18       | 15.9 (q)                      | 0.82 (3H, s)                   | 16.0 (q)                      | 0.82 (3H, s)                   |
| 19       | 14.1 (q)                      | 0.87 (3H, s)                   | 15.3 (q)                      | 1.12 (3H, s)                   |
| 20       | 51.0 (d)                      | 3.10 (1H, q, 6.5)              | 51.0 (d)                      | 3.10 (1H, q, 6.5)              |
| 21       | 17.8 (q)                      | 1.12 (1H, d, 6.5)              | 17.9 (q)                      | 1.13 (3H, d, 6.5)              |
| N-Me     | 34.6 (q)                      | 3.26 (3H, s)                   | 34.6 (q)                      | 3.22 (3H, s)                   |
| N$_1$-H  | -                             | 8.03 (1H, br.s)                | -                             | 5.95 (1H, d, 8.7)              |
| N$_3$-H  | -                             | 2.10 (1H, br.s)                | -                             | 2.20 (1H, br.s)                |
| 1′       | 168.0 (s)                     | -                              | 168.0 (s)                     | -                              |
| 2′       | 131.5 (s)                     | -                              | 131.5 (s)                     | -                              |
| 3′       | 131.2 (d)                     | 6.34 (1H, dd, 6.9, 1.2)        | 131.2 (d)                     | 6.34 (1H, q, 6.8)              |
| 4′       | 12.2 (q)                      | 2.40 (3H, d, 6.9)              | 12.2 (q)                      | 2.20 (3H, d, 6.8)              |
| 5′       | 13.9 (q)                      | 2.53 (3H, d, 1.2)              | 13.9 (q)                      | 2.53 (3H, s)                   |
| 1″       | -                             | 170.1 (s)                      | -                             | -                              |
| 2″       | -                             | 20.9 (q)                       | 20.9 (q)                      | 2.05 (3H, s)                   |
| 1‴       | -                             | 170.4 (s)                      | -                             | -                              |
| 2‴       | -                             | 21.3 (q)                       | 2.00 (3H, s)                  |                                |

CONCLUSIONS

Two antiplasmodial steroidal alkaloids have been isolated from marine sponge, Xestospongia sp and identified by spectroscopic methods as epoxysarcovagenine-D (1) and epoxypepakistamine-A (2). Compounds 1 showed stronger antiplasmodial activity than compound 2 with an IC$_{50}$ value of 0.013 μM, suggesting the presence of acetyl groups can decrease antiplasmodial
activity, whereas the presence an enone group can increase antiplasmodial activity.

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