SHORT REPORT

A C G publications

Rec. Nat. Prod. 10:5 (2016) 649-653

records of natural products

Cytotoxic and Antibacterial Activities of Constituents from *Calophyllum ferrugineum* Ridley

Nurul Iman Aminudin[1], Farediah Ahmad[*1], Muhammad Taher[2] and Razauden Mohamed Zulkifli[3]

[1]*Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia*
[2]*Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200, Kuantan, Pahang, Malaysia*
[3]*Department of Bioscience and Health Sciences, Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, 81310, Skudai, Johor, Malaysia*

(Received November 20, 2015; Revised January 12, 2016; Accepted January 13, 2016)

Abstract: This study evaluated the chemical composition of *Calophyllum ferrugineum*, cytotoxicity against human breast cancer (MCF-7) and human lung carcinoma (A-549) cell lines as well as antibacterial activities against two *Gram*-positive bacteria, *S. aureus and B. subtilis* and two *Gram*-negative bacteria, *P. aeruginosa* and *E. coli*. Phytochemical investigations of the bark extract yielded isoapetalic acid **(1)**, apetalic acid **(2)**, 6-hydroxy-2-methoxyxanthone **(3)** and *ent*-epicatechin **(4)**. Meanwhile, betulinic acid **(5)**, protocatechuic acid **(6)** and amentoflavone **(7)** were isolated from the leave extract. Isoapetalic acid **(1)** and apetalic acid **(2)** exhibited cytotoxic activities towards both cancer cell lines and both *Gram*-positive bacteria. Compounds **(3-7)** were inactive or showed moderate activities towards cytotoxic and antibacterial tests. This study presents the first report on the phytochemicals investigation from *C. ferrugineum* and all compounds are reported for the first time from this source.

Keywords: *Calophyllum ferrugineum*; cytotoxic activities; antibacterial activities.

1. Plant Source

Calophyllum is one of the genera from Guttiferae family and normally used as timber. *Calophyllum ferrugineum* is an evergreen tree that grows in lowland or colline mixed dipterocarp forest up to 30 meters tall. It can be found around the Southeast Asia especially in Malaysia. The bark is used traditionally as a decoction for a mother after childbirth [1]. This paper describes the first isolation and characterization of pure chemical constituents from the barks and leaves of *C. ferrugineum*. The cytotoxic and antibacterial activities of all chemical constituents were also evaluated. The plant was collected from Lenggong, Perak with a voucher specimen number

[*] Corresponding author: E- Mail:farediah@kimia.fs.utm.my (F. Ahmad), Phone +6075534137

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 03/01/2016 EISSN: 1307-6167

SK2587/14. The plant was identified by Dr. Shamsul Khamis and deposited at the herbarium of Universiti Putra Malaysia.

2. Previous Studies

A bioactivity-guided isolation of the ethyl acetate extract of an endophytic fungus 9PR2 isolated from internal root tissue of *C. ferrugineum* was reported [2]. The extract of 9PR2 was tested for cytotoxic effect against murine leukaemic cell line P388 by MTT assay. It led to the isolation of seven known polyesters identified as (+)-6-hydroxymellein, 15G256V, 15G256α-1, 15G256α-2, 15G256n, 15G256α, and 15G256β from the fungi based on dereplication process using HPLC.

3. Present Study

The air-dried barks (2.70 kg) and leaves (2.30 kg) of *C. ferrugineum* were macerated with dichloromethane, ethyl acetate, and methanol for three days respectively. The extracts were filtered and concentrated under reduced pressure to yield dichloromethane bark extract (127.16 g, 9.79%), ethyl acetate bark extract (8.86 g, 0.33%), methanol bark extract (208.40 g, 7.72%), dichloromethane leave extract (65.91 g, 2.87%), ethyl acetate leave extract (21.40 g, 0.93%) and methanol leave extract (178.61 g, 7.77%). The dichloromethane bark extract (2 g) was purified by using CC (*n*-hexane: Et_2O, 9:1) to afford 460 fractions. Fractions 85 – 105 were pooled together to give isoapetalic acid **(1)** (187 mg, 9.35%) as yellow gum [3] while fractions 230 – 440 were combined to yield apetalic acid **(2)** (427 mg, 21.37%) as yellow gum [4].

The ethyl acetate bark extract (8.86 g) was fractionated by VLC eluted with *n*-hexane-$CHCl_3$-EtOAc in increasing polarity by 10% to give seven major fractions. The purification of the fifth fraction (0.22 g) by CC (*n*-hexane: EtOAc, 9:1) gave 200 subfractions. Further purification of subfractions 78 – 150 (23.5 mg) by sephadex LH-20 eluted with MeOH in isocratic mode yielded 6-hydroxy-2-methoxyxanthone **(3)** (2.9 mg, 0.03%) as a pale yellow amorphous [5]. Compound **(3)** was previously reported as synthetic compound and this is the first time it was isolated from natural sources. The sixth fraction (0.36 g) was subjected to CC (*n*-hexane: EtOAc, 9:1) to give 500 fractions. The purification of subfractions 460 – 495 by sephadex LH-20 with MeOH in isocratic mode and solidification in the mixture of *n*-hexane and EtOAc furnished *ent*-epicatechin **(4)** (165 mg, 1.87%) as a pale brown amorphous [6, 7].

The fractionation of the dichloromethane leave extract (50 g) with VLC using *n*-hexane-$CHCl_3$-EtOAc as eluent systems in increasing polarity by 10% gave seven major fractions. The sixth fraction (0.85 g) was purified by CC (*n*-hexane: EtOAc, 9:1) to afford 406 subfractions. Subfractions 131 – 175 were mixed and washed with cold *n*-hexane to produce betulinic acid **(5)** (130 mg, 0.25%) as white solid [8]. The ethyl acetate leave extract (20 g) was fractionated by VLC with *n*-hexane/EtOAc as eluents in 10% stepwise gradient to yield nine major fractions. The seventh fraction (0.86 g) was subjected to CC (*n*-hexane:EtOAc, 3:2) to furnish protocatechuic acid **(6)** (36 mg, 0.18%) as a yellow needle [9]. The purification of the eighth fraction (2.27 g) over CC (*n*-hexane:EtOAc, 3:7) gave amentoflavone **(7)** (381 mg, 1.91%) as yellow amorphous [10].

Cytotoxic activity: The cytotoxic activity was evaluated by MTT colorimetric assay. The percentage of cell viability for both cell lines after treatment with all compounds for 24 hours was compared with the untreated control cell. The IC_{50} value acted as a parameter for a cytotoxic activity where it indicated 50% cell inhibition by the compound. Isoapetalic acid **(1)** and apetalic acid **(2)** gave similar IC_{50} values against A-549 cell lines at 249.04 and 249.43 μM, respectively (Table 1). In contrast, apetalic acid **(2)** was more toxic towards MCF-7 cell lines with IC_{50} values 100.36 μM compared to isoapetalic acid **(1)** at 151.60 μM. However, the mechanism exerted by both compounds towards both cell lines was not investigated in this study. It has been reported that other derivatives of chromanone carboxylic acid such as calophynic acid, brasiliensic acid, and inophylloidic acid, previously isolated from *C. inophyllum* were found to exhibit significant cytotoxic activities against KB cell line with IC_{50} values between 9.7 – 11.0 μM [11]. It signifies the potential of chromanone carboxylic acid

derivatives unique to *Calophyllum* species to be further investigated as a potent cytotoxic agent. Meanwhile, amentoflavone displayed cytotoxic activity against MCF-7 cell lines at IC_{50} 176.80 µM. This finding was supported by a study that reported amentoflavone showed significant cytotoxicity against MCF-7 at 150 µM and as a potent apoptosis-inducing agent in MCF-7 *via* mitochondria-dependent pathway [12]. On the other hand, *ent*-epicatechin **(4)** and protocatechuic acid **(6)** displayed more than 80% cell viability at the highest concentration tested thus, were devoid to the cytotoxic test of both cell lines.

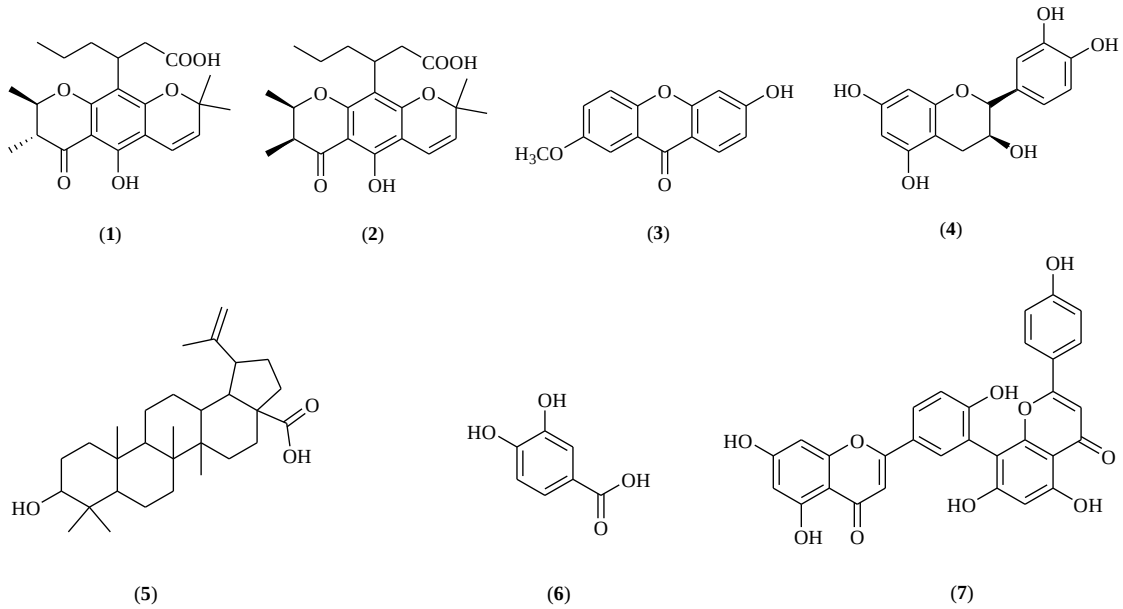

Table 1. Cytotoxic activity of the tested samples.

Compounds	MCF-7		A-549	
	IC (%)[a]	IC_{50} (µM)[b]	IC (%)[a]	IC_{50} (µM)[b]
1	26.64 ± 0.74[c]	151.60 ± 4.30	47.68 ± 1.80[c]	249.04 ± 5.21
2	3.79 ± 1.02[c]	100.36 ± 1.07	47.60 ± 1.19[c]	249.43 ± 3.24
3	NT	NT	NT	NT
4	98.98 ± 1.51[c]	ND	88.97 ± 6.86[c]	ND
5	56.76 ± 1.94[c]	ND	44.81 ± 1.72[c]	99.18 ± 5.12
6	94.95 ± 0.64[c]	ND	80.34 ± 3.12[c]	ND
7	47.29 ± 0.59[c]	176.80 ± 2.43	64.99 ± 5.69[c]	ND

[a] Cell viability at 100 µg/mL (highest concentration tested) as mean ± SD of triplicate experiments; [b] IC_{50} is defined as the concentration where the cell viability is reduced to 50% compared to the control group. IC_{50} is reported in µM unit; [c] The result is statistically significant different compared to control, $p < 0.05$; NT = Not tested; ND = Not Determined

Antibacterial activity: The antibacterial activity of all compounds was determined by minimum inhibition concentration (MIC) in serial broth microdilution. Two *Gram*-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* and two *Gram*-negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli* were employed for this study. The tetrazolium (INT) colorimetric microplate assay method was chosen as it enhances sensitivity and accuracy of MIC determination since the formation of violet formazan derivatives by living bacteria can be quantified and yield greater reproducible result [13]. The MIC result as tabulated in Table 2 displayed isoapetalic acid **(1)** and apetalic acid **(2)** that exhibited antibacterial activities against *Gram*-positive bacteria, *S. aureus* and *B. subtilis* both at 31.25 µg/mL. According to Rios [14], pure compounds with MIC value < 100 µg/mL is considered to be active. No inhibition was observed against *P. aeruginosa* and *E. coli*. It indicates that both compounds were more selective towards *S. aureus* and *B. subtilis*, *Gram*-positive

bacteria tested in this study. The presence of *n*-pentyl with carboxylic acid moiety at end-terminal substituent in the structure increases the lipophilic character of the compound. *Gram*-positive microorganisms have low lipid content in the cell wall, thus, permit the high activity of more lipophilic compounds to reach their active site causing its disruption [15]. The previous report on the isolation of six chromanone acids from *C. brasiliense* demonstrated moderate-to-strong antibacterial activity especially to *Gram*-positive bacteria [16], thus, supports the findings. The structure activity study of a series of lipophilic *N*-acyldiamines towards antibacterial activity showed that the best results obtained were from compounds having 10 – 12 carbons alkyl chains [17]. Both flavonoids; *ent*-epicatechin **(4)** and amentoflavone **(7)** showed moderate inhibition towards all bacteria in the range of 62.5 – 500 µg/mL.

Table 2. Minimum inhibition concentration (MIC)[a] of the tested samples.

Compounds	*Gram* positive		*Gram* negative	
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
1	250	31.25	>1000	>1000
2	31.25	31.25	>1000	>1000
3	NT	NT	NT	NT
4	500	500	125	250
5	>1000	>1000	>1000	>1000
6	NT	NT	NT	NT
7	125	500	62.5	62.5
Streptomycin Sulphate[b]	12.5	1.56	50	0.78

[a] Minimum inhibition concentration (µg/mL) in triplicate; [b] Streptomycin sulphate as standard control; NT = Not tested

Acknowledgements

The authors would like to thank the Ministry of Higher Education Malaysia for financial support through GUP2526.06H34 as well as Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia and Department of Pharmaceutical Technology, Kulliyyah of Pharmacy, International Islamic University Malaysia for the technical support and research facilities.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] P. F. Stevens (1980). Journal of the Arnold Arboretum. Edwards Brothers, Inc., Ann Arbor, Michigan, 117-424.

[2] S. Sultan, S. Adnan, A. L. I. Shah, L. I. N. Sun, K. Ramasami, A. Cole, J. Blunt, M. H. G. Munro and J. F. F. Wber (2011). Bioactive fungal metabolites of 9PR2 isolated from roots of *Callophyllum ferrugineum*, *Int. J. Pharm. Pharm. Sci.* **3**, 5-7.

[3] M. H. Reyes, M. C. Basualdo, F. Abe, J. M. Estrada, C. Soler and R. R. Chilpa (2004). HIV-1 Inhibitory Compounds from *Calophyllum brasiliense* Leaves, *Biol. Pharm. Bull*. **27**, 1471-1475.

[4] Y. S. Hen, L. W. Ang, T. K. Halil and Y. K. Uo (2004). Chromanones and dihydrocoumarins from *Calophyllum blancoi*, *Chem.Pharm. Bull.* **52**, 402-405.

[5] A. Peter and H. Per (1972). Xanthone studies III. Synthesis of some hydroxy and methoxy substituted xanthones, *Dansk Tidsskr Farm*. **46**, 133-148.

[6] B. A. Shamuratov, S. M. Mavlyanov, D. N. Dalimov and M. K. Allaniyazova (2003). Polyphenols from certain *Gossypium hirsutum* varieties, *Chem. Nat. Compd.* **39**, 597-598.

[7] J. Z. Deng, D. J. Newman and S. M. Hecht (2000). Use of COMPARE analysis to discover functional analogues of bleomycin, *J. Nat. Prod.* **63**, 1269-1272.

[8] C. Venkata, S. Prakash and I. Prakash (2012). Isolation and Structural Characterization of Lupane Triterpenes from *Polypodium Vulgare*, *Res. J. Pharm. Sci.* **1**, 23-27.

[9] H. L. Zhang, A. Nagatsu, H. Okuyama, H. Mizukami and J. Sakakikabara (1998). Sesquiterpene Glycosides from Cotton Oil Cake, *Phytochemistry* **48**, 665-668.
[10] J. R. Hanrahan, M. Chebib, N. L. M. Davucheron, B. J. Hall and G. A. R. Johnston (2003). Semisynthetic preparation of amentoflavone: A negative modulator at GABAA receptors, *Bioorg. Med. Chem. Lett.* **13**, 2281-2284.
[11] M. C. Yimdjo, A. G. Azebaze, A. E. Nkengfack, A. M. Meyer, B. Bodo and Z. T. Fomum (2004). Antimicrobial and cytotoxic agents from *Calophyllum inophyllum*, *Phytochemistry* **65**, 2789-2795.
[12] J. Pei, C. Liu, Y. Hsu, L. Lin, S. C. Wang, J. G. Chung, D. T. Bau and S. S. Lin (2012). Amentoflavone induces cell-cycle arrest and apoptosis in MCF-7 human breast cancer cells via mitochondria-dependent pathway, *In Vivo.* **26**, 963-970.
[13] P. Masoko, J. Picard and J. N. Eloff (2007). The antifungal activity of twenty-four southern African *Combretum* species (Combretaceae), *South African J. Bot.* **73**, 173-183.
[14] J. L. Ríos and M. C. Recio (2005). Medicinal plants and antimicrobial activity, *J. Ethnopharmacol*. **100**, 80-84.
[15] G. L. Biagi, M. C. Guerra, A. M. Barbaro and M. F. Gamba (1970). Influence of Lipophilic Character on the Antibacterial Activity of Cephalosporins and Penicillins, *J. Med. Chem.* **13**, 511-516.
[16] F. Cottiglia, B. Dhanapal, O. Sticher and J. Heilmann (2004). New Chromanone Acids with Antibacterial Activity from *Calophyllum brasiliense*, *J. Nat. Prod.* **67**, 537-541.
[17] C. Diniz, M. Le, C. G. D. Almeida, G. D. Garbois and L. M. Amaral (2010). Relationship between structure and antibacterial activity of lipophilic N –acyldiamines, *Biomed. Pharmacother.* **64**, 287-290.