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Phillipsins A and B from *Zingiber phillippsii* Mood & Theilade in Borneo

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Abstract: Two new aromatic compounds, phillipsins A (**1**) and B (**2**) were isolated from the Bornean wild ginger *Zingiber phillippsii* Mood & Theilade, and were characterized based on spectroscopic data (HRESI-MS, FTIR as well as 1D and 2D NMR). These metabolites also exhibited strong antifungal activity against selected fungi.

Keywords: *Zingiber phillipsii*; ginger; antifungal; chemotaxonomical marker; Borneo.

1. Introduction

The native species of ginger *Zingiber phillippsii* belongs to the family Zingiberaceae and is distributed throughout Southeast Asian countries, and it is traditionally believed to contain high medicinal value [1]. This species is widely used in traditional herbal medicine by the local Dusun community in Sabah, Malaysia [2,3]. It is a known that ginger has been used by indigenous people in Borneo for various treatment including respiratory and gastrointestinal diseases. In addition, local traditional knowledge also attributes ginger to have antiemetic, anti-flatulence, anti-diarrhea, cardiotonic and expectorant as well as to cure swollen body and snake bite [4,5]. Generally, ginger has been reported to contain diverse bioactive secondary metabolites from terpenoids to aromatic compounds [6-11]. The secondary metabolites isolated from ginger have been reported to exhibit antifungal potentials [10,11]. Therefore, we decided to test the isolated compounds against marine Oomycetes (lower fungi) that infects and causes disease outbreak in mud crab juveniles, *Scylla tranquebarica*, that have severely affected the mud crab production in Sabah, Malaysia [12]. The

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possible and potent causative agents for this issue were the lower fungi of genera *Lagenidium* and *Haliphthoros* [13]. In addition, there are no scientific reports for the chemical constituents of *Z. phillippsii*. Therefore, as part of our continuous effort to search for novel bioactive compounds from natural sources [14], we have isolated and elucidated chemical structures of two new compounds, phillipsins A (**1**) and B (**2**) from *Z. phillippsii*. Herein, we describe the isolation, structural determination and antifungal potential of these new metabolites.

2. Materials and Methods

2.1. General Experimental Procedures

Polarimeter AUTOPOL IV (Rudolph Research Analytical) was used to measure the value of optical rotations. FTIR spectrometer (Thermo Nicolet Nexus) was used to obtain IR data. ECA 600 NMR spectrometer (JEOL) was used to acquire spectra in $CDCl_3$ with TMS as the internal standard. LCMS-IT-TOF (Shimadzu) was used to obtain high resolution mass spectra. Column chromatography as well as preparative and analytical TLC were performed utilizing normal phase silica gel (Merck, Kieselgel 60, 70-230 mesh), normal phase silica gel precoated glass plate (Merck Kieselgel 60 F_{254}) and silica gel precoated aluminum sheet (Merck, Kieselgel 60 F_{254}), respectively.

2.2 Biological Materials

Specimens of *Z. phillippsii* were collected from Tudan Village, Sabah, Malaysia (05º86.730ˈN, 116º32.591ˈE) on September 2015 and identified by the second author. The voucher specimen (BORH1050JK) was stored in the BORNEENSIS Collection of Institute for Tropical Biology and Conservation, University of Malaysia Sabah.

2.3 Extraction and Isolation

Partially dried ginger rhizome (200 g) was rinsed in three changes of distilled water, air dried, chopped and soaked in 70% aqueous ethanol for 5 days. The resulting ethanol extract was filtered, concentrated, and liquid-liquid extraction between ethyl acetate (EtOAc)/H_2O. The organic layer was washed by double H_2O and moisture was removed under anhydrous Na_2SO_4. After evaporation step, a dark green oil (687.0 mg) was collected. This crude extract was separated by conventional column chromatography using gradient solvent system utilizing combination of hexane and EtOAc to produce five fractions, while total of 77.0 mg of fraction 2 eluted by hexane-EtOAc (8:2) and further purified *via* preparative TLC with toluene to acquire **1** (14.4 mg) and **2** (10.8 mg).

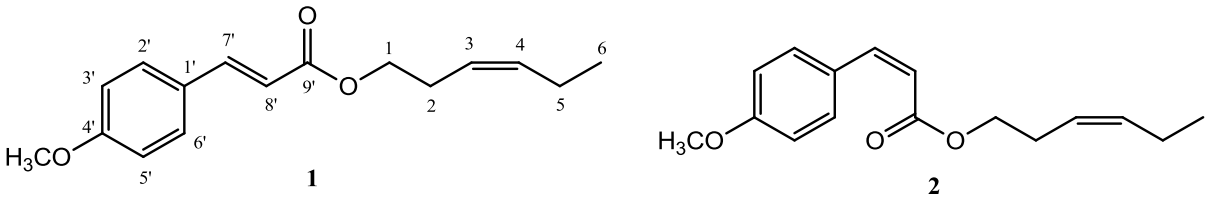

Figure 1. Structures of compounds **1** and **2**.

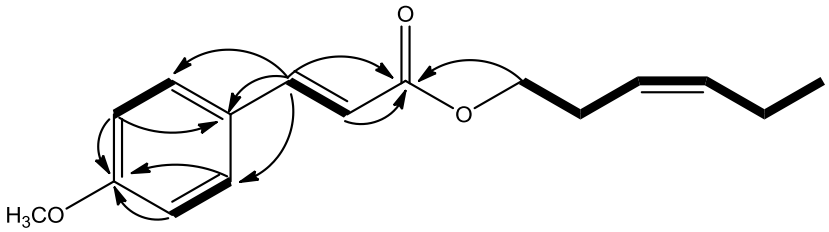

Figure 2. ^{1}H-^{1}H COSY and selected HMBC correlation of compound **1**.

Phillipsin A (1): colorless oil; $[\alpha]_D^{25}$: +5.3 (c 0.30, $CHCl_3$); IR (neat) ν_{max} 1706 and 1603 cm^{-1}; ^{1}H and ^{13}C NMR data, Table1; HRESI-MS *m/z* 261.1478 $[M + H]^+$ (calculated for $C_{16}H_{21}O_3$, 261.1485) and 283.1282 $[M + Na]^+$ (calcd for $C_{16}H_{20}O_3Na$, 283.1305).

Phillipsin B (2): colorless oil; $[\alpha]_D^{25}$: +13.3 (c 0.15, $CHCl_3$); IR (neat) ν_{max} 1699 and 1612cm^{-1}; ^{1}H and ^{13}C NMR data, Table1; HRESI-MS *m/z* 261.1486 $[M + H]^+$ (calculated for $C_{16}H_{21}O_3$, 261.1485) and 283.1284 $[M + Na]^+$ (calcd for $C_{16}H_{20}O_3Na$, 283.1305).

2.4 Biological Assay

Antifungal bioassay was conducted based on the slight modification from standard method reported by Munchan et al. [15]. The minimum inhibitory concentration (MIC) of fungistatic effect on hyphae were carried out by incorporating the compound solutions (100, 50, 25, 12.5 μg/mL) onto PYGS agar in petri dish followed by agar block inoculation of fungi strains *Fusarium moniliforme* NJM 8995, *Fusarium oxysporum* NJM 0179, *Fusarium solani* NJM 8996, *Haliphthoros milfordensis* IPMB 1603, *Haliphthoros sabahensis* IPMB 1402 and *Lagenidium thermophilum* IPMB 1401 *via* advance edge cut with No. 2 cork borer. The MIC was determined visually as the lowest concentration showing no hyphal growth when they were incubated at 25 ^{o}C for 3 and 7 days. The control was sterilized seawater solution without compounds.

3. Results and Discussion

3.1. Structure Elucidation

Partially dried specimens of *Z. phillippsii*, were extracted in ethanol, after chromatographic techniques, phillipsins A (**1**) (2.1 % on crude extract weight) and B (**2**) (1.6 %) were afforded from crude extract as shown in Figure 1. Compound **1** was isolated as colourless oil. The molecular formula of **1** was elucidated as $C_{16}H_{20}O_3$ with seven degrees of unsaturation as deduced from HRESI-MS, *m/z* 261.1478 $[M + H]^+$ (calcd for $C_{16}H_{21}O_3$, 261.1485) and 283.1282 $[M + Na]^+$ (calcd for $C_{16}H_{20}O_3Na$, 283.1305). The IR spectrum displayed absorption bands indicating carbonyl (1706 cm^{-1}) and aromatic (1603 cm^{-1}) functionalities. The ^{1}H and ^{13}C NMR spectra (Table 1) showed signals of methoxyl protons at δ3.84 (3H, s, 4` -OMe) and two pairs of aromatic protons at $\delta$7.47 (2H, d, $J$ = 8.9 Hz, H-3` and 5` ) and 6.90 (2H, d, $J$ = 8.9 Hz, H-2` and 6` ). In addition, these two pairs of benzylic protons were one bond correlated to two pairs of overlapped carbon signals at $\delta$130.4 and 115.0 suggested the presence of a benzene system with para substitution. While the remaining carbon signals were carbonyl ($\delta$168.1, C-9`), four olefinic methines, three methylenes (including a hydroxymethylene carbon (δ64.6, C-1)) and an aliphatic methyl group as attributed by HSQC and DEPT-135. Construction of planar structure of **1** based on ^{1}H-^{1}H COSY correlations and key HMBC

correlations are shown in Figure **2**. The cross peaks of ^{1}H-^{1}H COSY revealed the connectivity for the four partial structures H-2'/H-3', H-5'/H-6', H-7'/H-8' and H_2-1/H_2-2/H-3/H-4/H_2-5/H_3-6. The HMBC cross peak between 4'-OMe and C-4' were important key to confirm 1,4-disubstituted phenyl moiety. The two partial structures of C-7' to C-8' and C-1 to C-6 were connected through the ester linkage from HMBC interactions of H-7', H-8' and H_2-1 to C-9'. The downfield shift of H_2-1 resonance at δ4.19 suggested directly bonded to an oxygenated atom. Whereby, the side chain hex-3-en-1-yl propanoate unit was attached to C-1' based on HMBC correlations of H-7' to C-1', C-2' and C-6'; and H-2' and H-6' to C-7'.

The relative stereochemistry was determined by NOESY experiments and coupling constant values. The large coupling constant values between H-7'/H-8' was 15.8 Hz, suggested *E*-configuration double bonds at C-7'/C-8'. The lack of NOE correlations between H-7'/H-8' further supported this configuration. While, olefin at C-3/C-4 was determined as *Z*-configuration based on coupling constant $^{3}J_{3\text{-}4}$ = 10.7 Hz. Thus, the structure of **1** was identified as (*E*)-(*Z*)-3-(4-methoxyphenyl) hex-3-en-1-yl acrylate.

Table 1. ^{1}H and ^{13}C NMR data for **1** and **2** (600 and 150 MHz in $CDCl_3$, δ in ppm, *J* in Hz).

	δ_C		δ_H (mult., *J* in Hz)	
Position	**1**	**2**	**1**	**2**
1	64.6	64.4	4.19 t (6.9)	4.12 t (6.9)
2	27.6	27.4	2.46 q (6.9)	2.39 q (6.9)
3	124.5	124.5	5.38 td (6.9, 10.7)	5.31 td (6.9, 10.3)
4	135.3	135.2	5.52 td (6.9, 10.7)	5.50 td (6.9, 10.3)
5	21.3	21.3	2.09 quintet (6.9)	2.05 quintet (6.9)
6	15.0	14.9	0.98 t (6.9)	0.96 t (6.9)
1'	127.9	128.1		
2'	130.4	132.8	7.47 d (8.9)	7.68 d (8.9)
3'	115.0	114.1	6.90 d (8.9)	6.87 d (8.9)
4'	162.0	161.1		
5'	115.0	114.1	6.90 d (8.9)	6.87 d (8.9)
6'	130.4	132.8	7.47 d (8.9)	7.68 d (8.9)
7'	145.1	144.0	7.63 d (15.8)	6.84 d (12.4)
8'	116.3	117.8	6.30 d (15.8)	5.82 d (12.4)
9'	168.1	167.1		
4'-OMe	56.1	56.0	3.83 s	3.83 s

Compound **2** was obtained as a colorless oil. The molecular formula of $C_{16}H_{20}O_3$ was obtained on the basis of high resolution mass spectrum with $[M + H]^+$ ion at *m/z* 261.1486 (calcd for $C_{16}H_{21}O_3$, 261.1485) and $[M + Na]^+$ psedomolecular ion at *m/z* 283.1284 (calcd for $C_{16}H_{20}O_3Na$, 283.1305). Similar significant fragment ions in the mass spectrum, comparable absorption bands in the IR spectra and similar chemical shifts in the ^{1}H and ^{13}C NMR spectra (Table 1) indicated **1** and **2** to be stereoisomers. Comparison of 1D NMR data (Table 1) of **2** with those of **1**, revealed the double bond at C-7'/C-8' had *Z*-configuration instead of *E*-configuration was due to smaller coupling constant $^{3}J_{7'\text{-}8'}$ = 12.4 Hz. Furthermore, H-7' showed NOE correlation to H-8', supported the *Z*-configuration of double bond at C-7'/C-8'. Whereas, double bond at C-3/C-4 had *Z*-configuration was due to $^{3}J_{3\text{-}4}$ = 10.3 Hz. Hence, compound **2** was reported as (*Z*)-(*Z*)-3-(4-methoxyphenyl) hex-3-en-1-yl acrylate.

Compounds **1** and **2** were screened against six fungal strains from marine such as *F. moniliforme, F. oxysporum, F. solani, H. milfordensis, H. sabahensis* and *L. thermophilum* as shown in Table 2. The MIC indicated the fungistatic effect on hyphae by these compounds. Metabolites **1** and **2** were most active against *H. milfordensis* and *L. thermophilum* at concentrations of 25 µg/mL.

Table 2. MICs of compounds **1** and **2** against six strains of marine fungi.

	MIC (µg/mL)	
Strains	**1**	**2**
F. moniliforme	> 50	> 50
F. oxysporum	> 50	> 50
F. solani	> 50	> 50
H. milfordensis	25	25
H. sabahensis	50	50
L. thermophilum	25	25

Positive control: Itraconazole with MIC 3.2 µg/mL.

The genus *Zingiber* consists of 210 species worldwide but not all species have been chemically evaluated. Among the chemically studied species, various chemical structures have been determined including, diarylheptanoids, flavonoids, phenolics, phenylbutenoid and terpenoids. In our study, a total of two aromatic new compounds **1** and **2** were isolated as its major metabolites from *Z. phillippsii* that may be used for their chemical defense. The structures of isolated chemicals were closely related to the gingerol and shogaol analogues [16,17]. These compounds will be used as an important chemotaxonomical marker for the East Malaysian *Z. phillippsii*. Hence, the present study represents an important milestone in chemotaxonomical evaluation of the genus *Zingiber*.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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