Antibacterial *Porphyromonas gingivalis* ATCC 33277 Terpenoid from Sarang Semut (*Myrmecodia pendants*)

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A new steroid glycoside (1), along with one known triterpenoid (2) and a new sesquiterpenoid (3) has been isolated from the ethyl acetate extract of Sarang Semut (*Myrmecodia pendants*). The structures of the new and known compounds were established on the basis of extensive 1D and 2D NMR spectral data. The bioactivity evaluation was conducted using the inhibition zone of compounds (mm) using Kirby-Bauer method at concentrations of 2000 and 5000 ppm for each against pathogenic oral bacteria *Porphyromonas gingivalis* were 11.1 and 12.3 mm, respectively.

**Keywords:** Sarang Semut, *Myrmecodia pendants*; *Porphyromonas gingivalis*; antibacterial activity; terpenoid

Sarang Semut (*Myrmecodia pendants*) is widely used in West Papua as herb with broad range of therapeutic values [1]. This plant is a member of Rutaceae family with five genus, however, only two of which have association with ants. They are *Myrmecodia* (forty five species) and *Hypnophytum* (twenty six species). From those species, only *Hypnophytum formicarum*, *Myrmecodia pendants* and *Myrmecodia tuberosa* are considered to have medicinal values [2].

Research data from previous analysis result of crude extract of Sarang Semut showed that the extract has antioxidant activity [3-4]. Ethyl acetate fraction of *M. pendants* (50 µg mL\(^{-1}\)) showed the highest activity in lymphocytes proliferation thus sarang semut tubers are potential to be development as immunomodulatory agents [1]. Previous study by Soekmantno et al. (2010) have proved that the extract of *M. pendants* has anticancer activity in both human cervix (HeLa) and canine mammary tumor (MCM) cell lines with IC\(_{50}\) 27.61 ppm (HeLa) and 54.57 ppm (MCM-B2), respectively [2]. This effect may be the result of phenolic compounds especially flavonoids contained in extract [2-5]. Terpenoid have been isolated from Sarang Semut of Papua had capability to inhibit the growth of ovarian cancer cell lines (SKOV-3) with IC\(_{50}\) of 481±5 µM for 48 hours [6].

However, the antibacterial activity of the Sarang Semut and its active components against oral periodontal pathogens has not been evaluated. Thus, this study mainly aimed to isolate and elucidate antibacterial agents from the Sarang Semut against periodontal oral pathogen. Our chemical investigation isolated one new steroid glycoside (1) and one known triterpenoid (2) and new sesquiterpenoid (3) from the Sarang Semut. Their structures were elucidated as 6’-O-tridecanoyl-3-O-β-D-glucosyl-sitosterol (1), betulin (2) and phoroglucinol coupled sesquiterpene (3) (Fig. 1) by spectroscopic data analyses (IR, ES-MS, 1D-NMR, and 2D-NMR).

This report describes their isolation, structural elucidation, and antibacterial activity against *Porphyromonas gingivalis* ATCC 33277.

The ethyl acetate of Sarang Semut was subjected to multiple chromatographic steps, using silica gel G60 and ODS RP-18 to afford terpenoid (1-3) (Figure 1).

Compound 1 was isolated as white amorphous powder. TLC analysis on Kiesel gel 60 F\(_{254}\) 0.25 mm plate (Merck) in n-hexane-ethyl acetate = 40:60 (v/v), RF = 0.25; ES-MS m/z 773.340 (M+H\(^+\)) calcd. for C\(_{46}\)H\(_{63}\)O\(_{12}\), together with seven degrees of unsaturation. The IR spectrum showed hydroxyl groups at 3404 cm\(^{-1}\), a carbonyl ester (1722 cm\(^{-1}\)) and hemimethyl (1465, 1379 cm\(^{-1}\)). The \(^1\)H- and \(^13\)C-NMR spectral data are shown in Table 1. The \(^13\)C-NMR spectrum showed forty eight carbon signals which, according to the DEPT 135° spectrum, represented seven primary, twenty three secondary, fourteen tertiary, and four quartenary carbons. In the \(^13\)C-NMR spectrum showed twenty nine resonances attributed to a sitosterol skeleton, including double bond resonances at δ 140.4 and 122.4, and a hydroxymethylene signal at δ 79.7. Six carbon signals [δ 101.4, 76.1, 74.1, 73.7, 70.2, 63.3] were due to d-glycose, and others [δ 175.0, 34.4, 32.1, 29.9-29.4, 25.1, 22.9, 14.3] attributed to fatty acid esters. The \(^1\)H-NMR spectrum show signals for seven tertiary methyl groups at δ 0.67 (s, 3H), 0.80 (d, 3H, J = 6.5), 0.81 (d, 3H, J = 6.5), 0.84 (t, 3H, J = 1.3), 0.88 (m, 3H), 0.91 (d, 3H, J = 6.5), 1.00 (s, 3H), and an anomic proton signal of d-glycose at δ 4.37 (d, 1H, J = 7.8), which indicated the β-linkage of d-glycose with sitosterol [7.8]. The \(^1\)H-NMR spectrum also exhibited the presence of a long chain fatty acid ester, according to signals at δ 1.47 (m, 2H) and a secondary methyl group at δ 1.25 (m, 2H).

All these data suggested that compound 1 was a steroid glycoside with a long-chain fatty acid ester (Fig. 1). The complete structural determination was further achieved by analyses of HMQC and HMBC data. In the HMBC spectrum, the framework of revealed that the structures of the two compounds are closely related, the main differences are the position of fatty acid ester and number of fatty acid unit. In order to clarify the position of fatty acid ester unit, the HMBC experiment was carried out, proton at δ\(_H\) 4.9 (2H, dd, 4.55, 4.55) was correlated to C-1’ (δ\(_C\) 175.0) confirmed the ester group attaching to C-6’ of d-glucose (Fig. 1).
The ester group attaching on structure of compound same with (7S*,165*,185*,19R*)-7,18-dihydroxy-19-0-(4-methyl-6-E), 8(E)-hexadecadienoyl)16,18-dimethyl-10-phenyl-[11]-cytochalasia-6(12),13(1E)-diene-1,2-dione [10]. Based on HMOC experiment was carried out, number of carbons at fatty acid ester compound 1 was odd-numbered, carbons at δC 175.0, 34.4, 32.1, 29.9-29.4, 25.1, 22.9, 14.3 suggested as tridecanoyl ester. This fatty acid ester was new fatty acid for steroid glycoside, previously fatty acid ester usual range of straight-chain even-numbered [9,11]. Therefore, the structure of compound 1 was established as 6\(\beta\)-O-tridecanoyl-3-\(\alpha\)-β-neoglucosyl-sitosterol (1) (Fig. 1). This is a new sterol glucoside reported for the first time from this plant.

Compound 2 (Betulin) was isolated as white amorphous powder. TLC analysis on Kiesel gel 60 F254, 0.25 mm plate (Merck) in n-hexane-acetone = 80:20 (v/v), Rf 0.37; IR (KBr) \(\nu_{max}\) cm\(^{-1}\): 3415, 2973, 1628, 1451, 1381 and 1161; ES-MS m/z 447.700 [M+H]\(^+\) calculated for C\(_{27}\)H\(_{40}\)O\(_6\). The IR spectrum of 3 indicated the presence of carboxyl at 1628 cm\(^{-1}\). From an inspection of 1D-NMR data and the HMOC spectrum, 3 was found to possess a 2-methylenyl side chain (δ\_\(H\) 4.5 (1H, m), 1.12 (6H, m); δ\_C 166.5, 77.3, 29.4, 28.9), a phosphoroglucon unit (δ\_\(H\) 5.8 (1H, s); δ\_C 163.5, 163.4, 162.1, 109.0, 101.64, 95.0), one methylene (δ\_\(H\) 2.62 (1H, m), 2.56 (1H, m); δ\_C 28.0), three tertiary methyl [δ\_\(H\) 1.12 (3H, s), 1.37 (3H, J = 6.5), 1.70 (3H, s); δ\_C 9.86, 16.8, 18.5], a terminal double bond [δ\_\(H\) 4.50 (1H, m), 4.60 (1H, m); δ\_C 111.8, 149.5] as well as an oxygenated carbon at δC 71.5. The data suggested a phloroglucinol-coupled sesquiterpenoid for compound 3. The above accounted for seven out of eight double bond equivalents, which indicated the presence of one ring in compound 3. In the HMBC spectrum presence correlation of H-6’ (δ\_\(H\) 5.8) with C-2’ (δ\_C 101.6), C-4’ (δ\_C 109.3) and C-5’ (δ\_C 162.1) as well as H-10 (δ\_\(H\) 2.5) with C-3’ (δ\_C 163.4), C-4’ (δ\_C 109.3) and C-5’ (δ\_C 162.1) located methyn at ring aromatic and the C-10 methylene at positions 6’ and 4’, respectively, and also allowed the assignment of three hydroxyls at C-1’, C-3’ and C-5’. Consequently, the 2-methylenyl substituent could only be placed at C-2’. Thus, a grandiol was established based on this analysis data above, which was also supported by comparison with the NMR data of grandinol [15]. The connections of the two structural fragments, quartenary carbons, and the other functional groups were mainly achieved by the HMBC spectrum. The correlations of H-14 with C-1, C-2 and C-6 suggested attachment of Me-14 to C-1. An isopropenyl group was attached to C-4 by the HMBC correlations of H-12 with C-4, C-11 and C-13 and H-13 with C-4, C-11 and C-12. Based on analysis of NMR data as well as by comparison with previously reported papers, the structure of compound 3 was therefore elucidated as phloroglucinol sesquiterpenoid (3). This is a new sesquiterpenoid reported for the first time from this plant.

For evaluate the bioactivity of the compounds, the antibacterial activities against P. gingivalis ATCC 33277 of all the isolates (1-3)
from the tuber of *M. pendans* were conducted using disk diffusion method. The Kirby-Bauer disk diffusion susceptibility test was used to determine the sensitivity or resistance of *P. gingivalis* to compounds. Chlorhexidine was used as a positive control. Susceptibility of isolates (1-3) against *P. gingivalis* can see inhibit zone of sample on growth bacteria. From the antibacterial results as showed in Table 1, the compound 3 was active against *P. gingivalis*, but the compounds 1 and 2 were inactive. When considering of the inhibition zone values at the micromolar level, it is possible to observe that chlorhexidin is about potent than compound 3. Nevertheless, several adverse effects are associated with regular use of chlorhexidin. The reinforces the great importance of compound 3 as a prototype or lead compound for the development of novel and safe bioactive compounds for control of periodontitis.

**Table 1:** Antibacterial activity of compound 1-3 against *P. gingivalis* ATCC 33277

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Inhibition Zone of compounds (mm) at Concentrations (μg/mL)</th>
<th>5000</th>
<th>2000</th>
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<td>3</td>
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<tr>
<td>Chlorhexidin*</td>
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*standard*  
**not yet**

**Experimental**

**General:** NMR spectra recorded on a 500 MHz FT-NMR spectrometer (Varian ECA 500 JOEL, Japan) (500 (1H) and 125 (13C)); δ in ppm rel. to TMS as internal standard, 1 Hz in IR spectra were obtained from a Perkin Elmer Spectrum One FT-IR spectrometer (Buckinghamshire, England). ES-MS spectrometer (UPLC MS/MS TQD type, Waters); in m/z. Column chromatography (CC): silica gel (SiO2, 200-300 mesh; Merck, Darmstadt, Germany) and ODS was a LiChroprep RP-18 (Merck). TLC: Kiesel gel 60 F254 and RP-18 F254S (Merck). For antibacterial assay, laminar air flow, incubator Memmert, autoclave machine HVE-50 Hirayama, jar and ELISA reader Diagnostic Automation Inc.

**Plant material:** Dried of Sarang Semut *Myrmecodia pendans* was collected from from Papua island, Indonesia and identified by Mr Joko Kusmoro (Padjadjaran University), Laboratory of Plants Taxonomi, Department of Biology, Faculty of Mathematic and Natural Science, Padjadjaran University, Sumedang, Indonesia.

**Extraction and isolation:** The air-dried tuber of Sarang Semut (1.5 kg) plants was extracted with 100% ethyl acetate (3x3 L) at 40°C on heating mantle of Soxhlet extractor. This method was chosen to yield thermostable compounds as similar with empirical experiences of local people who use it after boiling process. The extract was evaporated to yield a residue (20 g). Ethyl acetate extract was subjected to column chromatography on stationary phase silica gel 60 eluting with 10% gradient of n-hexane-ethyl acetate, to yield 11 fractions (A-K). Fraction H (0.45 g) was subjected to column chromatography on stationary phase silica gel 60 eluting with 5% gradient of n-hexane-ethyl acetate, to yield 21 fractions (H01-H21). Fraction H08 (71 mg) was subjected to column chromatography on stationary phase silica gel 60 eluting with 5% gradient of n-hexane-ethyl acetate, to yield compound 1 (23 mg). Fraction H15 (71 mg) was subjected to an RP-C18 column, eluting with isocratic solvent of methanol-water (90:10 v/v) to yield 2 (20 mg). Fraction F (2.10 g) was subjected to column chromatography on stationary phase silica gel 60 eluting with 2.5% gradient of n-hexane-ethyl acetate, to yield 20 fractions (F01-F20). Fraction F09 (196.2 mg) was subjected to an RP-C18 column, eluting with 2.5% gradient of methanol-water to yield compound 3 (5 mg).

**6-O-tridecanoyl-3-O-β-d-glucosyl-sitosterol**

White amorphous powder.

**References**


