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Natural Products from Stem Bark of *Calophyllum andersonii*

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ABSTRACT

Phytochemical study on the stem bark of *Calophyllum andersonii* has resulted in the isolation of five xanthones, namely (1) caloxanthone I, (2) pyranojacareubin, (3) macluraxanthone, (4) caloxanthone C, and (5) euxanthone. In this study, the compounds were subjected to various spectroscopic analyses including FT-IR, GC-MS, 1D and 2D NMR for structural elucidations. Furthermore, these xanthones were obtained for the first time from *Calophyllum andersonii,* a plant never reported before. All four extracts, namely hexane, chloroform, ethyl acetate and methanol extracts of the plant showed moderate inhibitions against *Bacillus subtilis*.

Keywords: Anti-microbial, *calophyllum andersonii*, xanthones

INTRODUCTION

The genus *Calophyllum* falls under the family of Clusiaceae. *Calophyllum* is known to contain rich amounts of secondary metabolites such as xanthones, coumarins and triterpenes (Kashman et al. 1992; Patil et al., 1993). This genus also produces some chromanones and steroids (Su et al., 2008). Although this genus has been studied extensively, researchers are still looking for new potential lead compounds to be used for drug discovery studies. In fact, new xanthones and coumarins are still being discovered from the *Calophyllum* species (Aminudin, Ahmad, Taher, & Zulkifli, 2016; Li et al., 2016). Moreover, novel

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phenolic compounds with new carbon skeletons have also been discovered lately (Nguyen et al., 2016). The genus is also well-known by its medicinal properties such as anti-HIV (Kashman et al., 1992), anti-microbial (Kawamura, Muhamud, Hashim, Sulaiman, & Ohara, 2012; Morel et al., 2001;), anti-cancer (Guilet et al., 2001; Mah et al., 2012), as well as anti-oxidant (Alkhamaiseh, Taher, Ahmad, Susanti, & Ichwan, 2011).

MATERIALS AND METHOD

Plant Material

The 2 kg stem bark of Calophyllum andersonii was collected from the Semenggok Forest Reserve in Semenggok, Kuching, Sarawak, Malaysia. An identification process was carried out on the plant sample by Dr Vivien Jong and a voucher specimen (DV01) was deposited at the Herbarium, Centre of Applied Science Studies, Universiti Teknologi Mara Sarawak Branch, Kuching, Malaysia.

General

A Leica Galen III instrument was used in determining the melting points. The infrared spectra were obtained on a Perkin-Elmer 100 Series FT-IR spectrometer using universal attenuated total reflection technique. Electron-ionised mass spectrometry was conducted using a Shimadzu GCMS-QP 5050A spectrometer at 80 to 200°C. The column used in the experiment was SGE BPX5 of dimensions 30 m x 0.25 mm I.D x 0.25 μm film thickness. The UV spectra were recorded in ethanol using a Shimadzu UV-160A, UV-Visible Recording Spectrophotometer. NMR spectral analyses were carried out using JEOL 500MHz and 400MHz FT-NMR spectrometers, using $CDCl_3$ as well as acetone-d_6 as solvents and tetramethylsilane (TMS) as internal standard.

Extraction and Isolation

The dried stem bark of *Calophyllum andersonii* was ground into a total of two kg of fine powder. The extraction process on *Calophyllum andersonii* was carried out using four solvents consecutively, which were hexane, chloroform, ethyl acetate and methanol. The duration for each extraction was 72 hours. This was repeated three times for each solvent before the next solvent of higher polarity was introduced accordingly. The extracts were then concentrated by the removal of the solvents under reduced pressure. The yields of the extracts were 36.2 g of hexane extract, 73.0 g of chloroform extract, 14.4 g of ethyl acetate extract and 123.0 g of methanol extract. The crude extracts of *Calophyllum andersonii* were subjected to column chromatography using a stepwise gradient solvent system (hexane/chloroform, chloroform/ethyl acetate, ethyl acetate/methanol). The collected fractions were then monitored by TLC and fractions with similar characteristics were combined and further purified. The hexane and chloroform extracts that were subjected to gravity column chromatography resulted in 34 and 43 fractions respectively. The

third fraction from the hexane extract was subjected to a column packed with Sephadex Lipophilic LH-20 with methanol as the eluting solvent. This resulted in calaxanthone C (4). Then, fraction 6 from the hexane extract was purified using a small gravity column with chloroform as eluting solvent to obtain caloxanthone I (1). Fractions 16-23 from the hexane extract were combined and subjected to gravity column chromatography. This in turn caused seven fractions. Fraction 3 and 4 were then combined and purified to obtain pyranojacareubin (2). Fraction 25 and 26 from the hexane extract were combined and further purified with Sephadex Lipophilic LH-20 in methanol to yield macluraxanthone (3). Lastly, fraction 16 from the chloroform extract was subjected to gravity column chromatography with chloroform/ethyl acetate-80:20 as the eluting solvent to give four fractions. The second and third fractions were combined and further purified with Sephadex Lipophilic LH-20 with methanol as eluting solvent to obtain euxanthone (5).

Caloxanthone I (1): Whitish yellow amorphous powder. EIMS *m/z* (% intensity): 460[M^+] (34), 445 (100), 417 (19), 405 (12), 215 (12), 187 (15). IR λ_{max} cm^{-1}, uATR: 3336, 2970, 1591, 1450. ^1H NMR (400MHz, $CDCl_3$) and ^{13}C NMR (100MHz, $CDCl_3$): refer Table 1.

Table 1
^1H NMR (400MHz, $CDCl_3$), ^{13}C NMR (100MHz, $CDCl_3$) and HMBC correlations for caloxanthone I (1)

Position	δ_H	δ_C	*HMBC*
1		155.9	
2		104.6	
3		158.0	
4		107.6	
4a		154.1	
5a		145.3	
5		132.4	
6		144.6	
7		117.7	
8	7.45 (*s*, 1H)	113.4	
8a		114.5	
9		180.7	
9a		103.0	
10	6.73 (*d*, 1H, *J* = 8.3Hz)	115.9	C-3, C-12
11	5.58 (*d*, 1H, *J* = 8.3Hz)	127.3	C-2, C-12
12		78.0	
13	1.47 (*s*, 6H)	28.4	C-11, C-12 & 14
14			
15	6.42 (*d*, 1H, *J* = 10.3Hz)	121.5	C-6, C-7, C-8, C-17
16	5.71 (*d*, 1H, *J* = 10.3Hz)	130.9	C-7, C-17
17		78.8	
18	1.52 (*s*, 6H)	28.5	C-16, C-17& 19
19			
1’	3.51 (*d*, 2H, *J* = 5.7Hz)	21.6	C-3, C-4, C-4a, C-2’, C-3’
2’	5.28 (*t*, 1H, *J* = 5.7Hz)	122.4	
3’		131.6	
4’	1.86 (*s*, 3H)	25.9	C-2’, C-3’
5’	1.68 (*s*, 3H)	17.9	C-2’, C-3’
1-OH	13.23 (*s*, 1H)		
5-OH	5.47 (*s*, 1H)		C-5, C-5a

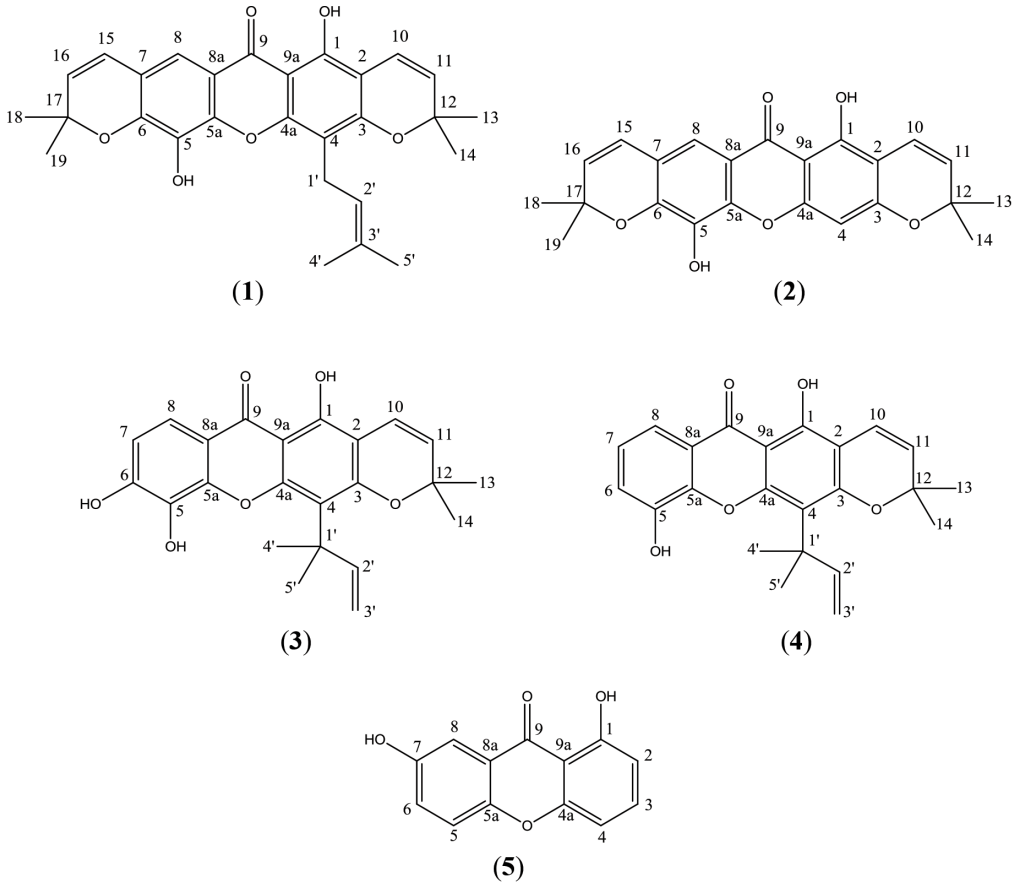

Figure 1. Structures of Caloxanthone I (**1**), Pyranojacareubin (**2**), Macluraxanthone (**3**), Caloxanthone C (**4**) and Euxanthone (**5**)

Pyranojacareubin(2): Yellowish white crystal; m.p. 260-261°C (Lit. 259-260°C, Monache*et al*., 1984). EIMS *m/z* (% intensity): 392[M^+] (18), 377 (100), 361 (11), 347 (8), 181 (27). IR λ_{max} cm^{-1}, uATR: 3232, 2958, 1602, 1462, 1284. ^{1}H NMR (500MHz, $CDCl_3$): 13.28 (*s*, 1H, OH-1), 7.46 (*s*, 1H, H-8), 6.71 (*d*, *J*= 10.1Hz, 1H, H-10), 6.42 (*d*, *J* = 9.6Hz, 1H, H-15), 6.41 (*s*, 1H, H-4), 5.71 (*d*, *J* = 10.1Hz, 1H, H-16), 5.58 (*d*, *J* = 9.2Hz, 1H, H-11), 5.54 (*s*, 1H, 5-OH), 1.52 (*s*, 6H, H-18 &19), 1.46 (*s*, 6H, H-13 &14).^{13}C NMR (125MHz, $CDCl_3$): 180.3 (C-9), 160.5 (C-3), 157.7 (C-1), 156.9 (C-4a), 145.1 (C-5a), 144.8 (C-6), 132.1 (C-5), 131.0 (C-16), 127.6 (C-11), 127.4 (C-15), 117.8 (C-7), 115.5 (C-10), 114.7 (C-8a), 113.5 (C-8), 104.8 (C-2), 103.3 (C-9a), 95.4 (C-4), 79.0 (C-17), 78.2 (C-12), 28.5 (C-18 & 19), 28.4 (C-13 &14).

Macluraxanthone (3): Yellow crystal needles; m.p. 202-204 °C (Lit. 200-201 °C, Wolfrom et al., 1964). EIMS *m/z* (%

intensity): 394[M^+] (38), 379 (100), 337 (11), 325 (26). IR λ_{max} cm^{-1}, uATR: 3174, 2927, 1589, 1448. ^{1}H NMR (400MHz, $CDCl_3$): 13.51 (*s*, 1H, 1-OH), 7.76 (*d*, *J* = 8.3Hz, 1H), 6.93 (*d*, *J* = 8.3Hz, 1H), 6.76 (*d*, *J* = 10.1Hz, 1H), 6.72 (*dd*, *J* = 17.4 & 11.0Hz, 1H), 6.26 (*s*, 1H, 6-OH), 5.92 (*s*, 1H, 5-OH), 5.60 (*d*, *J* = 10.1Hz, 1H), 5.18 (*dd*, *J* = 17.3 & 1.8Hz, 1H), 5.04 (*dd*, *J* = 11.0 & 1.8Hz, 1H), 1.63 (*s*, 6H), 1.50 (*s*, 6H). ^{13}C NMR (100MHz, $CDCl_3$): 180.8 (C-9), 159.0 (C-3), 156.9 (C-2'), 156.8 (C-1), 154.1 (C-4a), 149.0 (C-6), 144.6 (C-5a), 131.1 (C-5), 127.2 (C-11), 117.5 (C-8), 116.1 (C-10), 113.7 (C-8a), 113.1 (C-4), 112.8 (C-7), 105.6 (C-2), 103.3 (C-3'), 103.1 (C-9a), 78.3 (C-12), 41.5 (C-1'), 28.2 (C-4' & 5'), 28.0 (C-13 & 14).

Caloxanthone C (4): Fine yellow needles: m.p. 201-203°C (Lit. 201.5°C, Iinuma, Tosa, Tanaka, & Yonemori, 1994). EIMS *m/z* (% intensity): 378[M^+] (29), 363 (100), 335 (8), 154 (13). IR λ_{max} cm^1, uATR: 3441, 2936, 1606, 1426, 1283. ^{1}H NMR (400MHz, $CDCl_3$): 13.42 (*s*,1H, 1-OH), 7.69 (*dd*, *J* = 6.4 & 1.8Hz, 1H, H-8), 7.24 (*d*, *J* = 7.3 & 1.8Hz, 1H, H-6), 7.22 (*t*, *J* = 6.4Hz, 1H, H-7), 6.77 (*d*, *J* = 8.2Hz, 1H, H-10), 6.69 (*dd*, *J* = 14.7 & 8.2Hz, 1H. H-2'), 6.39 (*s*, 1H, 5-OH), 5.62 (*d*, *J* = 8.2Hz, H-11), 5.22 (*d*, *J* = 14.7Hz, 1H, H-3'a), 5.06 (*d*, *J* = 8.2Hz, 1H, H-3'b), 1.64 (*s*, 6H, H-4' & 5'), 1.51 (*s*, 6H, H-13 & 14). ^{13}C NMR (100MHz, $CDCl_3$): 181.4 (C-9), 159.4 (C-3), 156.7 (C-1), 155.8 (C-2'), 154.0 (C-4a), 145.4 (C-5), 144.2 (C-5a), 127.4 (C-11), 124.2 (C-7), 120.5 (C-8a), 119.7 (C-6), 116.1 (C-8), 116.0 (C-10), 113.2 (C-4), 105.6 (C-2), 104.1 (C-3'), 103.6 (C-9a), 78.4 (C-12), 41.4 (C-1'), 28.3 (C-13 & 14), 28.0 (C-4' & 5').

Euxanthone (5): Fine yellowish orange needles; m.p. 228-229°C (Lit. 226-229°C, Fujita, Liu, Ueda, & Takeda, 1992). EIMS *m/z* (% intensity): 228 [M^+] (100), 200 (19), 144 (15), 115 (23). IR λ_{max} cm^{-1}, uATR: 3452, 2924, 1610, 1477, 1232. ^{1}H NMR (400MHz, $CDCl_3$): 12.69 (*s*, 1H, 1-OH), 8.96 (*s*, 1H, 7-OH), 7.67 (*t*, *J* = 8.3Hz, 1H, H-3), 7.58 (*d*, *J* = 2.7Hz, 1H, H-8), 7.50 (*d*, *J* = 9.2Hz, 1H, H-5), 7.41 (*dd*, *J* = 9.3 &3.7Hz, 1H, H-6), 6.97 (*d*, *J* = 8.3Hz, 1H, H-4), 6.74 (*d*, *J* = 8.3Hz, 1H, H-2). ^{13}C NMR (100MHz, $CDCl_3$): 182.1 (C-9), 161.9 (C-1), 156.5 (C-4a), 154.1 (C-7), 150.2 (C-5a), 137.0 (C-3), 125.3 (C-6), 121.0 (C-8a), 119.4 (C-5), 109.7 (C-2), 108.3 (C-9a), 106.9 (C-4).

Anti-Microbial Activity

Agar diffusion disc method was used for the anti-microbial test. Tests were carried out on six bacteria, which were *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Serratia marcencens and Salmonella choleraesuis*. For the growth media, Mueller-Hinton agar (MHA) was used. Chlorhexidine (0.5mg/ml) was used as the positive control and dimethyl sulfoxide (DMSO) as the negative control. The culture was standardised to 0.5 MacFarland standards. The streaking of microbes was carried out using a sterile spreader on a petri-dish with MHA prepared and solidified. A paper disc was then positioned on the petri-dish followed

by the sample extract (10 µl) dropped onto it. The concentrations of the sample extracts were 10 mg per 1.0 ml of DMSO for every extract (hexane, chloroform, ethyl acetate, methanol). After that, the petri-dish was incubated at 37°C for 24 hours in an inverted position. The clear inhibition zone was measured around the paper disc as the area where no microbes were growing. The tests were repeated three times to obtain the mean values and standard deviations.

RESULTS AND DISCUSSION

Caloxanthone I (1) was obtained from the hexane and chloroform extracts. The compound appeared as a whitish yellow amorphous powder.

The EIMS spectrum of the compound exhibited a molecular ion peak at *m/z* 460, which corresponded to the molecular formula $C_{28}H_{26}O_6$. The IR analysis gave several bands that are common in all xanthones, which are at 3336, 2970, 1591and 1450cm^{-1}.

From the ^{1}H NMR spectrum, a chelated hydroxyl group (1-OH) and an aromatic proton (H-8) were revealed from two deshielded signals at δ_H 13.23 and δ_H 7.45 respectively. Besides, a pair of doublets at δ_H 6.73 (J = 8.3Hz, 1H, H-10) and δ_H 5.58 (J = 8.3Hz, 1H, H-11) showed the presence of a pyrano ring. Similarly, the occurrence of two doublets at δ_H 6.42 (J = 10.3Hz, 1H, H-15) and δ_H 5.71 (J = 10.3Hz, 1H, H-16) were due to another pyrano group in the structure. The presence of a prenyl moiety was justified by a series of proton signals which resonated at δ_H 3.51 (*d*, J = 5.7Hz, 2H, H-1'), δ_H 5.28 (*t*, J = 5.7Hz, 1H, H-2'), δ_H 1.86 (*s*, 3H, H-4') and δ_H 1.68 (*s*, 3H, H-5').

From the ^{13}C NMR spectrum, it was observed that there were 28 carbons in the structure. The DEPT spectrum revealed six methines, one methylene, six methyl groups and 15 quaternary carbons.

The position of one of the pyrano rings at C-2 and C-3 was confirmed from their long range correlations to H-11 and H-10 respectively. For another pyrano ring attached to C-6 and C-7, their ^{2}J and ^{3}J correlations to H-6, H-7 and H-8 confirmed this assignment. The correlations occurring between H-1' and C-3, C-4, C-4a supported that the prenyl side chain was attached to C-4.

Based on the information obtained from the NMR analysis, the compound was elucidated to be caloxanthone I (1) that was previously isolated from the plant *Calophyllum apetalum* (Iinuma et al., 1997).

Pyranojacareubin (2) was obtained from the hexane and chloroform extracts as yellowish white crystals with melting point at 260 to 261°C. The EIMS spectrum recorded a molecular ion peak at *m/z* 392, a molecular weight which corresponds to the molecular formula $C_{23}H_{20}O_6$. The IR spectrum showed absorptions at 3232, 2958, 1602 and 1462 cm^{-1}.

From the ^{1}H NMR, two hydroxyl protons were revealed by two separate signals at δ_H 13.51 (*s*, 1H, 1-OH) and δ_H 5.54 (*s*, 1H, 5-OH). Two pairs of doublets

at δ_H 6.71 (*d*, *J* = 9.7Hz, 1H, H-10), δ_H5.58 (*d*, *J* = 9.7Hz, 1H, H-11) and δ_H 6.42 (*d*, *J* = 9.9Hz, 1H, H-15), δ_H5.71 (*d*, *J* = 9.9Hz, 1H, H-16) indicated the presence of two pyrano rings in the structure.^{13}C NMR spectrum showed that there are 23 carbons present in the structure whereas the DEPT spectrum showed that there are six methines, four methyl groups and 13 quaternary carbons. Several ^3J correlations also proved that the two pyrano rings are attached to C-2 and C-3, C-6 and C-7 respectively. As a result, the compound was elucidated to be pyranojacareubin (2) that was previously isolated from *Rheedia gardneriana* (G. D. Monache, F. D. Monache, Waterman, Crichton, & Lima, 1984).

Macluraxanthone (3) was isolated from the hexane extract as yellow crystal needles with melting point of 202-204°C. The EIMS spectrum showed a molecular ion peak at *m/z* 394, which corresponds to the molecular formula $C_{23}H_{22}O_6$. The IR spectral analysis showed absorption at 3174, 2927, 1589 and 1448 cm^{-1}.

From the ^{1}H NMR, a chelated hydroxyl group was revealed by the occurrence of a downfield singlet at δ_H13.52 (*s*, 1H, 1-OH). Other than that, a pair of doublets at δ_H 6.76 (*d*, *J* = 10.0 Hz, 1H, H-10) and δ_H 5.60 (*d*, *J* = 10.0 Hz, 1H, H-11) pointed to the existence of a pyrano ring in the structure. The ^{13}C NMR spectrum revealed that there are 23 carbons in the structure. The DEPT spectrum showed that there are five methines, one methylene, four methyl groups and 13 quaternary carbons. From the HMBC, long range correlations proved that the pyrano ring is attached to C-2 and C-3. ^3J correlations between H-2' and C-4 justified the position of the prenyl moiety. The NMR data of the compound corresponds to the literature data of the same compound isolated from *Maclura pomifera* Raf. (Wolfrom et al., 1964).

Caloxanthone C (4) was isolated from the hexane extract of *Calophyllum andersonii* as yellow needles melting at 201-203°C. The EIMS recorded the molecular ion peak at *m/z* 378, a molecular weight that corresponds to the molecular formula $C_{23}C_{22}O_5$. The IR spectrum showed absorption bands at 3441, 2936, 1606 and 1426 cm^{-1}.

A chelated hydroxyl proton that is responsible for a rather deshielded signal at δ_H 13.42 (*s*, 1H, 1-OH) was observed from the ^{1}H NMR spectrum. Similarly, the presence of a pyrano ring was revealed by a pair of doublets at δ_H 6.77 (*d*, *J* = 8.2Hz, 1H, H-10) and δ_H 5.62 (*d*, *J* = 8.2Hz, 1H, H-11). A prenyl moiety was discovered from a series of signals at δ_H 6.69 (*dd*, *J* = 14.7 & 8.2Hz, 1H. H-2'), δ_H5.22 (*d*, *J* = 14.7Hz, 1H, H-3'a), δ_H5.06 (*d*, *J* = 8.2Hz, 1H, H-3'b) and δ_H1.64 (*s*, 6H, H-4' & 5'). The ^{13}C NMR indicated that there are 23 carbons in the structure. On the other hand, the DEPT spectrum showed that there are six methines, one methylene, four methyl groups and 12 quaternary carbons. ^3J correlations between H-4', H-5' and C-4 justified the suggested position of the prenyl moiety. Long range correlation between H-10 and C-3, H-11 and C-2 proved that the pyrano ring is

indeed attached to the suggested position. Thus, the compound was elucidated and confirmed as caloxanthone C (4) by comparison to data of compounds isolated from *Calophyllum inophyllum* (Iinuma et al., 1994).

The final compound, euxanthone (5) was isolated from the chloroform extract as yellowish orange needles with melting point recorded at 228 to 229°C. The molecular ion peak observed from the EIMS spectrum was recorded at *m/z* 228 which corresponds to the molecular formula $C_{13}H_8O_4$. From the IR spectrum, absorption bands at 3452, 2924, 1610 and 1477 cm^{-1} were observed.

From the ^1H NMR spectrum, a chelated hydroxyl proton was observed from the signal at δ_H 12.69 (*s*, 1H, 1-OH). A total of 13 carbons were deduced from the ^{13}C NMR. From the DEPT spectrum, there are six methines and 7 quaternary carbons in the structure. From the HMBC, hydroxyl proton of 1-OH is ^2J correlated to C-1 and ^3J correlated to C-2, further proving the position of the hydroxyl group. Lastly, the NMR data of the compound corresponds to literature data of euxanthone (5) isolated from *Polygala tenuifolia* (Fujita et al., 1992).

All the extracts were also tested for their antimicrobial activities against six bacterial strains, namely *bacillus subtilis*, *staphylococcus aureus, pseudomonas aeruginosa, escherichia coli, serratia marcencens and salmonella choleraesuis*. The clear inhibition zones observed on *B. subtilis* are around 10 mm across all extracts, which are comparable to the inhibition of the standard, which is 10.6 ± 0.2 mm. Hence, all the four extracts tested can be said to be moderately active against *B. subtilis*. However, all the extracts showed negative results on all the other bacterial strains that were tested. The activities of the plant extracts against the *B. subtilis* are believed to be contributed by the abundant presence of xanthones. The results are tabulated in Table 2.

Table 2
Anti-microbial activities of extracts against target microbes via disc diffusion method

Samples	Target microbes					
	I	II	III	IV	V	VI
Hexane extract	9.0 ± 0.82	-	-	-	-	-
Chloroform extract	10.3 ± 0.47	-	-	-	-	-
Ethyl acetate extract	10.3 ± 0.47	-	-	-	-	-
Methanol extract	9.6 ± 0.54	-	-	-	-	-
Chlorhexidine	10.6 ± 0.54	12.0 ± 0.0	8.3 ± 0.47	11.0 ± 0.82	11.3 ± 0.47	10.6 ± 0.54

Note: All results measured are in millimetre (mm)
Values presented are means of triplicate determinations ± standard deviation (SD)
The concentrations of samples are 10 mg/ml and 0.5mg/ml for the standard
I: *Bacillus subtilis*
II: *Staphylococcus aureus*
III: *Pseudomonas aeruginosa*
IV: *Escherichia coli*
V: *Serratia marcencens*
VI: *Salmonella choleraesuis*
-: No bacterial effect

CONCLUSION

The phytochemical study conducted on *Calophyllum andersonii* has resulted in the isolation of five xanthones: (1) caloxanthone I, (2) pyranojacareubin, (3) macluraxanthone, (4) caloxanthone C, and (5) euxanthone. All these compounds were isolated from the stem bark of *Calophyllum andersonii* for the first time. This is also a first report on this plant. The clear inhibition zones of all the extracts on *B. subtilis* were around 10 mm. This is comparable to that of the standard at 11 mm. Therefore, all four extracts can be regarded as moderately active against *bacillus subtilis*. Thus, the plant can be studied further for its anti-microbial and biological properties as there is potential for it to contribute to the development of new drugs.

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REFERENCES

Alkhamaiseh, S. I., Taher, M., Ahmad, F., Susanti, D., & Ichwan, S. J. A. (2011). Antioxidant and cytotoxic activities of calophyllum rubiginosum. *International Journal of Phytomedicine, 3*(2), 157-163.

Aminudin, N. I., Ahmad, F., Taher, M., & Zulkifli, R. M. (2016). Incrassamarin A-D: Four new 4-substituted coumarins from calophyllum incrassatum and their biological activities. *Phytochemistry Letters*, *16*, 287-293.

Fujita, T., Liu, D. Y., Ueda, S., & Takeda, Y. (1992). Xanthones from polygala tenuifolia. *Phytochemistry, 31*(11), 3997-4000.

Guilet, D., Helesbeux, J. J., Seraphin, D., Sevenet, T., Richomme, P., & Brunetone, J. (2001). Novel cytotoxic 4-phenylfuranocoumarins from calophyllum dispar. *Journal of Natural Products, 64*(5), 563-568.

Iinuma, M., Ito, T., Tosa, H., Tanaka, T., Miyake, R., & Chelladurai, V. (1997). New linear pyranoxanthones from calophyllum apetalum. *Heterocycles, 45*(2), 299-307.

Iinuma, M., Tosa, H., Tanaka, T., & Yonemori, S. (1994). Two new xanthones in the underground part of calophyllum inophyllum. *Heterocycles, 37*(2), 833-838.

Kashman, Y., Gustafson, K. R., Fuller, R. W., McMahon, J. B., Currens, M. J., Buckheit, J. R., ... & Boyd, M. R. (1992). The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, Calophyllum lanigerum. *Journal of Medicinal Chemistry, 35*(15), 2735-2743.

Kawamura, F., Muhamud, A., Hashim, R., Sulaiman, O., & Ohara, S. (2012). Two antifungal xanthones from the heartwood of Calophyllum symingtonianum. *Japan Agricultural Research Quarterly, 46*(2), 181-185.

Li, Z. L., Li, Y., Qin, N. B., Li, D. H., Liu, Z. G., Liu, Q., & Hua, H. M. (2016). Four new coumarins from the leaves of Calophyllum inophyllum. *Phytochemistry Letters*, *16*, 203-206.

Mah, S. H., Ee, G. C. L., Teh, S. S., Rahmani, M., Lim, Y. M., & Go, R. (2012). Phylattrin, a new cytotoxic xanthone from Calophyllum soulattri. *Molecules, 17*(7), 8303-8311.

Morel, C., Séraphin, D., Teyrouz, A., Larcher, G., Bouchara, J. P., Litaudon, M., ... & Bruneton, J. (2002). New and antifungal xanthones from Calophyllum caledonicum. *Planta Medica, 68*(01), 41-44.

Monache, G. D., Monache, F. D., Waterman, P. G., Crichton, E. G., & Lima, R. A. D. (1984). Minor xanthones from Rheedia gardneriana. *Phytochemistry*, *23*(8), 1757-1759.

Nguyen, L. T. T., Lai, N. T., Nguyen, L. T., Nguyen, H. T., Nguyen, D. M., & Nguyen, L. H. D. (2016). Thoreliolides A and B, two polyisoprenylated benzoylphloroglucinol derivatives with a new carbon skeleton from the fruits of Calophyllum thorelii. *Tetrahedron Letters, 57*(25), 2737-2741.

Patil, A. D., Freyer, A. J., Eggleston, D. S., Haltiwanger, R. C., Bean, M. F., Taylor, P. B., ... & Bartus, H. R. (1993). The inophyllums, novel inhibitors of HIV-1 reverse transcriptase isolated from the Malaysian tree, Calophyllum inophyllum Linn. *Journal of Medicinal Chemistry*, *36*(26), 4131-4138.

Su, X. H., Zhang, M. L., Li, L. G., Huo, C. H., Gu, Y. C., & Shi, Q. W. (2008). Chemical constituents of the plants of the genus Calophyllum. *Chemistry and Biodiversity, 5*(12), 2579-2608.

Wolfrom, M. L., Dickey, E. E., McWain, P., Thompson, A., Looker, J. H., Windrath, O. M., & Komitsky Jr, F. (1964). Osage orange pigments. XIII. Isolation of three new pigments from the root bark. *The Journal of Organic Chemistry*, *29*(3), 689-691.