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Isolation of a Morphinan Alkaloid and a Methoxybenzoic Acid with The Investigation on The Antibacterial Effect of *Alphonsea cylindrica* King Leaves

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Alphonsea cylindrica King belongs to the family Annonaceae, which is traditionally used to treat rheumatism, bruises, and edema. Investigation of its antibacterial properties was carried out on hexane and dichloromethane crude extracts using agar diffusion method and minimum inhibitory concentration against ESKAPE pathogens. Both extracts showed positive results in inhibiting *E. faecium*, *S. aureus*, and *K. pneumoniae*, while the dichloromethane extract showed further inhibition against *P. aeruginosa*. A morphinan alkaloid, *O*-methylpallidinine (**1**), along with a methoxybenzoic acid, isovanillic acid (**2**), were isolated from the dichloromethane leaf extract of the plant and elucidated via NMR spectroscopy, FTIR spectroscopy, UV-vis spectroscopy, and GC-MS, and compared with the reported data. Both compounds are reported for the first time from *A. cylindrica*, while *O*-methylpallidinine was isolated as natural product for the first time from the family Annonaceae. Both **1** and **2** showed low activity against *S. aureus* with an IC_{50} value of 371.82 μg/mL and 385.37 μg/mL, respectively. This is the first report on the antibacterial activity of compound **1**. The results can be used as future references for the discovery of morphinans and the potential of *A. cylindrica* as an antibacterial source.

Key words: *Alphonsea cylindrica* King; antibacterial activity; *O*-methylpallidinine; isovanillic acid

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Alphonsea cylindrica King, also known as 'mempisang', is a tree that can grow up to 10 meters tall, which can be discovered in the lowland forests in Malaysia and Thailand [1, 2]. It belongs to the genus *Alphonsea*, which belongs to the big family Annonaceae. Traditionally, *Alphonsea* species are medicinally used to treat rheumatism [3] and edema [4], as well as painkillers [5] and febrifuge [2]. Researchers have reported that the *Alphonsea* species possess various properties, such as anticancer [6, 7], anti-larvicidal [8], anti-inflammatory [9], antileshmanial [10], antitrypanosomal [11], antibacterial, and antifungal [7]. Several researchers have isolated different types of alkaloids from *Alphonsea* species. Aldulaimi *et al*. [6] had isolated oxoaprorphines known as atherospermidine and liriodenine, as well as an azafluorenone known as kinabaline from the bark of *A. elliptica*. The isolated alkaloids showed positive results in inhibiting breast cancer cell lines. Xie Ning *et al*. [3] isolated mollisine, an oxoaporphine alkaloid, from the bark of *A. Mollis.* Suman *et al.* [12] had also successfully isolated aporphine alkaloid, named crebanine, from *A. sclerocarpa*, which showed *in vitro* cytotoxicity against K562 blood cancer cells. By referring to these studies, *Alphonsea* species have a huge potential as a source for alkaloid compounds.

Currently, extensive phytochemical and pharmacological studies on *A. cylindrica* are still lacking with only limited studies had been done on the plant [13, 14]. Talip *et al.* [14] had successfully isolated different types of alkaloids from the bark of *A. cylindrica*, known as azafluorenone (kinabaline), oxoaporphine (*O*-methylmoschatoline), and 4, 5-dioxoaporphine (*N*-methylouregidione) alkaloids. The isolated compounds have shown inhibition ability against bacteria such as *S. aureus*, *P. aeruginosa*, and *B. cereus*. Aldulaimi *et al.* [13] further isolated two isoquinoline alkaloids, named iraqiine and kareemine, from the bark of *A. cylindrica*, which possessed positive antioxidant activity. The potential of obtaining alkaloids from different parts of *A. cylindrica* has driven further investigations and studies on this plant. Hence, the present work aims to discover potential antibacterial alkaloids from the leaves of *A. cylindrica*, along with investigation on their antibacterial activities.

In this study, a morphinan alkaloid, *O*-

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methylpallidinine (**1**), along with a methoxybenzoic acid, isovanillic acid (**2**), were isolated from the dichloromethane leaf extract of *A. cylindrica* with the aid of silica gel 60 and Sephadex LH-20 column chromatography. This is the first report that has isolated morphinan from A. cylindrica, while O-methylpallidinine (1) is also isolated for the first time as a natural product from the family Annonaceae as it had only been isolated and reported by Vecchietti *et al.* [15] from *Ocotea acutangula* in 1981. Although morphinan is commonly used as pain-reliever [16], researchers have gained positive results on the bioactivity of morphinan alkaloids. Qing *et al.* reported positive antiviral activity from morphinan alkaloids such as FK-3000, tannagine, and sinoacutine against Herpes Simplex [17]. Carraz *et al.* also obtained positive antimalarial activity from their isolated morphinan named tazopsine [18]. Therefore, investigation on the antibacterial activity was carried out on the hexane and dichloromethane leaf extracts as well as the isolated compounds by agar diffusion method and minimum inhibitory concentration method using ESKAPE pathogens as the inhibition targets.

MATERIALS AND METHODS

1. Plant Materials

A. cylindrica with herbarium number TM1049 was identified and collected from Kuala Lipis, Pahang by botanical researchers of University Pendidikan Sultan Idris. The voucher specimen was kept at the Herbarium at University Pendidikan Sultan Idris in June 2018.

2. Methods

2.1. General

Nuclear magnetic resonance (NMR) spectra were obtained from JEOL ECX (500 MHz). The mass spectra were recorded using Agilent 5975C Gas Chromatography-Mass Spectrometry (GC-MS). The infrared (IR) spectra were obtained from Shimadzu IRTracer-100. The ultraviolet-visible (UV-Vis) spectrum was obtained by using Hitachi UH530 Spectrophotometer. Thin-layer chromatography (TLC) was carried out by using Merck's aluminum supported silica gel 60 F_{254} plates which would be visualised under UV-Vis light (254 and 365 nm). The identification of alkaloids was confirmed by spraying Dragendorff's reagent on the TLC plates. Silica gel column chromatography (CC) was prepared by using silica gel 60 of 70 – 230 mesh and 230 – 400 mesh depending on the weight of the fractions. Sephadex LH-20 CC was prepared by soaking Sephadex LH-20 in methanol.

2.2. Extraction and isolation

Dried and powdered leaves of *A. cylindrica* (2 kg) were extracted by Soxhlet extraction using hexane, dichloromethane (DCM), and methanol (MeOH) to obtain three crude extracts that consisted of different polarities. The extracts were then concentrated by using a rotary evaporator, which yielded 32, 23, and 65 g respectively. The DCM crude extract (20 g) was injected into silica gel 60 (70 – 230 mesh) CC and eluted with hexane/DCM/MeOH of different ratios (100:0:0 – 0:0:100, v/v). The elution resulted in 8 subfractions (F1 – F8).

Subfraction F4 was then subjected to silica gel 60 (230 – 400 mesh) CC and eluted with hexane/DCM/MeOH (50:50:0 – 0:50:50, v/v) to obtain 23 subfractions (4A – 4W). Subfraction 4Q was subjected to silica gel 60 (230 – 400 mesh) CC and eluted with DCM/MeOH (100:0 – 50:50, v/v) to obtain 36 fractions ($4Q_1$ – $4Q_{36}$). Subfraction $4Q_{20}$ was subjected to silica gel 60 (230 – 400 mesh) CC and eluted with hexane/DCM/MeOH (50:50:0 – 0:50:50, v/v) to obtain **1** (5 mg).

Subfraction F5 was chromatographed over silica gel 60 (70 - 230 mesh) CC and eluted with (100:0:0 – 0:0:100, v/v) to obtain 10 subfractions (5-1 – 5-10). Subfraction 5-9 was passed over silica gel 60 (230 – 400 mesh) CC and eluted with DCM/MeOH (100:0 – 50:50, v/v) to obtain 18 subfractions (5-9-1 to 5-9-18). Subfractions 5-9-19 was rechromatographed with Sephadex LH-20 CC and eluted with MeOH/CH_2Cl_2 (100:0 – 0:100, v/v) to afford **2** (7.2 mg).

Table 1. Solvent composition used in silica gel 60 CC

Solvent Composition	Ratio	Volume used (mL) *
Hexane	100	100
Hexane: DCM	90:10	100
Hexane: DCM	80:20	100
Hexane: DCM	70:30	100
Hexane: DCM	60:40	100
Hexane: DCM	50:50	100
Hexane: DCM	40:60	100
Hexane: DCM	30:70	100
Hexane: DCM	20:80	100

Hexane: DCM	10:90	100
DCM	100	200
DCM: MeOH	99:1	200
DCM: MeOH	98:2	200
DCM: MeOH	97:3	200
DCM: MeOH	96:4	200
DCM: MeOH	95:5	200
DCM: MeOH	90:10	200
DCM: MeOH	80:20	200
DCM: MeOH	70:30	200
DCM: MeOH	60:40	200
DCM: MeOH	50:50	200
Methanol	100	300

* Volume used varied with the polarity and weight of the selected extracts

Table 2. Solvent composition used in Sephadex LH-20 CC

Solvent Composition	Ratio	Volume used (mL) *
MeOH	100	100
MeOH: CH_2Cl_2	9:1	100
MeOH: CH_2Cl_2	8:2	100
MeOH: CH_2Cl_2	7:3	100
MeOH: CH_2Cl_2	6:4	100
MeOH: CH_2Cl_2	1:1	100
MeOH	100	300

* Volume used varied with the polarity and weight of the selected extracts

O-methylpallidinine (**1):** Reddish brown solid; $[\alpha]^{25}_D$: -36.4 (c 7.86 x 10^{-4} M, CH_2Cl_2); IR ν_{max} 3314, 2939, 1724, 1647, 1161, 1018 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ε), nm: 243 (3.80), 260 (3.66), 415 (0.50), 662 (0.07); ^1H ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) data see Table 3; GC-MS *m/z* 355.0 (calcd for $C_{21}H_{26}NO_4$, 356.43)

Isovanillic acid (**2**): Colorless needles; $[\alpha]^{25}_D$: 25.0 (c 4.28 x 10^{-3} M, CH_2Cl_2); IR ν_{max} 3486, 2917, 1680, 1598, 1205 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ε), nm: 230 (2.31), 260 (3.19), 288 (2.09); ^1H ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) data see Table 4; GC-MS *m/z* 168.2 (calcd for $C_{21}H_{26}NO_4$, 168.15)

2.3. Agar diffusion assay

The antibacterial properties of *A. cylindrica* were tested by using the agar diffusion method [19] as previously described by Talip *et al.* [14] with slight modifications. The DCM crude extract was dissolved in 10% dimethylsulfoxide (DMSO) [20] and solutions with concentrations of 40, 20, 10, and 5 mg/mL were prepared. 6 mm discs were placed in the prepared crude extract solutions. Test bacterial isolates were grown in nutrient broth with shaking in a shaking incubator overnight at 200 rpm at 37ºC. Agar plates were then spread with the prepared bacterial broths using sterile cotton swabs. The discs were then placed on the plates and sealed with parafilm. The plates were then placed in the incubator at 37ºC overnight. After the incubation, the zone of inhibition of each disc was measured to determine the antibacterial activity.

2.4. *In vitro* antibacterial assay

The Minimum Inhibition Concentration (MIC) method [21] was used to determine the minimum concentration of the crude extracts that had inhibited the bacteria in the agar diffusion test. The standard procedures and measures were as described by Clinical and Laboratory Standards Institute (CLSI) [22] with slight modification. The antibacterial activity of the isolated compounds was also tested via this method. The IC_{50} of the crude extracts and isolated compounds was also determined from the tests. Two-fold serial dilutions for the crude extracts and isolated compounds were carried out in sterilized 96-well plates. The bacterial broth was prepared as 0.5 McFarland's standard using UV-visible Spectrophotometer after overnight incubation at 37ºC. The MIC and IC_{50} of the crude extracts and isolated compounds were determined by measuring the optical density at 620 nm using a plate reader.

RESULTS AND DISCUSSION

O-methylpalldinine **(1)** and isovanillic acid (**2**) were isolated from the dichloromethane leaf extract of *Alphonsea cylindrica* King (*A. cylindrica*) via chromatography methods. The compounds were

elucidated by spectroscopic methods and compared with previously reported data.

1. Structure elucidation

1.1. Structure elucidation of *O*-methylpallidinine (1)

Compound 1 was isolated as a light orange amorphous solid and was sprayed with Dragendorff's reagent on a TLC plate to confirm its alkaloid properties. The GC-MS spectrum of compound 1 resulted in a molecular ion peak at m/z 355.0, which confirmed the molecular formula of $C_{20}H_{25}NO_5$. In the ^{1}H-NMR spectrum (Table 3), three aromatic protons were indicated with δ_H of 6.83 (J = 8.6 Hz, H-1), 6.87 (J = 8.6 Hz, H-4), and 7.49 (H-8), which suggested the presence of an aromatic ring [23]. The downfield signal of H-8 resulted from the deshielding effect due to the presence of methoxy group at C-7 [13]. The intense singlets at δ_H 3.93, 3.87, and 3.65 indicated the presence of three methoxy groups, which were assigned as 2-OCH_3, 3-OCH_3, and 7-OCH_3, respectively [24]. The peak at δ_H 2.45 suggested the presence of *N*-methyl carbon attached to C-9 and C-16, which was later confirmed by the HMBC correlation between H-9 to C-NCH_3. The ^{13}C spectrum (Table 3) indicated the presence of 17 carbons. The ring-A of the morphinan structure could be accurately depicted with the HMBC correlation between H-1 to C-2/C-11 and H-4 to C-3/C-12, with two substituted methoxy groups via the correlation between 2-O$\underline{C}H_3$ to C-2, 3-O$\underline{C}H_3$ to C-3, H-1 to C-2, and H-3 to C-4. The ring-B of compound **1** was depicted by the correlation of H-9 to C-13/C-NCH_3 and H-10 to C-11/C-12. The nitrogen bridge was confirmed by the correlation between H-9 to C-13/C-16, H-15 to C-12, and H-16 to C-15. The ketone at ring-C was positioned at C-6 (δ_C 194.4), verified through HMBC correlations of H-5 to C-6. The remaining methoxy group and aromatic proton at ring-C were verified by the HMBC correlation of H-5 to C-6, as well as H-8 to C-7. The structure of compound **1** was further confirmed by ^{1}H-^{1}H COSY correlation between H-15 to H-16, H-9 to H-10, and H-14 to H-9/H-10. Although it has been isolated and reported by Vecchietti et al. [15], the complete ^{1}H and ^{13}C NMR data as well as the correlation of HMBC and COSY of compound 1 were not specified by the previous study as Vecchietti et al. who had only reported some of ^{1}H NMR data.

Table 3. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **1**

Positions	**1**		References [15]
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	6.83 d (8.6)	111.3	6.32 s
2		147.5	
3		151.0	
4	6.87 d (8.6)	123.8	6.62 s
5	2.07 m	27.2	
	1.25 d		
6		194.4	
7		151.1	
8	7.49 s	123.9	6.85 s
9	2.93 d (5.7)	56.4	
10	2.80 dd (17.8, 5.7)	27.9	
	3.08 d (18.3)		
11		130.5	
12		133.7	
13		38.1	
14	2.36 s	42.4	
15	1.85 d (13.2)	33.3	
	2.15 dd (12.9, 4.3)		
16	2.57 d	46.7	
2-OCH_3	3.93 s	60.7	3.89 s
3-OCH_3	3.87 s (5.2)	55.8	3.85 s
7-OCH_3	3.65 s	54.9	3.69 s
N-CH_3	2.45 s	40.9	2.37 s

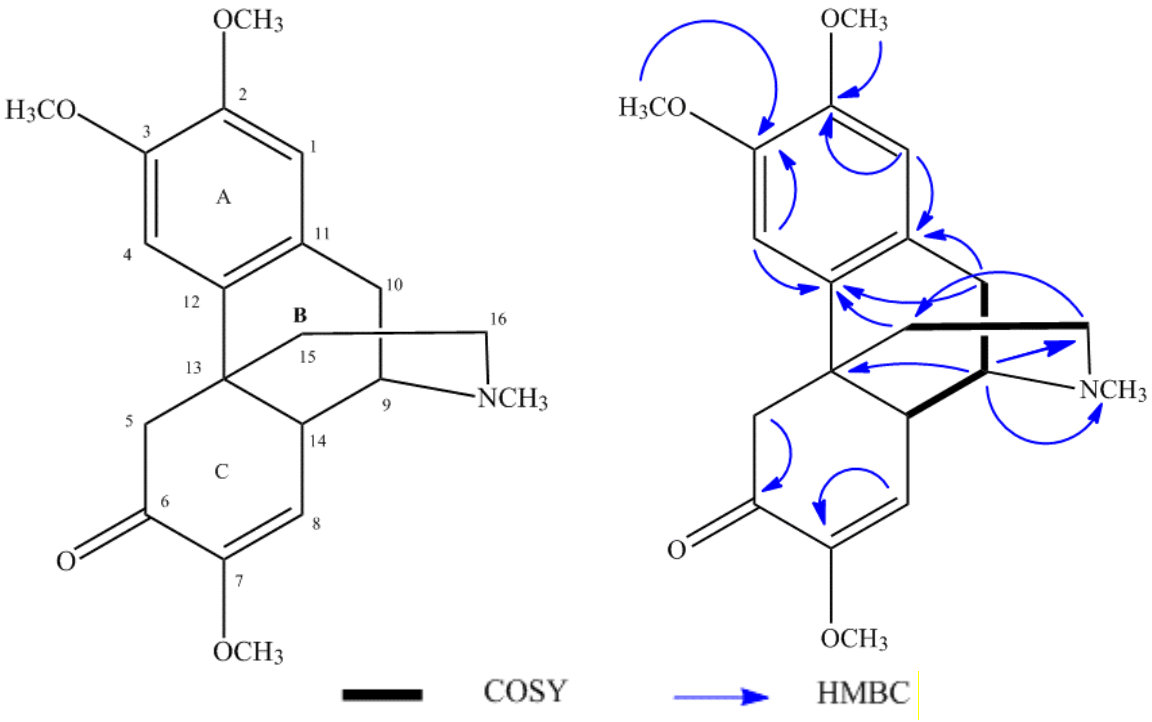

Figure 1. Chemical structure and selected HMBC and COSY of **1**

1.2. Structure elucidation of isovanillic acid (2)

Compound **2** was isolated as colorless needles. The GC-MS spectrum of compound **2** showed a molecular ion peak at *m/z* 168.2, which verified the molecular formula of $C_8H_8O_4$. In ^{1}H-NMR spectrum (Table 4), the presence of an aromatic ring was indicated by three aromatic protons with δ_H 7.59 (J = 1.7 Hz, H-2), 6.97 (H-5), and 7.71 (H-6) [14]. The intense singlet at δ_H 3.96 indicated a methoxy group, assigned as 4-OCH_3, which was verified through the HMBC correlation of 4-O<u>C</u>H$_3$ to C-4. The presence of COOH at position 1 (δ_C 170.7) and OH group at position 3 (δ_C 150.7) were verified via medium adsorption at 3486 cm^{-1}, indicating the presence of intramolecular H-bond within the compound. In ^{1}H-NMR spectrum (Table 4), the presence of an aromatic ring was indicated by three aromatic protons with δ_H 7.59 (J = 1.7 Hz, H-2), 6.97 (H-5), and 7.71 (H-6) [14].The structure of compound **2** was accurately depicted by the HMBC correlation of H-2 to C-2/C-4/C-1-COOH, H-5 to /C-4, and H-6 to C-1/C-1-COOH. The structure of compound **2** was further confirmed by ^{1}H-^{1}H COSY correlation between H-2 to H-6 and H-5 to H-6/4-O<u>C</u>H$_3$. The signals from the ^{1}H and ^{13}C-NMR spectra were identical to the previously reported data by Xiang *et al.* [25].

Table 4. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **2**

Positions	**2**		Reference [25]	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		121.2		122.9
2	7.59 d (1.7)	112.0	6.92 d (1.6)	115.5
3		150.7		148.1
4		146.2		152.0
5	6.97 d	114.2	7.58 d (8.4)	113.5
6	7.71 dd	125.1	7.61 dd (8.4)	124.8
1-COOH		170.7		167.8
4-OCH_3	3.96 s	56.1	3.93 s	56.3

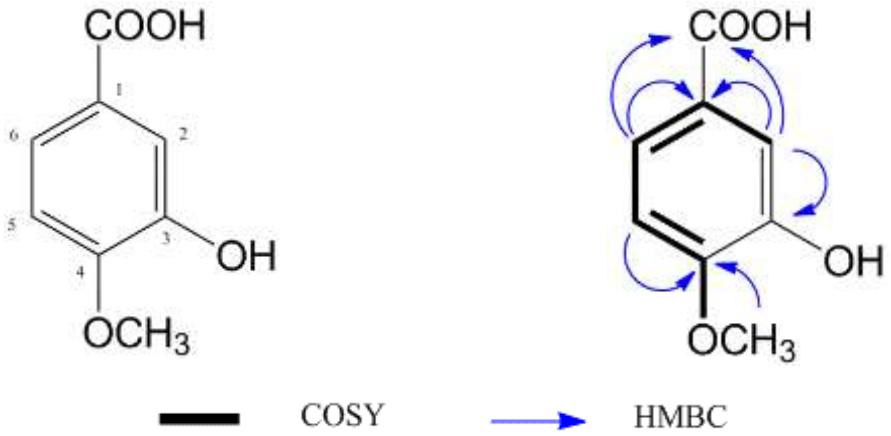

Figure 2. Chemical structure and selected HMBC and COSY of **2**

2. Antibacterial properties

2.1. Antibacterial properties of *A. cylindrica* King on ESKAPE bacteria

The hexane and dichloromethane leaf extracts of *A. cylindrica* were tested for antibacterial activity by the agar diffusion method on selected pathogens by using 40, 20, 10, and 5 mg/mL as the testing concentrations. Both crude extracts showed moderate inhibitory effects against *E. faecium*, *S. aureus*, and *K. pneumoniae* at 5 mg/mL, while the dichloromethane extract showed further inhibition on *P. aeruginosa* (Table 5). The antibacterial properties of the hexane and dichloromethane leaf extracts were further examined by using the minimum inhibitory concentration method (MIC) on *E. faecium*, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* to determine the minimum concentration of the crude extracts to inhibit the respective bacteria and also calculate the IC_{50} for each of the bacteria. The MIC values are summarized in Table 6. The MIC results showed that both the hexane and dichloromethane leaf extracts were most effective against *S. aureus*, which recorded IC_{50} values of 2.898 ± 0.085 mg/mL and 0.679 ± 0.060 mg/mL, respectively.

Table 5. Diameter of the inhibition zone for hexane and dichloromethane leaf extracts of *A. cylindrica* King against ESKAPE bacteria at 5 mg/mL

Bacteria	Inhibition zone (mm) ± SE*	
	Hexane extract	Dichloromethane extract
E. faecium	7.67 ± 0.580	15.67 ± 0.652
S. aureus	9.33 ± 0.580	9.67 ± 0.335
K. pneumoniae	14.00 ± 1.000	10.33 ± 0.878
A. baumanii	6.00 ± 0.000	6.00 ± 0.000
P. aeruginosa	6.00 ± 0.000	7.67 ± 0.335
*K. aerogenes***	6.00 ± 0.000	6.00 ± 0.000

*SE = standard error

**Previously named as *Enterobacter*

Table 6. MIC and IC_{50} of hexane and dichloromethane leaf extracts of *A. cylindrica* King against *E. faecium*, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa*

Bacteria	Hexane extract		Dichloromethane extract	
	MIC (mg/mL) ± RSD (%)	IC_{50} (mg/mL) ± SE, RSD (%)	MIC (mg/mL) ± RSD (%)	IC_{50} (mg/mL) ± SE, RSD (%)
E. faecium	5.00 ± 0.00	3.304 ± 0.118 (3.58)	2.50 ± 0.00	1.915 ± 0.070 (6.33)
S. aureus	5.00 ± 0.00	2.898 ± 0.085 (2.93)	1.25 ± 0.00	0.679 ± 0.060 (15.2)
K. pneumoniae	5.00 ± 0.00	4.218 ± 0.212 (5.03)	1.25 ± 0.00	1.216 ± 0.048 (6.79)
P. aeruginosa	-*	-*	5.00 ± 0.00	2.657 ± 0.072 (4.71)

* MIC test was not carried out since the extract did not show any inhibition effect in the agar diffusion test

2.2. Antibacterial properties of isolated compounds on *S. aureus*

Compounds **1** and **2** were also tested for their antibacterial activities via MIC test on *S. aureus* using 500 µg/mL as the starting concentration. The results showed that both compounds **1** and **2** possessed weak inhibition properties against *S. aureus* at MIC = 500 µg/mL, while IC_{50} = 371.82 µg/mL and 385.37 µg/mL, respectively. This is the first report on the antibacterial activity of compound **1**. The weak inhibition effect of compound **2** was similar to the previous report by Kuete *et al*. [26]

CONCLUSION

A known morphinan alkaloid, *O*-methylpallidinine, and a methoxybenzoic acid, isovanillic acid, were isolated from the dichloromethane leaf extract of *A. cylindrica*. Both compounds are for the first time to be isolated from *A. cylindrica*, while *O*-methylpallidinine is the first time to be isolated as natural product from the family Annonaceae. The hexane and dichloromethane leaf extracts from *A. cylindrica* King showed positive results in inhibiting *E. faecium*, *S. aureus*, and *K. pneumoniae*, while the dichloromethane extract showed further inhibition against *P. aeruginosa*. The isolated compounds showed weak inhibition activity against *S. aureus*. Therefore, extensive phytochemical and pharmacology studies on *A. cylindrica* are necessary since it can be regarded as a medicinal plant with a potential source of antibacterial alkaloids from its leaves.

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REFERENCES

1. Hanum, I. F., Ibrahim, A. Z., Khamis, S., Nazre, M., Lepun, P., Rusea, G., Lajuni, J. J. and Latiff, A. (2001) An annotated checklist of higher plants in Ayer Hitam forest reserve, Puchong, Selangor, *Pertanika Journal of Tropical Agricultural Science*, **24(1)**, 63–78.

2. Wong, T. M. (2002) *A Dictionary of Malaysian Timbers*, Forest Research Institute Malaysia, Malaysia.

3. Xie, N., Xu, R., Zhong, S. and Zhao, S. (1994) Chemical investigation on *Alphonsea mollis* Dun., *Journal of China Pharmaceutical University*, **25(4)**, 205–207.

4. Attiq, A., Jalil, J. and Hussain, K. (2017) Annonaceae: Breaking the wall of inflammation, *Frontiers in Pharmacology*, **8**, 752.

5. Yang, N., Xie, N. and Zhi, F. (1999) Study on the major alkaloids of *Alphonsea monogyna*, *Journal of China Pharmaceutical University*, **30(3)**, 171–173.

6. Aldulaimi, A. K. O., Azziz, S. S. S. A, Bakri, Y. M., Nafiah, M. A., Awang, K., Aowda, S. A., Litaudon, M., Hassan, N. M., Naz, H., Abbas, P., Hashim, Y. Z. H. and Majhool, A. A. (2018) Alkaloids from *Alphonsea elliptica* barks and their biological activities, *Journal of Global Pharma Technology*, **10(8)**, 270–275.

7. Doddapaneni, S. J. D. S., Amgoth, C., Kalle, A. M., Suryadevara, S. N. and Alapati, K. S. (2018) Antimicrobial and anticancer activity of AgNPs coated with *Alphonsea sclerocarpa* extract, *3 Biotech*, **8(3),** 1–9.

8. Weerasinghe, A., Puvanendran, S., Wickramasinghe, A., Karunaratne, D. N., Wijesundara, S. and Karunaratne, V. (2013) Potent bioactivities of the endemic annonaceae heighten its dire conservation status, *Journal of the National Science Foundation of Sri Lanka*, **41(4)**, 345–350.

9. Bakri, Y. M., Talip, M. A. and Azziz, S. S. S. A. (2017) A mini review on *Alphonsea* sp. (Annonaceae): traditional uses, biological activities and phytochemistry, *Journal of Applied Pharmaceutical Science*, **7(10)**, 200–203.

10. Jahn, M. S. R., Getha, K., Ilham, A. M., Norhayati, I., Haffiz, J. M. and Amyra, A. S. (2018) *In Vitro* anti-leismanial activity of Malaysian medicinal and forest plant species, *Journal of Tropical Forest Science*, **30(2)**, 234–241.

11. Norhayati, I., Getha, K., Haffiz , J. M., Ilham, A. M., Sahira, H. L., Syarifah, M. M. S. and Syamil, A.M. (2013) *In vitro* antitrypanosoma activity of Malaysian plants, *Journal of Tropical Forest Science*, **25(1)**, 52–59.

12. Suman, J. D. S. D., Chander A., Surya, N. S., Padmavathi, C. H., Madhavi, J. and Krishna, S. A. (2018) Antioxidant and anticancer activities of an aporphine alkaloid isolated from *Alphonsea sclerocarpa*, *Journal of Phytopharmacology*, **7(1)**, 51–55.

13. Aldulaimi, A. K. O., Azziz, S. S. S. A, Bakri, Y. M., Nafiah, M. A., Awang, K., Aowda, S. A. and Litaudon, M. (2019) Two new isoquinoline alkaloids from the bark of *Alphonsea cylindrica* King and their antioxidant

activity, *Phytochemistry Letters*, **29(5)**, 110–114.

14. Talip, M. A., Azziz, S. S. S. A., Wong, C. F., Awang, K., Naz, H., Bakri, Y. M., Ahmad, M. S. and Litaudon, M. (2017) New azafluorenone derivative and antibacterial activities of *Alphonsea cylindrica* barks, *Natural Product Sciences*, **23(3)**, 151–156.

15. Vecchietti, V., Casagrande, C., Ferrari, G., Danieli, B. and Palmisano, G. (1981) Alkaloids of *Ocotea acutangula*, *Journal of Chemistry Society, Perkin Transactions 1*, 578–581.

16. Fossati, E., Narcross, L., Ekins, A., Falgueyret, J, Vincent, J. and Martin, J. (2015) Synthesis of morphinan alkaloids in *Saccharomyces cerevisiae*, *PLoS ONE*, **10(4)**, 1–15.

17. Qing, Z., Yang, P., Tang, Q., Cheng, P., Liu, X., Zheng, Y., Liu, Y. and Zeng, J. (2017) Isoquinoline alkaloids and their antiviral, antibacterial, and antifungal activities and structure-activity relationship, *Current Organic Chemistry*, **21**, 1–15.

18. Carraz, M., Jossang, A., Franetich, J., Siau, A., Ciceron, L., Hannoun, L., Sauerwein, R., Frappier, F., Rasoanaivo, P., Snounou, G. and Mazier, D. (2006) A plant-derived morphinan as a novel lead compound active against malaria liver stages, *PLoS Medicine*, **3(12)**, 513.

19. Hauck, O., Allen, N., Lees, G. C., Rowe, H. and Verran, J. (2010) Preliminary studies into wash-fast antimicrobial treatments of polyester, *Medical and Healthcare Textiles*, 122–125.

20. Madikizela, B., Ndhlala, A. R., Finnie, J. F. and Staden, J. V. (2013) *In Vitro* antimicrobial activity of extracts from plant used traditionally in South Africa to treat tuberculosis and related symptoms, *Evidence-Based Complementary and Alternative Medicine*, **2013**, 840719.

21. Lambert, R. J. W. and Pearson, J. (2000) Susceptibility testing : accurate and reproducible Minimum Inhibitory Concentration (MIC) and Non-Inhibitory Concentration (NIC) values, *Journal of Applied Microbiology*, **88**, 784–790.

22. CLSI. M02-A11 (2012) *Performance standards for antimicrobial disk susceptibility tests: Approved standard – Eleventh edition*, Clinical and Laboratory Standards Institute, Wayne, PA 19087.

23. Tan, S. P., Ali, A. M., Nafiah, M. A., Awang K. and Ahmad K. (2015) Isolation and Cytotoxic investigation of new carbazole alkaloids from *Murraya koenigii* (Linn.) Spreng, *Tetrahedron*, **71(23)**, 3946–3953.

24. Tan, S. P, Lim, S. M., Wong, M. K., Lim, C. Y. and Nafiah, M. A. (2020) Chemical constituents of *Murraya koenigii* berries, *Chemistry of Natural Compounds*, **56(5)**, 962–963.

25. Xiang, M., Su, H., Hu, J. and Yan, Y. (2011) Isolation, identification and determination of methyl caffeate, ethyl caffeate and other phenolic compounds from *Polygonum amplexicaule var. sinese*, *Journal of Medicinal Plants Research*, **5(9)**, 1685–1691.

26. Kuete, V., Metuno, R., Ngameni, B., Tsafack, A. M., Ngandeu, F., Fotso, G. W., Bezabih, M., Etoa, F., Ngadjui, B. T., Abegaz, B. M. and Beng, V. P. (2007) Antimicrobial activity of the methanolic extracts and compounds from *Treculia obovoidea* (Moraceae), *Journal of Ethnopharmacology*, **112**, 531–536.