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Antimalarial Evaluation of the Chemical Constituents Isolated from *Dendrocalamus asper*

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Abstract: Bamboo shoots of *Dendrocalamus asper* is essential for human health because of the high content of dietary fiber, low sugar, negligible amount of fat, and rich vitamin and mineral content. The bamboo shoots' chemical constituents were evaluated against the *P. falciparum* strain 3D7 as a potential antimalarial drug. One new metabolite, (11*Z*,13*E*,17*E*,19*Z*)-dimethyl-15,16-dibutoxytriconta-11,13,17,19-tetraenedioate (**1**) along with four known compounds; *β*-sitosterol (**2**), methyl-4-hydroxybenzoate (**3**), 1-methoxy-4-(methoxymethyl)benzene (**4**) and 4-hydroxybenzaldehyde (**5**) were isolated from the crude extract of *Dendrocalamus asper* using chromatographic methods: MPLC, UPLC/MS, analytical and preparative HPLC. Among these, compounds **1**, **3,** and **4** showed promising antimalarial activity with IC_{50} between 0.8-2.2 µg/mL. The molecular docking between the most potent compound **3** and dihydrofolate reductase-thymidylate synthase (DHFR-TS) was done to understand and explore the ligand-receptor interactions and hypothesize the compound's refinements.

Keywords: *Dendrocalamus asper,* antimalarial, *P. Falciparum,* Molecular docking, dihydrofolate reductase-thymidylate synthase (DHFR-TS).

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INTRODUCTION

Malaria is a tropical blood-borne protozoan disease caused by parasites of the genus Plasmodium, and spread by female *Anopheles* mosquitoes. There are five types of *Plasmodium* causing malaria *viz*; *P. ovale, P. knowlesi, P. malariae, P. vivax, and P. falciparum* (1). The World Health Organisation (WHO) reported that in 2018, there were 228 million malaria cases that occurred worldwide. It resulted in 405,000 deaths and approximately 70% of the global dealths involving children under-five from malaria (2). Even though Malaysia does not have any domestic malaria (indigenous) cases recorded since 2018, it remains one of the most significant health challenges to other southeast

Asian countries (2). The main drawback of malaria's current treatment are the development of multiple drug resistance and the non-specific targeting to intracellular parasites. This, in turn results in the requirement of high-dose anti-parasitic drugs and subsequent intolerable toxicity. Hence, there is a need for novel chemotherapeutic agents.

The tropical rain forest is rich in biological and chemical resources, which have a huge potential as defense agents against pests, diseases, and predators (3). Two natural compounds, quinine, isolated from the stem of *Cinchona sp*. and artemisinin, isolated from the herbal plant *Artemisinin annua* are phenomenal, and have contributed greatly to reducing deaths due to malaria all over the world (4,5). Malaysia is one of the world's most thriving center of biodiversity with its tropical rainforests. Many of the rainforest plant based phytomedicines are used as an alternative treatment for malaria (6). Among these, the *Dendrocalamus asper* (bamboo) from the family of Poaceae is a tall arborescent grass, which plays an essential role in construction, reinforcing fibers in paper, medicines, and food sources (7). Bamboo shoots are young, edible plants with health benefits, including healthy weight loss, antibacterial and anti-carcinogenic activities.

To date, no antimalarial studies have been reported on the bamboo plant. Therefore, this research reports new potential antimalarial agents, and phytochemical investigations of the extract from bamboo shoots of *Dendrocalamus asper*. The evaluation of the antimalarial activity of all the isolated compounds are herein reported.

EXPERIMENTAL SECTION

Plant material

Bamboo shoots of *Dendrocalamus asper* (DAPB52014) were collected at Pos Brook Village, Gua Musang, Kelantan, Malaysia. The bamboo shoots were identified by Assistant Botanist, Mr. Deraman, M., from South Kelantan Development Authority (KESEDAR).

General

All chemicals were obtained from QRec (Asia) and Merck (Germany). Thin-layer chromatography (TLC) were performed on alumina plates pre-coated with silica gel (Merck 60 F_{254}). The spots were determined under UV radiation (λ_{max} = 254 nm). SiO_2-MPLC was performed using CombiFlash Companion (Teredyne ISCO). Preparative reverse phase HPLC was performed using a Waters 600 pump system with Waters 2998 photodiode array detector and Senshu pack pegasil ODS column (20 X 250 mm). Analytical HPLC was performed using an Empower system with Waters 2996 detector with senshu pack column (4.6 X 250 mm) and Waters 717 plus autosampler. One- and two-dimensional nuclear magnetic resonance (NMR) spectra were recorded using a JEOL ECA-500 spectrometer at Chemical Biology Building, RIKEN, Wakoshi, Saitama, Japan using $CDCl_3$ and methanol-d_4 as solvents. Infrared (IR) spectrum was recorded on a Perkin-Elmer System FTIR-ATR spectrometer at the School of Chemical Sciences, USM. The chemical ionization mass spectrometry (CI-MS) was recorded by using a Bruker Micro TOF-QII LCMS at the Department of Chemistry, National University of Singapore. The measurement was carried out in positive-ion mode. Mass resolution: 17,500 (FWHM), temperature compensated, mass range: 50-20,000 *m/z*, acquisition rate: 20 Hz (2 GHz sampling rate).

Extraction, separation, and isolation

The dried bamboo shoot (1.29 kg) was extracted with three different types of solvents, one after the other on the same bamboo shoot. First, *n*-hexane was used in the extraction. After the extraction, the bamboo shot was evaporated to dryness. It was then extracted with dichloromethane. After which the sample was evaporated to dryness and finally extracted with methanol. The recovered extractants were evaporated using a rotary evaporator to obtain the crude extract. The dry weight of *n*-hexane extract (BSH) was 19.4 g, the dichloromethane extract (BSD) was 30.9 g, and the methanol extract (BSM) was 80.9 g. **Figure 1** shows the structure of compounds isolated from the *n*-hexane and dichloromethane extracts.

n-hexane extract (BSH)

BSH extract (16.5 g) was separated by SiO_2-MPLC eluted with *n*-hexane:acetone stepwise gradient (100:0, 99:1, 98:2, 95:5, 90:10, 80:20, 50:50, 0:100) to obtain 8 fractions. Fr.5 (2.08 g) was subjected to the SiO_2-MPLC with *n*-hexane/ethyl acetate gradient system (ethyl acetate: 0–100%) to afford five fractions (Fr.5.1 to Fr.5.5). Fr.5.4 (200.7 mg/ 432.3 mg) was subjected to the SiO_2-MPLC using *n*-hexane/ethyl acetate in a stepwise gradient (ethyl acetate 0-100%) to afford six fractions (Fr.5.4.1 to Fr.5.4.6). Fr.5.4.5 (18.2 mg) was purified by preparative reverse phase HPLC using an isocratic solvent system of MeCN/H_2O (80/20) to yield compound **1** (t_R 12.5 min, 2.1 mg, 0.013%) as a yellowish oil.

Further analysis of Fr.5.4.3 afforded compound **2** (27.0 mg, 0.164%) as a white solid. Fr.6 (1.53 g) was subjected to the SiO_2-MPLC using hexane/ethyl acetate gradient system (ethyl acetate: 0–100%) to afford five fractions (Fr.6.1 to Fr.6.5). Purification of Fr.6.3 (50.5 mg) by preparative reverse phase HPLC using an isocratic

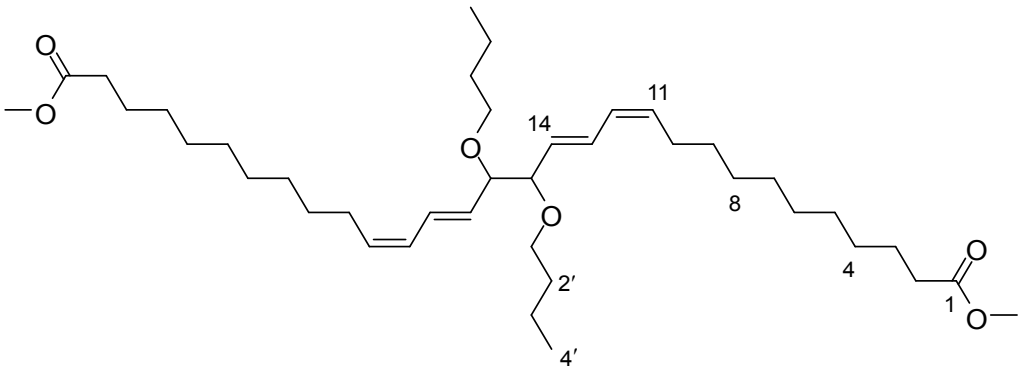
solvent system of MeCN/H_2O (20/80) yielded compound **3** (t_R 42.1 min, 3.9 mg, 0.024%) as a white powder. This fraction also afforded compound **4** (t_R 21.6 min, 3.7 mg, 0.022%) as a white powder through the preparative reverse phase HPLC using an isocratic solvent system of MeCN/H_2O (20/80).

From spectroscopic analyses, compound **1** was found to be a new compound. While compounds **2**, **3** and **4** were identified as *β*-sitosterol, methyl-4-hydroxybenzoate, and 1-methoxy-4-(methoxymethyl)benzene, respectively, by comparing with the spectroscopic data in the literature (8-11).

(11*Z*,13*E*,17*E*,19*Z*)-dimethyl-15,16-dibutoxytriconta-11,13,17,19-tetraenedioate (**1**) (t_R 12.5 min, 2.1 mg, 0.013%) as yellowish oil. FT-IR (ATR) v_{max} cm^{-1}: 3013 (C-H sp^2), 2927 (C-H sp^3), 1712 (C=O), 1221 (C-O), 1093 (C-H in plane bending), 751 (C-H out-of-plane bending); **^{1}H-NMR (500MHz, $CDCl_3$)**: δ_H 0.87 (3H, t, *J* = 6.5 Hz, 4′-C<u>H</u>$_3$), 2.15 (2H, q, *J* = 5.5 Hz and 6.9 Hz, 10-C<u>H</u>$_2$), 2.28 (2H, t, *J* = 7.5 Hz, 2-C<u>H</u>$_2$-), 3.62 (2H, s, 1′-OC<u>H</u>$_2$-), 3.64 (3H, s, -OC<u>H</u>$_3$), 4.13 (1H, m, 15-OC<u>H</u>-), 5.43 (1H, dt, *J* = 6.4 Hz and 10.9 Hz, H-11), 5.64 (1H, dd, *J* = 6.9 Hz and 15.2 Hz, H-14), 5.95 (1H, t, *J* = 11.5 Hz, H-12) and 6.46 (1H, dd, *J* = 10.9 Hz and 15.5 Hz, H-13); **^{13}C-NMR (125 MHz, $CDCl_3$)**; δ_C 14.3 (C-4′), 22.8 (C-3′), 25.1 (C-3), 25.6 (C-4), 28.0 (C-10), 29.3 (C-5), 29.4 (C-6), 29.5 (C-7), 29.6 (C-8), 31.7 (C-2′), 34.3 (C-2), 37.5 (C-9), 51.7 (-O<u>C</u>H$_3$), 70.8 (C-1′), 73.1 (C-15), 126.1 (C-13), 127.9 (C-12), 133.3 (C-11), 136.0 (C-14), and 174.5 (C-1); CI-MS: *m/*z 647.4 $[M+H]^+$ {calcd 646.5 for $(C_{20}H_{35}O_3)_2$} and *m/z* 585.2 $[M-31]^+$ {calcd 585.9 for $(C_{19}H_{32}O_2)_2$}.

Dichloromethane extract (BSD)

A part of BSD (5.12 g) was separated by SiO_2-MPLC using $CHCl_3$/MeOH in a stepwise gradient ($CHCl_3$/MeOH 100:0, 99:1, 98:2, 95:5, 90:10, 80:20, 50:50, 0:100) to obtain eight fractions. Fr.5 (414.2 mg) was subjected to the SiO_2-MPLC with $CHCl_3$/MeOH gradient system (methanol: 0–100%) to afford eight fractions (Fr.5.1 to Fr.5.8). Then, Fr.5.4 (0.414 g/ 2.380 g) was subjected to the SiO_2-MPLC using *n*-hexane/EtOAc in a stepwise gradient (EtOAc 0-100%) to afford eight fractions (Fr.5.4.1 to Fr.5.4.8). TLC analysis of Fr.5.4.3 yielded compound **5** (203.3 mg, 3.9707 %) as a white powder. Based on the spectral data and comparison with the literature report (12,13), compound **5** was identified as 4-hydroxybenzaldehyde.



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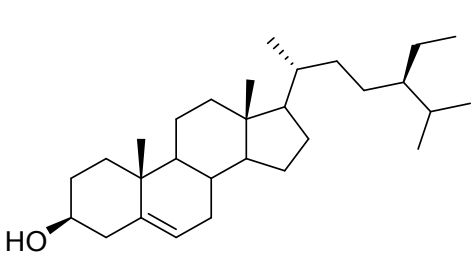

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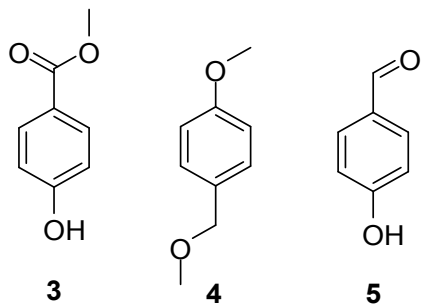


3 **4** **5**

Figure 1: Compounds **1-5** isolated from *Dendrocalamus asper*. Compound **1** to **4** is from the BSH extract and compound **5** is from the BSD extract.

Antimalarial activity

Plasmodium falciparum 3D7 were cultured at 37 °C under 5.0% CO_2 an 5% O_2 in 3% hematocrit t-type A human red blood cells (Japanese Red Cross Society) in RPMI1640 (Thermo Fisher Scientific), supplied with 0.4% glucose, 20 µg/mL hypoxanthine, 24 µg/mL gentamicin, and 0.25% AlbuMax-II [14,15]. The, *P. falciparum* growth assay was performed by suspending 100 µL of 0.3%-parasitized red blood cells (as above) and 2% hematocrit in a 96-well plate for 72 h; the plates were frozen overnight at -70 ⌗C and then thawed at room temperature for 4 h. An amount of 150 µL of the reaction mixture (166 mM sodium *L*-lactate, 166 µM 3-acetylpyridine adenine dinucleotide, 208 µM Nitro Blue Tetrazolium Chloride, 150 µg/mL diaphorase (22.5 U/mL), 0.8% Tween 20, 116 mM Tris-HCl, pH 8.0) was then freshly prepared and added into the wells to analyze LDH activity. After 10 minutes of incubation at room temperature, the plates were shaken to ensure mixing and the absorbance at 650 nm using a plate reader (PerkinElmer) was recorded.

Molecular docking

Molecular docking studies were performed using AutoDock v. 4.2.2 to identify appropriate binding modes and conformation of the ligand molecule. The crystal structure of *Plasmodium falciparum* DHFR-TS complexed with pyrimethamine (PDB code: 3QG2) was retrieved from the RCSB protein data bank as a PDB format (https://www.rcsb.org/structure/3QG2) (16). Preparation of protien was carried out following the steps described elsewhere (17). All hetero atoms and water molecules were deleted using PyMol(version 1.3) software packages whereas, energy minimization of the protein was carried out using Swiss-Pdb viewer software (version 4.1.0). The structures of all the ligands were drawn using Chemdraw Ultra 13.0 and converted into 3D structures using Hyperchem Pro 8.0 software (www.hyper.com). Autodock Tools (ADT) version 1.5.6 (www.autodock.scrips.edu) was used to prepare the molecular docking. The active site was considered as a rigid molecule, while the ligands were treated as being flexible. Finally, rigid docking simulation was performed by AutoDock software considering the center grid box size of 27x6x67 in the x, y and z coordinates. The best binding con-formation was selected from the docking log (.dlg) file for each ligand and further interaction analysis was done using PyMol and Discovery Studio Visualizer 4.0.

RESULTS AND DISCUSSION

Chemistry

Extraction followed by chromatographic fractionation of *Dendrocalamus asper* yielded one new compound; (11*Z*,13*E*,17*E*,19*Z*)-dimethyl-15,16-dibutoxytriconta-11,13,17,19-tetraenedioate **(1)**, and four known compounds; *β*-sitosterol **(2)** (18), methyl-4-hydroxybenzoate **(3)** (19,20), 1-methoxy-4-(methoxymethyl)benzene **(4)** (21) and 4-hydroxybenzaldehyde **(5)** (22) (**Figure 1**)**.** The last four known compounds have not been previously isolated from this plant species. The structure of **1** was elucidated by FTIR, 1D and 2D NMR, and CI-MS.

The FTIR of compound **1** exhibited transmission bands at 3013, and 2927 cm^{-1} due to the C-H sp^2 and C-H sp^3 stretching. Besides that, the transmission band at 1712 cm^{-1} indicated the presence of the carbonyl group (C=O) stretching of the methylated fatty acid ester moiety attached to compound **1**. Also, the band at 1221 cm^{-1} was attributed to the C-O bond stretching vibration, while the band at 1093 cm^{-1} was assigned to the in-plane bending of the C-H bond.

The ^{1}H NMR spectrum of compound **1** exhibits four signals at δ_H 6.46 ppm (1H, dd, *J* = 10.9 Hz and 15.5 Hz), 5.95 ppm (1H, t, *J* = 11.5 Hz), 5.64 ppm (1H, dd, *J* = 6.9 Hz and 15.2 Hz), 5.43 ppm (1H, dt, *J* = 7.5 Hz and 10.9 Hz) which represents the four vinylic protons, H-13, H-12, H-14, and H-11. H-11 and H-14 protons are bonded to an sp^3 hybridized carbon, C-10, and C-15, respectively. So, they are shielded and absorb upfield compared to H-12 and H-13. H-13 has two nearby non-equivalent protons that split its signal, the *trans* protons of H-12 and H-14. H-14 splits the H-13 signal into a doublet, and H-12 proton splits the doublet into two doublets, forming doublet of the doublet. At the same time, H-12 has two nearby non-equivalent protons, the *cis* proton H-11, and *trans* proton H-13. H-13 is more deshielded than H-12 due to the hydrogen in a *cis*-isomer being slightly more upfield and *trans* hydrogen being more downfield to the left of the spectrum. Two singlet signals located at δ_H 3.64 (3H) and δ_H 3.62 (2H) were assigned to the -OC$\underline{H_3}$ and 1′-OC$\underline{H_2}$. The ^{13}C NMR of compound **1** shows the presence of 20 signals. The signals for twelve methylene carbons (C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-1′, C-2′, and C-3′) can be observed at δ_C 34.3, 25.1, 25.6, 29.3, 29.4, 29.5, 29.6, 37.5, 28.0, 70.8, 31.7, and 22.8, respectively. Meanwhile, the signal for carbonyl carbon, C-1 showed a resonance at δ_C 174.5. The ^{1}H-^{1}H COSY and HMBC correlations of compound **1** are shown in **Figure 2**. The HMBC spectrum reveals the aliphatic proton, H-14 at δ_H 5.64, correlated with C-12 (δ_C 127.9) and C-15 (δ_C 73.1). The signal for methyl proton (4′-C$\underline{H_3}$) showed cross-peak with C-2′ (δ_C 31.7)

and C-3′ (δ_C 22.8). Besides, the singlet methoxy proton (-OC<u>H</u>$_3$) at δ_H 3.64 assigned to -O<u>C</u>H$_3$ (δ_C 51.7) showed HMBC correction with the carbonyl carbon, C-1 (δ_C 174.5).

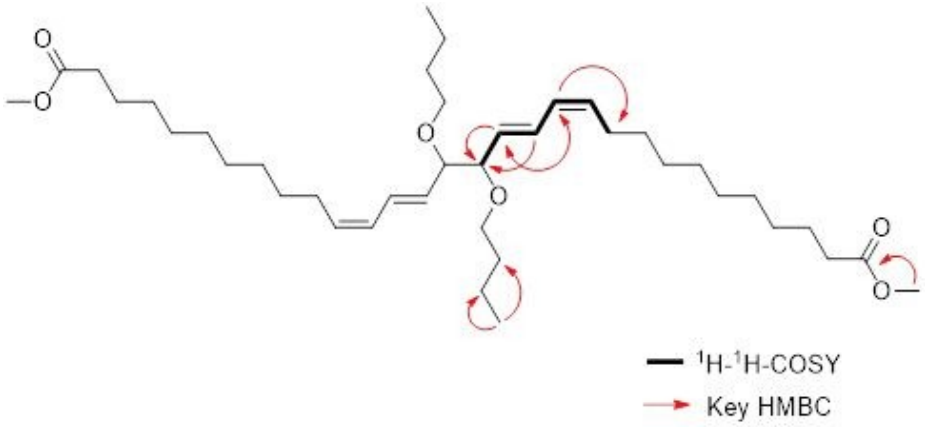


Figure 3. Key COSY/ HMBC correlations of compound **1**.

The nominal APCI-MS spectra of compound **1** were in good agreement with the molecular formula $(C_{20}H_{35}O_3)_2$ showing a base peak at *m/z* 646.4 $[M+H]^+$ {calcd 646.5 for $(C_{20}H_{35}O_3)_2$}. The ion peak at *m/z* 585.6 arise due to the $(C_{19}H_{32}O_2)_2$ fragment, by the loss of two methoxy radicals $[M+H-62]^+$ (**Figure 3**). Based on the spectroscopy data (IR, 1D- and 2D-NMR) and mass spectrometric data, compound **1** is a new diester isolated for the first time from the plant and its chemical name is (11*Z*,13*E*,17*E*,19*Z*)-dimethyl-15,16-dibutoxytriconta-11,13,17,19-tetraenedioate.

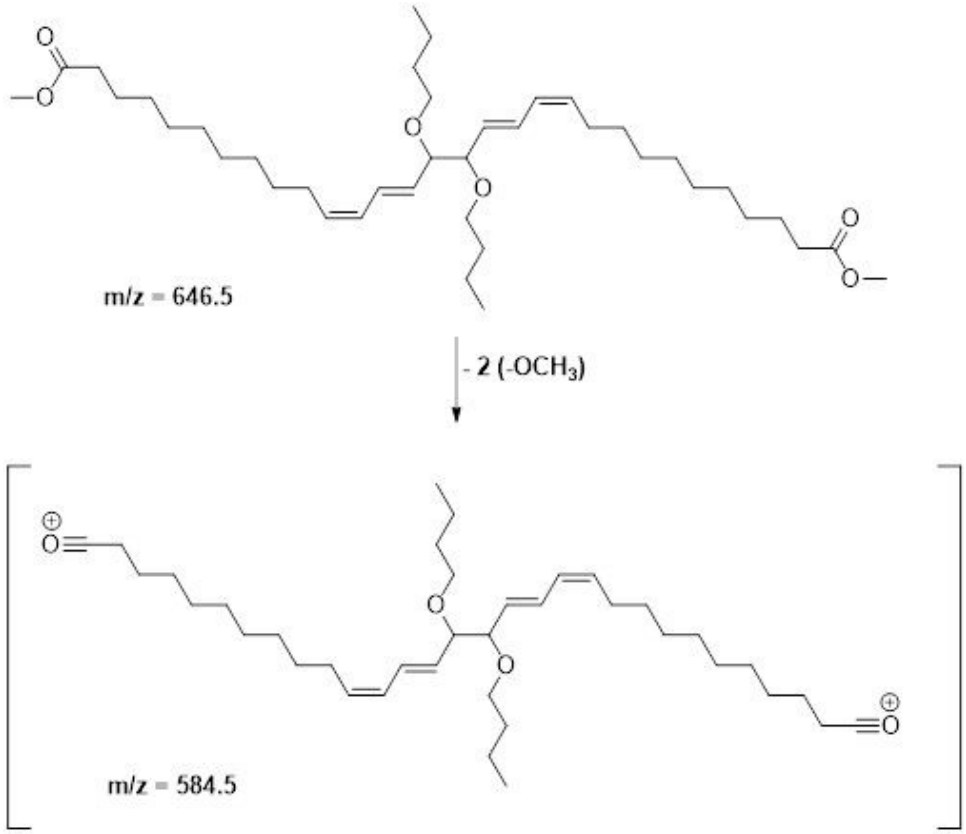


Figure 3. The fragmentation of compound **1** leading to the ion peak m/z 584.5.

Antimalarial activity

All the five isolated chemical constituents of *Dendrocalamus asper* were evaluated for their potential antimalarial properties against the *P. falciparum* strain 3D7 (**Table 2**). Compound **3** was the most active compound against the *P.*

falciparum with the IC_{50} value of 5 µM (~0.82 µg/mL). Meanwhile, compounds **1** and **4** showed moderate antimalarial activity with the IC_{50} values of 3 µM (~2.2 µg/mL) and 7 µM (~1.1 µg/mL), respectively. However, compounds **2** and **5** showed weak antimalarial activity with 50 % inhibition (IC_{50}) value > 24 µM (>10 µg/mL) and > 82 µM (>10 µg/mL), respectively.

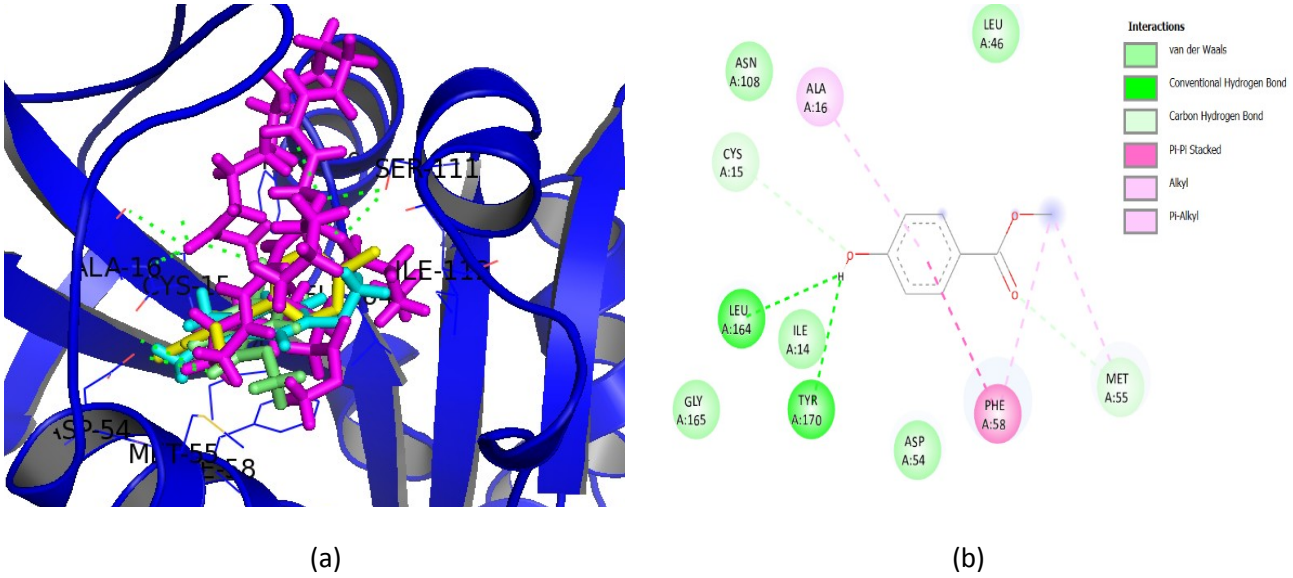
Table 2. Growth inhibitory activity of compounds **1**-**5** against *P. falciparum* 3D7.

Compound	IC_{50}	
	µg/mL	**µM**
1	2.2	3
2	>10	>24
3	0.8	5
4	1.1	7
5	>10	>82
Chloroquine (control)	0.01	0.05

Results are mean values of duplicate independent assays

Molecular docking studies

To gain further evidence regarding the mode of action of the potent compounds (i.e **1**, **3** and **4**) a molecular docking study was carried out on *P. falciparum* enzyme dihydrofolate reductase-thymidylate synthase (DHFR-TS), which is a potential drug target for malaria (16). All the three compounds **1** (-4.20 kcal/mol), **3** (-5.4 kcal/mol), and **4** (-5.2 kcal/mol) showed good interactions with the enzyme DHFR-TS in terms of binding interactions. It was also observed that all the three compounds **1**,**3**, and **4** occupied the same binding site and formed similar type of interactions with the active site residues (**Figure 4**). The results of binding studies of the most active compound **3** clearly indicate that the compound exhibited significant interactions at the active site by forming two hydrogen bonds with LEU164 and TYR170 residues (docking score of -4.16 kcal/mol). A π-π stacking was also observed between the phenyl ring of compound **3** and PHE58. The results of molecular docking studies revealed that the antimalarial activity of compound **1**, **3** and **4** might be due to DHFR-TS inhibition.



(a) (b)

Figure 4. (a) Docking poses of compound **1** (magenta), **3** (lime), **4** (cyan) and reference ligand pyrimethamine (yellow) at the active site of *P. Falciparum* DHFR-TS (PDB ID: 3QG2) showing hydrogen bondings (green). (b) 2D binding interactions of the most active compound **3** showing two hydrogen bonds (green) with LEU164 and TYR170 residues at the active site.

CONCLUSION

Five bioactive principles were isolated from bamboo shoot extracts, of which one new metabolite, compound **1,** and three known metabolites, which are compounds **2**-**4** were isolated from *n*-hexane extract, whereas one known metabolite, compound **5** was obtained from dichloromethane extract. Among these, compound **3** was highly active against *P. falciparum* with IC_{50} value of 0.82 µg/mL.

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