Anticancer Activities of Chemical Constituents from Leaves and Twigs of *Mitrephora winitii*

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Abstract: *The genus* Mitrephora *has been investigated, and its anti-inflammatory, anti-bacterial, and anti-parasitical activities were examined along with its potential as an anti-cancer cell line and inhibitor for platelet aggregation. In this work, air-dried leaves and twigs of* M. winitii *were grounded and extracted with n-hexane, ethyl acetate, and methanol, respectively. Chromatographic separations of these extracts led to the isolation of three known compounds and one new compound (compound* ***2****). The chemical structures of these were identified using the spectroscopic investigation of 1D- and 2D-NMR, and the resulting data confirmed these as stigmasterol (****1****), (3,4-dimethoxyphenyl)(5-(3,4-dimethoxyphenyl)-4-(hydroxymethyl)tetrahydrofuran-3-yl)methanol (****2****), diayangambin (****3****), and methyl-L-inositol (****4****). The chemical constituents were reported the first time in* M. winitii. *Compound* ***2*** *showed anti-cancer cell lines with* ED_{50} *13.07 µg/mL against KB cells and then was tested for cytotoxicity against MCF-7 cells with* ED_{50} *11.77 µg/mL.*

Keywords: Mitrephora winitii*; anticancer; extraction; extract*

■ INTRODUCTION

Within the pantropical family of shrubs, trees, and lianas, the *Annonaceae* family comprises an interesting group of medicinal plants. Consisting of roughly 130 genera and 2,500 species, the majority of these are found in Asia, Australia, and Pacific regions [1]. One of these is the genus *Mitrephora*, which is comprised of some 48 species found in Asia and Australia [2]. In Thailand, 12 species are found, and some plants in this genus have been used in the country for the production of folk medicine [3]. These genera have great potential for the treatment of cancers, bacterial infections, brain dysfunctions, and hypertension [1]. Phytochemical investigations have established that *Mitrephora* species contain diterpenoids, polyacetylene carboxylic acids/esters, fatty acids, lignans, sesquiterpenes, alkaloids [4-9], the diterpenoids and alkaloids have shown significant anti-microbial, anti-malarial, anti-platelet aggregation, and cytotoxic potential [10-12]. Interestingly, the *M. winitii* was the new source and reported on the antitumor and potent cytotoxic activities of the genus showed high activity anticancer agents.

The *M. winitii* [13] extracts with solvents (*n*-hexane, ethyl acetate, and methanol) and isolated by column chromatography was found to contain four compounds. They were identified using IR, NMR, and ESI-MS spectrometry to yield stigmasterol (**1**), (3,4-dimethoxyphenyl)(5-(3,4-dimethoxyphenyl)-4-(hydroxy

methyl)tetrahydrofuran-3-yl)methanol (**2**), diayangambin (**3**), and methyl-L-inositol (**4**). In this paper, we investigated the cytotoxicity of the *n*-hexane extract of this plant tested against a panel of two mammalian cancer cell lines. We also report on the isolation and characterization of one new, and three known compounds were found in *M. winitii*.

■ EXPERIMENTALSECTION

Materials

M. winitii leaves and twigs were collected from Lampang Province in Thailand in January 2011. *M. winitii* was confirmed by the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok, Thailand, where a voucher specimen (BKF16447) has been deposited. Silica gel (Merck 7734, Mesh 70-230) and TLC 60 PF_{254} sheets were purchased from Merck. All organic solvents used for extraction and chromatographic separation (CC) were distilled at their boiling point ranges of *n*-hexane, ethyl acetate, and methanol (laboratory grade), whereas AR grade solvents were used for crystallization were from Merck.

Instrumentation

The melting points of the extracted compounds were measured on a digital electrothermal melting apparatus, and the uncorrected results were recorded in degrees Celsius (°C). IR spectra were recorded on KBr disks using a Shimadzu 8900 FTIR spectrophotometer, whereas major bands (ν_{max}) were recorded at wavenumber (cm^{-1}) unit,^{1}H (400 MHz), and ^{13}C (100 MHz). NMR spectra were determined using either $CDCl_3$ or D_2O solution. Chemical shifts were recorded in δ values, which were referenced to TMS as the internal standard at δ 0.00 ppm. The signal of chloroform at δ 7.26 was used as a reference in the case of ^{1}H-NMR spectra and at δ 77.00 in the case of ^{13}C-NMR spectra. The instrument was achieved using a DPX on a Bruker AV 400 spectrometer for 1D and 2D determinations. Low resolution mass spectra were recorded on a Thermo Finnegan Polaris Q mass spectrometer at 70 eV (probe) for the EIMS. High-resolution mass spectra (made using the electrospray ionization mode, ESI-MS) were measured on a micro massQ-TOF-2^{Tm} (Waters) spectrometer. Column chromatography was conducted on silica gel 60 (Merck 7734, 70–230 mesh). TLC was performed on aluminum backed pre-coated silica gel 60 PF_{254} sheets, and detections were made using a UV detector at 254 and 365 nm.

Procedure

Extraction and isolation

Dried and powdered leaves and twigs of *M. winitii* (2.0 kg) were treated at room temperature with *n*-hexane, ethyl acetate, and methanol successively. The *n*-hexane extract (25.0 g) was subjected to silica gel (Merck 7734, 70–230 mesh) column chromatography (CC) and eluted in a gradient system with an increasing concentration of *n*-hexane/ethyl acetate to yield fifteen fractions (A1-A15). Fraction A7 was found to contain a solid. This was recrystallized with a solution of ethyl acetate/ethanol (3:1) to afford stigmasterol (**1**). Fraction A10 was subjected to further CC on silica gel, eluted with a gradient of *n*-hexane/ethyl acetate to afford 150 mg of (3,4-dimethoxyphenyl)(5-(3,4-dimethoxyphenyl)-4-(hydroxymethyl)tetrahydrofuran-3-yl)methanol (**2**). The ethyl acetate extract (24.0 g) was subjected to silica gel (Merck 7734, 70–230 mesh) column chromatography (CC), eluted in a gradient system with an increasing concentration of *n*-hexane/ethyl acetate and ethyl acetate/methanol to give ten fractions (B1-B10). Fraction B8 was subjected to further CC on silica gel, eluted with a gradient of ethyl acetate/methanol to afford 200 mg of diayangambin (**3**). The methanol extract (72.0 g) was subjected to silica gel (Merck 7734, 70–230 mesh) column chromatography and eluted in a gradient system with an increasing concentration of ethyl acetate/methanol to give ten fractions (C1-C10). Fraction C4 was subjected to further CC on silica gel and eluted with a gradient of ethyl acetate/methanol to give 300 mg of methyl-L-inositol (**4**) (Fig. 1).

Evaluation of cytotoxic activity

The cytotoxic activities of the compounds extracted from *M. winitii* were tested using the *in-vitro* sulforhodamine B (SRB) method. Ellipticine was used as

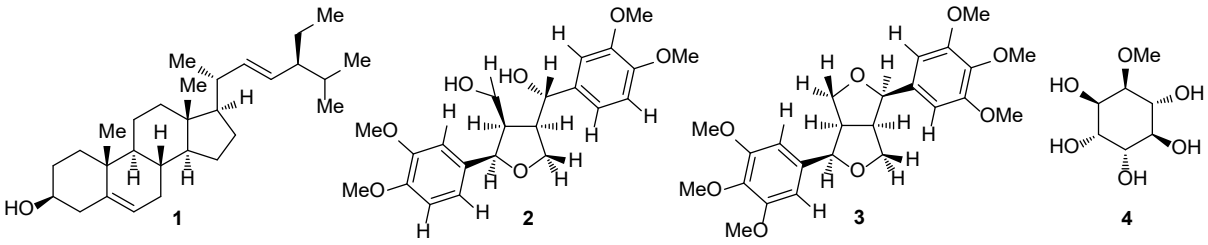

Fig 1. Structure of compounds from *M. winitii*

a positive control. Test samples were dissolved in dimethyl sulfoxide (DMSO) at a stock concentration of 3 mg/mL, and these were tested in triplicate with a final concentration of DMSO at 0.5%. The cancer cell lines were grown in a 96-well plate in the following media: P-388, in RPMI-1640 with 5% fetal bovine serum (FBS). The P-388, KB, HT29, MCF-7, A549, ASK, and HEK293 cell lines were cultured in MEM (minimum essential medium with Earle's salt and l-glutamine) with 10% FBS, while Lu-1 was grown in MEM with 5% FBS. After drug exposure was at 37 °C for 72 h (48h for P-388) with 5% CO_2 in air and 100% relative humidity, cells were then fixed with a final concentration of 10% trichloroacetic acid and stained with 0.4% SRB in 1% acetic acid. The bound and dried stain was solubilized with 10 mM Trizma base after removing the unbound dye by washing. Absorbance was read on a Fluostar optima BMG plate reader at a wavelength of 570 nm. The cytotoxic activity is expressed as a 50% effective dose (ED_{50}) [14].

The ED_{50} value was determined by:

$$\% \text{ Survival} = \frac{\text{OD (test sample)} - \text{OD (Day 0)}}{\text{OD (0.5\% DMSO control)} - \text{OD (Day 0)}}$$

Criteria of activity: Extracts having an ED_{50} < 20 µg/mL and pure compounds having an ED_{50} < 4 µg/mL = Active; No Response = ED_{50} > 20 µg/mL

■ RESULTS AND DISCUSSION

Structure Elucidation

Compound **1** was obtained as a white powder; mp 163–164 °C. From the EIMS spectrum $[M+H_2O+H]^+$ at m/z 395.37, this could be assigned the molecular formula $C_{29}H_{48}O$. The IR spectrum showed the broad absorption bands of a hydroxyl group at 3460 cm^{-1}. Absorption bands appearing at 2955 and 2870 cm^{-1} were due to C–H stretching. C–H bending showed weak absorption bands at 1465 cm^{-1} and 1377 cm^{-1}, and C=C stretching appeared as a weak absorption band at 1650 cm^{-1}. The medium absorption band at 1050 cm^{-1} was assigned to C–O stretching. The structure was further elucidated by examination with NMR techniques. The ^{1}H-NMR spectra of compound **1** showed the presence of six methyl signals, which appeared at δ 0.72 (3H, *s*, H-28), 0.81 (3H, *d*, *J*=5.0 Hz, H-27) 0.85 (3H, *d*, *J*=5.0 Hz, H-26), 0.87 (3H, *t*, *J*=5.0 Hz, H-24), 0.94 (3H, *d*, *J*=10.0 Hz, H-19), and 1.06 (3H, s, H-29). The spectra also showed protons at δ 5.03, 5.16, and 5.62 ppm, suggesting the presence of protons corresponding to a tri-substituted and a di-substituted olefinic bond. A comparison of the ^{1}H and ^{13}C-NMR spectral data to data in the literature, together with the melting point of the sample, points to the molecular structure of stigmasterol (**1**) [9,15].

Compound **2** was isolated as a colorless needle crystal; mp 126-127 °C. Its ESIMS gave a molecular ion peak $[M+2H^+]$ at *m/z* 406, consistent with the molecular formula $C_{22}H_{28}O_7$ (cal. for $C_{22}H_{28}O_7$, 404). In addition, the mass spectrum of the compound found *m/z* 406 $[M+2H^+]$. The key fragmentation ions in the mass spectrum were at 359, 324, 323, 249, and 151, which was useful in obtaining the structure of the compound (Fig. 2) [16]. The IR spectrum showed absorption bands attributable to hydroxyl at 3400 cm^{-1} and aromatics at 1617, 1589, and 1519 cm^{-1}. The absorption bands appearing at 2945 and 2850 cm^{-1} were due to C–H stretching. The C–H bending appeared as weak absorption bands at 1464 and 1375 cm^{-1}. In addition, the methoxy groups showed typical C–O stretching absorptions, which appeared at 1269 and in the range from 1234 to 1160 cm^{-1}. The ^{1}H-NMR signals at δ 6.85–6.93 ppm (6H) represented a tri-substituted phenyl moiety. Two oxygen bearing methylene protons were

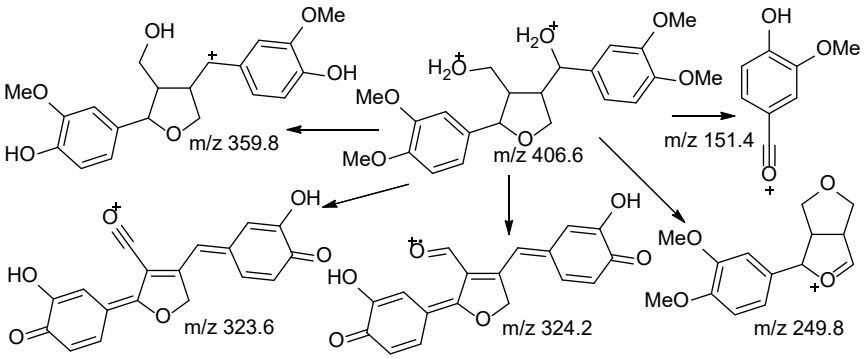

Fig 2. The mass spectral fragmentation of compound **2**

suggested at δ 3.36 ppm (2H, *m*, H-3a) and two nonequivalent methylene protons at δ 3.85 (1H, *dd*, *J*=5.0, 10.0 Hz, H-5$_\alpha$) and 4.13 (1H, *d*, *J*=10.0 Hz, H-5$_\beta$) ppm. Four methine protons were indicated at δ 2.92 (1H, *dd*, *J*=5.0, 10.0 Hz, H-4), 3.32 (1H, *m*, H-3), 4.45 (1H, *d*, *J*=10.0 Hz, H-4a), and 4.88 (1H, *d*, *J*=5.0 Hz, H-2) ppm. Four methoxy groups were also indicated at δ 3.89, 3.87, 3.88, and 3.91 (each 3H, *s*) ppm in the ^{1}H-NMR spectrum. Further spectral evidence was required to confirm the structure of **2**. The ^{1}H-^{1}H COSY showed coupling correlations through the sequence of H-2 to H-3, H-3 to H-3a, H-4 to H-5, and H-4 to H-4a for the connectivity of the protons to the structure. The connectivity of the aromatic carbon skeleton (e.g., C-5', C-6', C-5'', and C-6'') was also confirmed by the COSY correlations (Fig. 3). The HMBC spectrum showed crossed peaks between the aromatic signals (H-2', H-5', and H-6') and C-1', C-2', C-3', and C-4' and between H-6', H-2' and C-2, which indicated the aromatic ring was connected to C-2. Further, the aromatic signals (H-2'', H-5'', and H-6'') and C-1'', C-2'', C-3'', and C-4'' and between H-6'', H-2'' and C-4a indicated the aromatic ring was connected to C-4a (Fig. 3). The ^{1}H-^{13}C spectrum revealed signals from 22 atoms, and DEPT experiments also showed 16 protonated carbon signals, thereby revealing the presence of six quaternary carbons in the molecule. The presence of a trisubstitued phenyl ring was evident from the signals at δ 109.11, 111.14, 117.76, 131.02, 148.09 and 148.92 (a ring connected C-2) and signals at δ 109.26, 111.14, 118.47, 133.75, 148.78 and 149.29 (a ring connected C-4a). Additionally, oxymethine carbon signals were found at δ 82.07, 87.63, and methoxy carbons at δ 55.93, 55.96, and 55.97. A literature search revealed that *cis*- and *trans*- orientation of substituents at C-2 and C-3 give a signal of H-2 at δ 4.76 and 4.91 (*J*=4, 4.8 Hz), respectively. The H-2 signal of compound **2** (δ 4.88 and *J*=5.0 Hz) thus agreed well with the assignment of a *cis*-configuration. The relationship between the torsion angle and vicinal coupling constant ^3J is given theoretically by the Karplus equation: $^3J(HH) = P_1 \cos^2\phi + P_2\cos^2\phi + P_3 + \Sigma\Delta\chi_i\{P_4 + P_5 \cos^2(\xi_i\phi + P_6|\Delta\chi_i|)\}$ [17]. So, the relative configuration at H-4 and H-4a could be determined by the $^3J_{4,4a}$, H–C–C–H (10 Hz) coupling constant, which would indicate that the two protons were located on opposite sides with a dihedral angle of 180°. Furthermore, the H-4a showed a signal at δ 4.45 ppm

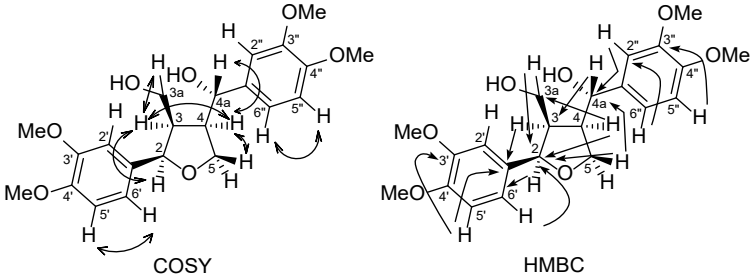

Fig 3. ^{1}H-^{1}H COSY correlations and the selected HMBC correlations of compound **2**

Table 1. ^{13}C and ^{1}H-NMR data of compound **2** and ^{1}H-^{1}H, ^{1}H-^{13}C correlations exhibited in the 2D NMR spectra in $CDCl_3$

Position	δ_C(ppm)	δ_H(ppm)	COSY	HMBC
1	"	"	"	"
2	82.07	4.88, *d*(5.0)	H-3	3, 3a, 1', 2', 6'
3	50.18	3.32, *m*	H-2, H-3a, H-4	2, 3a, 4, 4a
3a	69.73	3.36, *m*	H-3	2, 3, 4
4	54.50	2.92, *dd*(5.0,10.0)	H-3, H-4a, H-5	2, 4a, 1"
4a	87.63	4.45, *d*(10.0)	H-4	3, 4, 5, 1", 2", 6"
5	71.04	4.13, *d*(10.0) 3.85, *dd*(5.0,10.0)	H-4	2, 3, 4, 4a
1'	131.02			
2'	109.11	6.93, *s*		2, 1', 3', 4', 6'
3'	148.92			
4'	148.09			
5'	111.14	6.85, *m*	H-6'	2, 1', 2', 3', 4', 6'
6'	117.76	6.89, *m*	H-5'	2, 1', 2', 3', 4'
1"	133.75			
2"	109.26	6.92, *s*		4a, 1", 3", 4", 6"
3"	149.29			
4"	148.78			
5"	11.14	6.85, *m*	H-6"	1", 2", 3", 4", 6"
6"	118.47	6.90, *m*	H-5"	4a, 1", 2"
3'-OMe	55.96	3.89, *s*		3'
4'-OMe	55.93	3.87, *s*		4'
3"-OMe	55.97	3.88, *s*		3"
4"-OMe	55.96	3.91, *s*		4"

and *J*=10.0 Hz, giving a spectra data assignment to the *trans*- configuration. On the basis of the above data [18-19], the structure of **2** was determined to be (3,4-dimethoxyphenyl)(5-(3,4-dimethoxyphenyl)-4-(hydroxyl methyl)tetrahydrofuran-3-yl)methanol, which has not been previously reported. The resonances for the protons of the tetrahydrofuran skeleton between two propanyl groups showed nonequivalent for H-3/H-4, H-2/H-4a, and H_2-5/H_2-3a; therefore, requiring an unsymmetrical substitution stereochemistry of the furan system; especially the chemical shifts of the benzylic oxymethine protons H-2 (4.88, 5.0 Hz, *d*) and H-4a (4.45, 10 Hz, *d*) confirmed that two the propanyl groups substituent are asymmetrical in compound **2** [18-19]. When compared, the protons for the furan system of compound **3** showed the equivalents of H-7/H-7' and H-8/H-8'; therefore, requiring a symmetrical substitution stereochemistry for the aryl substituents and furan ring. Moreover, the few coupling constants and the chemical shifts of the benzylic oxymethine protons of H-7 and H-7', showed *J*=5.0 Hz, δ 4.92, and for bridge carbons, C-8/C-8' (49.43/49.43) confirmed that two aryl substituents are symmetry in compound **3** [20]. Support the absolute configuration of compound **2** was studied by electronic circular dichroism (ECD) spectroscopy. The optimized structure of compound was performed by the density functional theory (DFT) calculation at the B3LYP/6-31G (d,p) level of theory (Fig. 4). ECD spectra were carried out by using TD-DFT method at the CAM-B3LYP/6-311G++(d,p) including PCM model (MeOH) (Fig. 5). The rotary strengths of 80 excited states were calculated. All calculations were performed using Gaussian09 program package. Gaussian bandshape with a bandwidth of 0.25 eV was used to simulate ECD spectra.

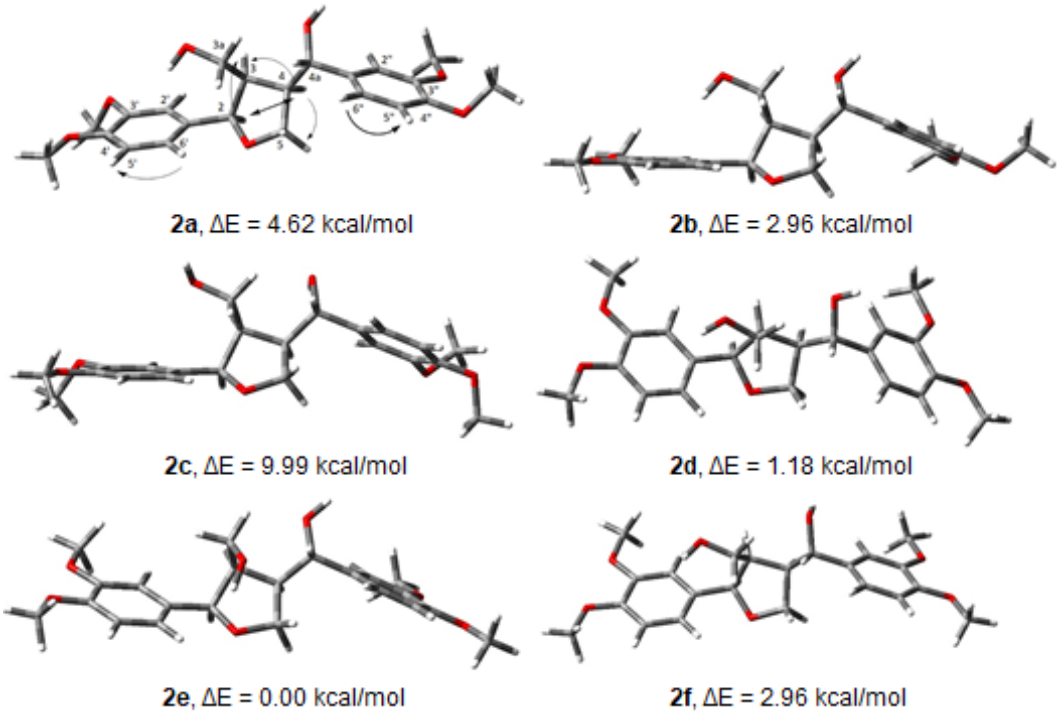

Fig 4. Optimized structures of compound **2** using B3LYP/6-31G(d,p)

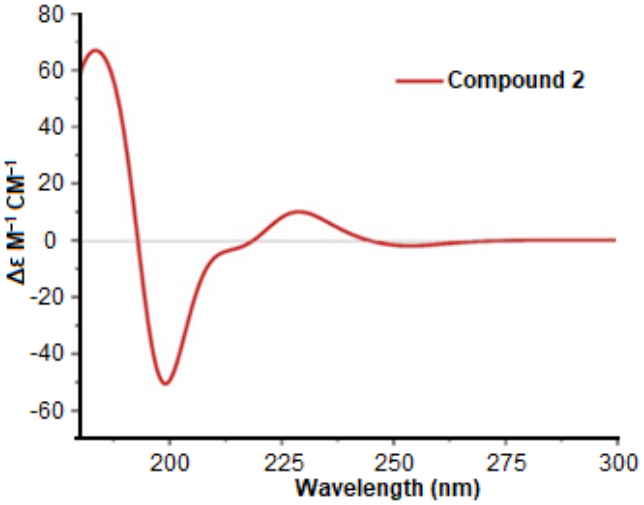

Fig 5. TDDFT calculated CD spectra of compound **2-2e** at Cam-B3LYP/6 311++G)d,p

The ECD curve was generated by SpecDis 1.64 (University of Wurzburg, Wurzburg, Germany) softwares. The proposed biosynthesis pathway correctly showed to reasonably assure the specific structure for the unknown compound **2** (Fig. 6) [21].

Medicinal plants are important sources of bioactive compounds in cancer suppression and treatment [1]. Plants of the *Mitrephora* genera were used to treat sickness in folk medicines [3]. The *in-vitro* sulforhodamine B (SRB) method assay to study the inhibition of cell viability on seven cancer cell lines P-388, KB, HT29, MCF-7, A549, ASK, and HEK293 by treatment of seven naturally occurring pure compounds, compared with an ellipticine drug. Compound **2** showed moderated anti-proliferative activity against KB and MCF-7 cell lines, with ED_{50} values of 13.07, and 11.77 µg/mL, respectively, which is reported here for the first time.

Compound **3** was obtained as a white needle crystal; mp 144–145 °C. Its EIMS gave a molecular ion peak $[M]^+$ at *m/z* 445, which was consistent with the molecular formula $C_{24}H_{30}O_8$ (cal. for $C_{24}H_{30}O_8^+$, 445). The mass showed the fragmentation characteristics described for liriosinol-*B* dimethyl ether. The EIMS spectrums showed fragmentation ions in the mass spectrum at *m/z* 249, 219, 195, 181, 177, and 165. The IR spectrum of the compound showed medium absorption bands at 1634, 1614, 1589, and 1509 cm^{-1}, which were characterized as aromatic C=C stretching. The strong absorption bands at 2935 and 2840 cm^{-1} were characterized as C–H stretching, while the corresponding bending vibrations appeared at 1422 and 1367 cm^{-1}. The

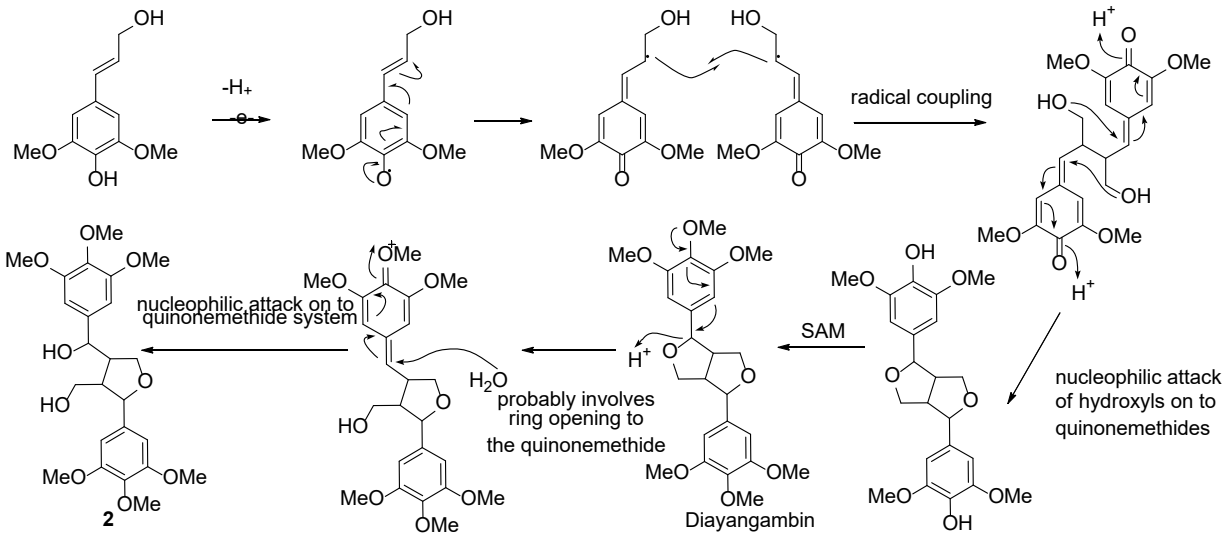

Fig 6. Proposed biosynthesis pathway of diayangambin and compound **2**

C–O stretching showed medium absorption bands at 1129 and 1004 cm^{-1}. The structure of compound **3** was further elucidated through 1D- and 2D-NMR experiments. The ^{1}H-NMR spectrum showed the presence of tetra-substituted aromatic protons at δ 6.62 (4H, *s*, H-2,6,2',6') ppm. A doublet of methine proton H-7,7' was observed at δ 4.92 (2H, *d*, *J*=5.0 Hz, H-7,7') ppm and the resonances at δ 3.90 (12H, *s*, 3,5,3',5'-OMe) and 3.87 (6H, *s*, 4,4'-OMe) ppm indicated a methoxy proton. A doublet of methylene protons ($H_{\alpha,\beta}$-9) on a tetrahydrofuran ring was observed at δ 3.74 (2H, *dd*, J_1=1.8 Hz, J_2=9.6 Hz, H_α-9,9') and 3.58 (2H, *dd*, J_1=6.7 Hz, J_2=9.6 Hz, H_β-9,9') ppm. The ^{13}C-NMR spectrum exhibited the resonances of a quaternary aromatic carbon at δ 153.22 (C-3,5,3',5'), 137.02 (C-1,1'), 134.58 (C-4,4'), and four aromatic methine carbons at δ 103.17 (C-2,6,2',6') ppm. Methine carbons were found at δ 84.08 (C-7,7') and 49.43 (C-8,8') ppm, and methylene carbons at δ 68.89 (C-9,9') ppm. Deshieldedoxymethyl carbons were indicated at δ 68.89 (C-9,9'), 60.89 (4,4'-OMe), and 56.10 (3,5,3',5'-OMe) ppm. The HMBC correlation of H-2 to C-4, C-6, and C-7, and H-7 to C-1, and C-9 confirmed an aromatic ring connecting to the tetrahydrofuran ring. These data were in accordance with those of diayangambin (**3**) [20,22].

Compound **4** was obtained as a white crystal; mp 190–192 °C. The IR spectrum showed the broad absorption band of a hydroxyl group at 3420 cm^{-1}. The absorption bands at 2940 and 2840 cm^{-1} were due to C–H stretching. In addition, the hydroxyl and methoxy groups showed typical C–O stretching absorptions, which appeared at 1146 cm^{-1} and in the range from 1119 to 1066 cm^{-1}. The structure of the compound was further elucidated by 1D- and 2D-NMR experiments. The ^{1}H-NMR displayed signals for protons of oxygenated carbons at δ 3.46 (1H, *m*, H-2), 3.48 (1H, *m*, H-3), 3.60 (1H, *m*, H-4), 3.92 (1H, *t*, *J*=5 Hz, H-5), and 4.13 (1H, *t*, *J*=5 Hz, H-6) ppm, while a methoxy group was indicated at δ 3.31 (3H, *s*, -OMe) ppm. One proton at δ 3.26 (1H, *m*, H-1) ppm was assigned to a methine proton adjacent to the oxygen of an ether group. The ^{13}C-NMR spectrum showed a resonance signal at δ 80.06 ppm, which was assigned to a carbon of ether, while the methoxy carbon showed at δ 56.79 ppm. The signals at δ 72.74, 71.83, 70.27, 71.28, and 67.03 ppm also indicated oxymethine carbons. The COSY spectrum showed correlations between H-1 and H-2, H-2 and H-3, H-3 and H-4, H-4 and H-5, H-5 and H-6, H-6 and H-1. The HMBC spectrum demonstrated the correlation of H-OMe to C-1, indicating the methoxy was connected at C-1, and this confirmed the position of the methoxy group. These data were in accordance with those recorded for methyl-L-inositol (**4**) [23-25].

■ CONCLUSION

The investigation focused on the phytochemical of medicinal plant together with biochemical evaluation.

The results presented four compounds derivative from *M. winitii* were carried out from crude extract of *M. winitii* found the compounds; stigmasterol (**1**), (3,4-dimethoxyphenyl)(5-(3,4-dimethoxyphenyl)-4-(hydroxy methyl)tetrahydrofuran-3-yl)methanol (**2**), diayangambin (**3**), and methyl-L-inositol (**4**). Chemical constituents were the first report isolated from *M. winitii*, in addition, compound 3,4-dimethoxyphenyl)(5-(3,4-dimethoxyphe nyl)-4-(hydroxymethyl)tetrahydrofuran-3-yl)methanol (**2**) was new the structure. Other than the compound **2** can effectively inhibit the growth of the KB and MCF-7 cancer cell lines; when compared with an ellipticine as the positive control.

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■ REFERENCES

[1] Florence, A.R., Joselin, J., Brintha, T.S.S., Sukumaran, S., and Jeeva, S., 2014, Preliminary phytochemical studies of select members of the family Annonaceae for bioactive constituents, *Biosci. Discovery*, 5 (1), 85–96.

[2] Mols, J.B., and Kessler, P.J.A., 2003, The genus *Miliusa* (Annonaceae) in the Austro-Malesian area, *Blumea*, 48 (3), 421–462.

[3] Rayanil, K., Limpanawisut, S., and Tuntiwachwuttikul, P., 2013, *Ent*-pimarane and *ent*-trachylobane diterpenoids from *Mitrephora alba* and their cytotoxicity against three human cancer cell lines, *Phytochemistry*, 89, 125–130.

[4] Lee, N.H.S., Xu, Y.J., and Goh, S.H., 1999, 5-Oxonoraporphines from *Mitrephora* cf. *maingayi*, *J. Nat. Prod.*, 62 (8), 1158–1159.

[5] Zgoda, J.R., Freyer, A.J., Killmer, L.B., and Porter, J.R., 2001, Polyacetylene carboxylic acids from *Mitrephora celebica*, *J. Nat. Prod.*, 64 (10), 1348–1349.

[6] Li, C., Lee, D., Graf, T.N., Phifer, S.S., Nakanishi, Y., Burgess, J.P., Riswan, S., Setyowati, F.M., Saribi, A.M., Soejarto, D.D., Farnsworth, N.R., Falkinham, J.O., Kroll, D.J., Kinghorn, A.D., Wani, M.C., and Oberlies, N.H., 2005, A hexacyclic*ent*-trachylobrane diterpenoid possessing an oxetane ring from *Mitrephora glabra*, *Org. Lett.*, 7 (25), 5709–5712.

[7] Deepralard, K., Pengsuparp, T., Moriyasu, M., Kawanishi, K., and Suttisri, R., 2007, Chemical constituents of *Mitrephora maingayi*, *Biochem. Syst. Ecol.*, 35 (10), 696–699.

[8] Li, C., Lee, D., Graf, T.N., Phifer, S.S., Nakanishi, Y., Riswan, S., Setyowati, F.M., Saribi, A.M., Soejarto, D.D., Farnsworth, N.R., Falkiham, J.O., Kroll, D.J., Kinghorn, A.D., Wani, M.C., and Oberlies, N.H., 2009, Bioactive constituents of the stem bark of *Mitrephora glabra*, *J. Nat. Prod.*, 72 (11), 1949–1953.

[9] Husain, K., Jamal, J.A., and Jalil, J., 2012, Phytochemical study of *Cananga odorata* (Lam) Hook.F. & Thomson & Thoms (Annonaceae), *Int. J. Pharm. Pharm. Sci.*, 4 (4), 465–467.

[10] Zgoda-Pols, J.R., Freyer, A.J., Killmer, L.B., and Porter, J.R., 2002, Antimicrobial diterpenes from the stem bark of *Mitrephora celebica*, *Fitoterapia*, 73 (5), 434–438.

[11] Mueller, D., Davis, R.A., Duffy, S., Avery, V.M., Camp, D., and Quinn, R.J., 2009, Antimalarial activity of azafluorenone alkaloids from the Australian tree *Mitrephora diviversifolia*, *J. Nat. Prod.*, 72 (8), 1538–1540.

[12] Moharam, B.A., Jantan, I., Jalil, J., and Shaari, K., 2010, Inhibitory effects of phylligenin and quebrachitol isolated from *Mitrephora vulpina* on platelet activating factor receptor binding and platelet aggregation, *Molecules*, 15 (11), 7840–7848.

[13] Royal Botanic Gardens, 1922, "*Mitrephora winitii*" in *Bulletin of Miscellaneous Information*, Springer, Berlin/Heidelberg, 227–228.

[14] Graidist, P., Martla, M., and Sukpondma, Y., 2015, Cytotoxic activity of *Piper cubeba* extract in breast cancer cell lines, *Nutrients*, 7 (4), 2707–2718.

[15] Chaturvedula, V.S.P., and Prakash, I., 2012, Isolation

of stigmasterol and β-sitosterol from the dichloromethane extract of *Rubus suavissimus*, *Int. Curr. Pharm. J.*, 1 (9), 239–242.

[16] Russell, G.B., and Fenemore, P.G., 1973, New lignans from leaves of *Macropiper excelsum*, *Phytochemistry*, 12 (7), 1799–1803.

[17] Haasnoot, C.A.G., de Leeuw, F.A.A.M., de Leeuw, H.P.M., and Altona, C., 1981, The relationship between proton-proton NMR coupling constants and substituent electronegativities. II–Conformational analysis of the sugar ring in nucleosides and nucleotides in solution using a generalized Karplus equation, *Org. Magn. Reson.*, 15 (1), 43–52.

[18] Jung, K.Y., Kim, D.S., Oh, S.R., Park, S.H., Lee, I.S., Lee, J.J., Shin, D.H., and Lee, H.K., 1998, Magnone A and B, Novel anti-PAF tetrahydrofuran lignans from the flower buds of *Magnolia fargesii*, *J. Nat. Prod.*, 61 (6), 808–811.

[19] Tulake, A., Jiang, Y. and Tu, P.F., 2012, Nine lignans from *Atemisia absinthium* L., *J. Chin. Pharm. Sci.*, 21, 360–364.

[20] Wang, T., Yuan, Y., Wang, J., Han, C., and Chen, G., 2012, Anticancer activities of constituents from the stem of *Polyalthia rumphii*, *Pak. J. Pharm. Sci.*, 25 (2), 353–356.

[21] Dewick, P.M., 2002, "The shikimate pathway: Aromatic amino acids and phenylpropanoids" in *Medicinal Natural Products: A Biosynthetic Approach*, 2nd Ed., John Wiley & Sons, Ltd., UK, 121–166.

[22] De León, E.J., Olmedo, D.A., Solis, P.N., Gupta, M.P., and Terencio, M.C., 2002, Diayagambin exerts immunosuppressive and anti-inflammatory *effects in vitro* and *in vivo*, *Planta Med.*, 68 (12), 1128–1131.

[23] Júnior, H.V.N., Cunha, G.M.A., Moraes, M.O., Luciana, M.F.D., Oliveira, R.A., Maia, F.D., Nogueira, M.A.S., Lemos, T.L.G., and Rao, V.S., 2006, Quebrachitol (2-*O*-methyl-L-inositol) attenuates 6-hydroxydopamine-induced cytotoxicity in rat fetal mesencephalic cell cultures, *Food Chem. Toxicol.*, 44 (9), 1544–1551.

[24] Sanseera, D., Niwatananun, W., Liawruangrath, B., Liawruangrath, S., Baramee, A., Trisuwan, K., and Pyne, S.G., 2012, Antioxidant and anticancer activities from aerial parts of *Acalypha indica* Linn, *Chiang Mai Univ. J. Nat. Sci.,* 11 (2), 157–168.

[25] Abraham, R.J., Byrne, J.J., Griffiths, L., and Koniotou, R., 2005, ^{1}H chemical shifts in NMR: Part 22—Prediction of the ^{1}H chemical shifts of alcohols, diols and inositols in solution, a conformational and solvation investigation, *Magn. Reson. Chem.*, 43 (8), 611–624.