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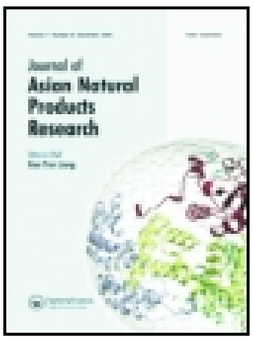

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Leucoxenols A and B, two new phenolics from Bornean medicinal plant *Syzygium leucoxylon*

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ABSTRACT

The medicinal plant, *Syzygium leucoxylon* or commonly known as Obah found in North Borneo was considered as traditional medicine by local committee. Two new phenolics, leucoxenols A (**1**) and B (**2**) were isolated and identified as major secondary metabolites from the leaves of *S. leucoxylon*. Their chemical structures were elucidated based on spectroscopic data such as NMR and HRESIMS. Furthermore, these compounds were active against selected strains of fungi.

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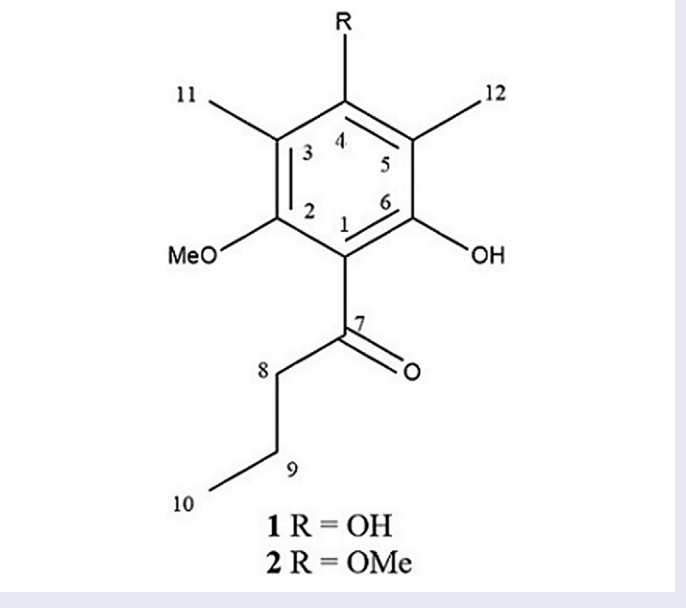

1 R = OH
2 R = OMe

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The supplemental data for this article can be accessed at https://10.1080/10286020.2018.1440391.

1. Introduction

Medicinal plants are important in pharmaceutical study due to their ability to synthesize diverse natural products that often exhibit potent biological activities [1,2]. One of the medicinal plants of the genus *Syzygium* that belong to the family Myrtaceae have been used as traditional medicine [3], and more than 1,200 species distributed in the tropical regions in all over the world [4,5]. *Syzygium leucoxylon* Korth is often used as traditional medicine to treat various ailments such as anti-flatulence, antiemetic, anti-diarrhea, expectorant, and cardiotonic effects as well as to cure swollen body and snake bite in local East Malaysian committee [3,6]. According to the survey conducted by Kulip, its fruits were used by the Dusun people to relieve stomach ache and headache. However, there is no in-depth investigation on the chemical constituents of this plant. In addition, its antifungal properties are not reported to date. Therefore, our recent investigation on a population of Bornean *S. leucoxylon* has led to isolation of two new phenolics, leucoxenols A (**1**) and B (**2**) as shown in Figure 1. Details of the isolation of these compounds, structural elucidation and their antifungal properties are described in this paper.

2. Results and discussion

Compound **1** was isolated as colorless oil, with $[\alpha]_D^{23}$ + 37.0 (*c* 0.2, $CHCl_3$). A molecular formula of $C_{13}H_{19}O_4$ and five degrees of unsaturation were determined for **1** based on HRESIMS $[M + H]^+$ ion at *m/z* 239.1276. The IR spectrum suggested the presence of a hydroxy and carbonyl groups (3407 and 1678 cm^{-1}). The ^{13}C NMR spectrum (Table 1) revealed the presence of 13 signals in the molecule whose multiplicities were determined by DEPT-135 and HSQC spectra to four methyls, two methylenes and seven quaternary carbons, including a carbonyl at δ_C 206.5 and six sp^2 quaternary carbons at δ_C 161.2, 159.0, 158.9, 108.8, 108.5 and 106.5. These distinct non-overlapping carbon signals have suggested a non-symmetrical benzene structure. While, the ^1H NMR spectrum (Table 1) revealed no olefinic proton signals were observed and indicated a 1,2,3,4,5,6-hexasubstituted aromatic ring.

The limited amount of information provided by ^1H–^1H COSY and HMBC data has made the structure determination become more difficult. Based on ^1H–^1H COSY spectrum, one spin system was determined from correlations of H_2-8/H_2-9/H_3-10 (Figure 2). Diagnostic correlations in HMBC spectrum (Figure 2) permitted construction of two partial structural units: a benzene system from correlations of H_3-11 to C-2, C-3 and C-4; and H_3-12 to C-4, C-5 and C-6, while another partial structure was a side chain which was determined from

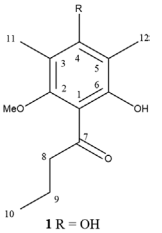

Figure 1. Structures of compounds **1** and **2**.

the cross peaks of H_3-10 to C-8 and C-9; and H_2-9 to C-7. In the benzene system, the C-2 position was substituted by methoxy moiety (δ_C 61.8; δ_H 3.70). This substitution was confirmed by the HMBC cross peak of 2-OMe to C-2. The aforementioned HMBC correlations have confirmed the position of tertiary methyls H_3-11 and H_3-12 at C-3 and C-5, respectively. While, the remaining two hydroxy moieties must be located at C-4 (δ_C 158.9) and C-6 (δ_C 161.2) based on the downfield of ^{13}C chemical shifts [7,8]. This assignment leaves an empty position at C-1 without substitution group, thus the side chain was attached to C-1. This conclusion was in good agreement with the HRESIMS measurement. Therefore, structure of **1** was established unambiguously.

Compound **2** was isolated as colorless oil, with $[\alpha]_D^{23}$ + 5.0 (*c* 0.2, $CHCl_3$). Its molecular formula was determined as $C_{14}H_{20}O_4$ based on HRESIMS $[M + H]^+$ ion at *m/z* 253.1402. The IR absorption bands at 3407 and 1677 cm^{-1} suggested the presence of hydroxy and carbonyl moieties in the molecule. Upon comparison of NMR data with those of **1**, revealed the structure of **2** was identical to those of **1** except the replacement of hydroxy group at C-4 in **1** by a methoxy group (δ_C 60.0; δ_H 3.73) in **2**. This finding was consistent with the HRESIMS data.

Crude extract showed hyphal and zoospore inhibition against *Lagenidium thermophilum* IPMB 1401 at MIC values of 160 µg/ml (Table 2). The result of toxicity on mud crab *Scylla tranquebarica* larva (zoea stage) showed no mortality occurred in mud crab larva for crude extract *S. leucoxylon* at 160 µg/ml and below for 1, 2 and 24 h (Table 3). Consequently, compounds **1** and **2** were screened against three strains of fungi *L. thermophilum* IPMB 1401, *Haliphthoros sabahensis* IPMB 1402 and *Haliphthoros milfordensis* IPMB 1603 as shown in Table 4. The *H. sabahensis* was described in 2017 [9], and this is the first report of natural products tested on this strain. The most active molecule was compound **1** with MIC 12.5 µg/ml against *L. thermophilum*. This may be due to the additional hydroxy group in **1**.

3. Experimental

3.1. General experimental procedures

An AUTOPOL IV automatic polarimeter (Rudolph Research Analytic, Hackettstown, USA) was used to measure the optical rotation at 25 °C. Infrared spectra were recorded on

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

		1		**2**
Position	δ_C	δ_H	δ_C	δ_H
1	108.8	–	111.6	–
2	159.0	–	158.8	–
3	108.5	–	115.2	–
4	158.9	–	163.3	–
5	106.5	–	115.6	–
6	161.2	–	160.9	–
7	206.5	–	207.3	–
8	44.6	3.04–3.06 m	44.9	3.07–3.09 m
9	18.3	1.70–1.71 m	18.1	1.71–.1.73 m
10	13.9	0.96 t (6.9)	13.9	0.97 t (6.9)
11	8.6	2.12 s	9.1	2.15 s
12	7.5	2.09 s	8.7	2.13 s
2-OMe	61.8	3.70 s	61.7	3.71 s
4-OMe			60.0	3.73 s

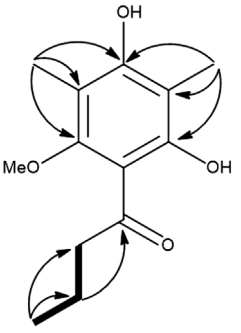

Figure 2. ^{1}H–^{1}H COSY and key HMBC correlations of **1**.

a Fourier transform infrared spectrophotometer (Thermo Nicolet, MA, USA). ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded on a JEOL ECA 600 FT-NMR (JEOL, Tokyo, Japan) using $CDCl_3$ with TMS as an internal standard. The high resolution mass spectrum was acquired via LCMS-IT-TOF (Shimadzu, Kyoto, Japan). Preparative TLC was performed with silica gel glass plates (Merck, Kieselgel 60 F_{254}), and column chromatography (CC) with silica gel (Merck, Kieselgel 60, 70–230 mesh, Bandar Sunway, Malaysia).

3.2. Biological material

A specimen of *Syzygium leucoxylon* was collected from Universiti Malaysia Sabah, in August 2015. The voucher specimen (BORH1059JK) was deposited in the BORNEENSIS Collection of the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. The scientific name of this plant was identified by the third author.

3.3. Extraction and isolation

The fresh leaves (1 kg wet wt) were chopped and extracted with EtOH at room temperature for 5 days. The resulting EtOH extract was concentrated and partitioned between EtOAc/H_2O. The EtOAc crude (600 mg) was subjected to column chromatography eluting with a gradient of hexane-EtOAc in an increasing polarity to obtain five fractions. The fraction 3

Table 2. Effects of leaf crude extract on hyphal growth and zoospore production of *L. thermophilum*.

	Exposure duration (h)		
Concentration (µg/ml)	**1**	**2**	**24**
0	+/+++	+/+++	+/+++
80	+/+++	+/++	+/++
160	+/+	+/–	–/–
320	+/+	+/–	–/–
640	+/+	+/–	–/–
1280	–/+	–/–	–/–
2560	–/–	–/–	–/–
5120	–/–	–/–	–/–
10,240	–/–	–/–	–/–

[a]Hyphal growth on PYGS agar. –: no growth, +: growth
[b]Number of zoospores released: + mean little amount, ++ mean fair amount and +++ mean excellent amount.

Table 3. The corrected cumulative mortality (%) of mud crab larva treated by the leaf crude extract.

	Exposure duration (h)		
Concentration (μg/ml)	**1**	**2**	**24**
0	0	0	0
80	0	0	0
160	0	0	0
320	0	0	25
640	0	0	40
1280	0	0	50
2560	65	80	100
5120	70	90	100
10,240	100	100	100

Table 4. The MIC (μg/ml) of 1 and 2 against three fungal strains.

	MIC (μg/ml)	
Strains	**1**	**2**
L. thermophilum	12.5	25
H. sabahensis	50	25
H. milfordensis	50	25

was applied on preparative TLC with hexane-EtOAc (85:15) as solvent system to isolate **1** (40.0 mg; 6.7%). While, compound **2** (18.8 mg; 3.1%) was purified from a portion of fraction 2 through preparative TLC with toluene. Yields were calculated as percentage of the crude.

3.3.1. Leucoxenol A (1)

Colorless oil; $[\alpha]_D^{23}$ + 37.0 (c 0.2, $CHCl_3$); IR (KBr) λ_{max} 3407 and 1678 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectral data: see Table 1; HRESIMS: *m/z* 239.1276 $[M + H]^+$ (calcd for $C_{13}H_{19}O_4$, 239.1278).

3.3.2. Leucoxenol B (2)

Colorless oil; $[\alpha]_D^{23}$ + 5.0 (c 0.2, $CHCl_3$); IR (KBr) λ_{max} 3407 and 1677 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectral data: see Table 1; HRESIMS: *m/z* 253.1402 $[M + H]^+$ (calcd for $C_{14}H_{21}O_4$, 253.1434).

3.4. Effects of leaf crude extract on hyphal growth and zoospore production

The leaf crude extract was diluted to different concentrations (80, 160, 320, 640, 1280, 2560, 5120 and 10,240 μg/ml) using sterilized seawater (SSW) in Petri dishes, respectively, while, SSW without extracts was served as the control. According to the methods in Lee et al. [10,11], agar blocks of the strain *L. thermophilum* were excised from the edge of the parent colony using No. 2 cork borer (5 mm in diameter) and subsequently immersed in each Petri dishes containing 30 ml of the test solutions for 1, 2 and 24 h. After each exposure time, two agar blocks were rinsed several times in SSW. Agar block was inoculated onto PYGS agar, the hyphal growth was observed after 48 h incubation at 25 °C. Another agar block was transferred to fresh SSW, the zoospore production was observed at 24 h with 25 °C incubation using an inverted microscope Olympus CKX 41.

3.5. Toxicity effects of leaf extract on larva mud crab (S. tranquebarica)

Healthy newly hatched mud crab larvae (zoea stage) from hatchery were rinsed with SSW containing each 500 µg/ml of streptomycin and ampicillin solution [12]. The negative control group is SSW. The experiments were carried out with the concentrations of crude extract at 80, 160, 320, 640, 1280, 2560, 5120 and 10,240 µg/ml. Total 20 individuals of mud crab larvae were exposed to each 30 ml of the test solutions in Petri dishes for 1, 2 and 24 h. After each exposure duration, the survival of mud crab larva was observed by naked eyes in Petri dishes for their movement and motility under an inverted microscope Olympus. While, inactive and non-motile mud crab larvae were considered dead. These dead ones were investigated if the cause was fungal infection [13]. The number of mud crab larva surviving at each exposure period was used to calculate the corrected mortality following Abbott's formula [14]:

$$Pt = (Po - Pc)/(100 - Pc) \times 100$$

Pt = percentage of corrected mortality (%); Po = mortality of test group; Pc = mortality of control group.

3.6. Antifungal bioassay

The minimum inhibitory concentration (MIC) of fungistatic effect on hyphae was performed by incorporating the compound solutions (100, 50, 25, 12.5 µg/ml) onto PYGS agar in petri dish followed inoculation of *L. thermophilum*, *H. sabahensis* and *H. milfordensis*. The procedure was modified from known method [15,16]. The MIC was determined visually as the lowest concentration showing no hyphal growth when they were incubated at 25 °C for 7 days.

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