

RESEARCH PAPER

Antibacterial Activity of Phytochemicals from *Garcinia parvifolia* Miq. and *Garcinia hombroniana* Pierre

Noor Amira Muhammad^{1*}, Norazah Basar², Shajarahunnur Jamil²

¹Faculty of Applied Sciences, Universiti Teknologi MARA, Perak Branch, Tapah Campus, Tapah Road, 35400, Perak, Malaysia

²Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia (UTM), 81310 Skudai, Johor, Malaysia

*Corresponding author: noora6359@uitm.edu.my

DOI: <https://doi.org/10.37134/jsml.vol7.5.2019>

Received: 1 June 2019; Accepted: 2 July 2019; Published: 10 July 2019

Abstract

Phytochemical studies on the stem bark of *Garcinia parvifolia* and pericarp of *Garcinia hombroniana* have been investigated. The crude extracts were purified using chromatographic techniques to obtain pure compounds named as rubraxanthone (**1**), parvixanthone G (**2**), cowanin (**3**), garcihombronane B (**4**), garcihombronane C (**5**) and stigmaterol (**6**). The structures of all compounds were characterized by spectroscopic methods. The extracts and phytochemicals were tested for their antibacterial activity using disc diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Acetone extract of the pericarp of *G. hombroniana* showed the most significant antibacterial activity with MIC and MBC values of 225 µg/mL, meanwhile compound (**3**) exhibited the most significant antibacterial activity compared to other xanthenes with MBC value of 225 µg/mL.

Keywords: Clusiaceae; *Garcinia parvifolia*; *Garcinia hombroniana*; antibacterial

INTRODUCTION

Genus *Garcinia* which belong to family Clusiaceae are well-known as a source of natural phenolic containing compounds such as xanthenes, biflavonoids, benzophenones and triterpenoids (Niu et al., 2012; Rukachaisirikul et al., 2005; Nguyen et al., 2011). The plant of interest in this study, *Garcinia hombroniana* Pierre and *Garcinia parvifolia* Miq. were discovered to have their traditional usage worldwide. *G. hombroniana* has been known as 'seashore mangosteen' or commonly known as 'manggis hutan'. In Malaysia, the roots and leaves were traditionally used to treat itchiness and treatment of skin diseases (John et al., 2008). *G. parvifolia* which known as 'kandis' was also documented medicinally used to treat fever (Kardono et al., 2006; Rukachaisirikul et al., 2005). Recently, studies on the antimicrobial activity of xanthenes were found only focused on *Staphylococcus aureus* strain (Iinuma et al., 1996). Therefore, in this research antibacterial investigation were tested towards four strain bacteria including *Enterococcus faecalis*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* which examine scientific approach of screening and determining minimum bactericidal concentration (MBC) of each extracts and phytochemicals. Antibacterial investigation on triterpenoids isolated from *G. hombroniana* were first reported in this study.

MATERIALS AND METHODS

General experimental procedures

Fractionation, isolation and purification were done by using silica gel as the stationary phase. Different types of silica gel were required for different usage. For the gravity column chromatography (CC), Keisegel silica with the size 70-230 mesh was used for column packing, whereas silica gel with size 230-400 mesh was used in preparing the stationary phase for vacuum liquid chromatography (VLC). Thin layer chromatography (TLC) was carried out on Merck F₂₅₄ precoated silica gel plate with 0.2 mm thickness. The spots on the TLC plate were visualized by UV lamp (254 nm) and sprayed with vanillin-sulphuric acid. The infrared (IR) spectra were obtained using Perkin-Elmer Spectrum Two (ATR) and Shimadzu 8000 series (FTIR). While the nuclear magnetic resonance (NMR) spectra for ¹H, ¹³C, DEPT, HMBC and HMQC were recorded using the Bruker Avance Spectrometer 400 MHz and deuterated chloroform (CDCl₃) and deuterated acetone (Me₂CO-d₆) as solvents. High resolution mass spectral data were obtained from UCL Spectrometry Service, London, UK. Ultraviolet (UV) analysis was performed by UV-Vis 1601 PC spectrophotometer in methanol. The chromatograms of gas chromatography mass spectrometry (GC-MS) were recorded using a Hewlett Packard Model 5989A mass spectrometer. GC-MS was equipped with Wiley Library Software.

Plant materials

The stem barks of *Garcinia parvifolia* Miq., pericarps and stem barks of *G. hombroniana* Pierre (SK 1961/11) were collected from Sekayu Forest Reserved in Kuala Berang, Terengganu, Malaysia in June 2011. The pericarps of *G. mangostana* Linn. were collected in May 2010 from Johor, Malaysia.

Extraction and isolation

The dried stem bark of *G. parvifolia* (600 g) was extracted sequentially using hot *n*-hexane, CH₂Cl₂ and MeOH for 8 hours each. The extracts obtained were *n*-hexane (GPH) (7.62 g, 0.01%), CHCl₃ (GPC) (68.58 g, 0.11%), and MeOH (GPM) (35.91 g, 0.06%). Each crude extract was then fractionated by vacuum liquid chromatography (VLC) to afford different fractions based on solvent polarity. The GPH (8.5 g) and GPC (8.5 g) were fractionated using sintered funnel (100 mm × 90 mm height) packed with silica gel 230-400 mesh (250 g) eluted with *n*-hexane/Et₂O/EtOAc and *n*-hexane/CH₂Cl₂/MeOH, respectively with polarity increasing gradually to give few fractions. Fraction GPC14 (1.53 g, 0.02%) from VLC was introduced into a column (2.5 cm diameter × 45 cm height) packed with silica gel 70-230 mesh (40 g) eluted by *n*-hexane and CH₂Cl₂ increasing polarity gradually. Subfraction GPC14-134 showed a single spot identified as rubraxanthone (**1**). Fraction GPC18 (2.80 g, 0.04%) was chromatographed over silica gel (50 g), column size (2.0 cm diameter × 45.0 cm length) eluted with *n*-hexane and CH₂Cl₂ to give five subfractions. Subfraction GPC18-50 was then recolumn (1.0 cm diameter × 15 cm length) to yield parvixanthone G (**2**). Fraction GPH16 (2.87 g) from the *n*-hexane crude extract was subjected to column chromatography to give nine subfractions. Subfraction GPH1612 (0.10 g) was then purified (1.0 cm × 15 cm length) using CC packed with silica gel (5.0 g) eluted by *n*-hexane and CH₂Cl₂ to give a minor xanthone, cowanin (**3**).

The powdered pericarp (690 g) and stem barks (900 g) of *G. hombroniana* was shaken consecutively with *n*-hexane, CH₂Cl₂, acetone and CHCl₃ each for 24 hours to yield the crude extracts as *n*-hexane (GHHP) (4.62 g, 0.01%), CH₂Cl₂ (GHDP) (13.33 g, 0.02%), acetone (GHAP) (4.92 g, 0.01%), and CHCl₃ (GHCS) (16.10 g, 0.02%). Fraction GHDP14 (0.43 g)

was purified using CC packed with SiO₂ and eluted with *n*-hexane and EtOAc to give 100 fractions. Fraction 11-16 were combined to give a pale yellow solid, named as garcihombrone B (**4**). Fraction GHDP11 (0.54 g) was subjected to CC and eluted with *n*-hexane and EtOAc to give 200 fractions. Fractions 78-93 (0.09 g) were combined and purified by preparative TLC to yield garcihombrone C (**5**). The GHHP crude extract (2.0 g) was purified by CC packed with SiO₂ and eluted with *n*-hexane and Et₂O to give 200 fractions. Fractions 97-144 were combined and recrystallized from cold *n*-hexane to give stigmasterol (**6**).

Antibacterial assay

Four bacteria were used in the antimicrobial assay named as *Enterococcus faecalis* (ATCC19433), *Bacillus subtilis* (ATCC6633), *Escherichia coli* (ATCC10536), *Klebsiella pneumoniae* (ATCC13883), which are two gram-positive and two gram-negative bacteria, respectively. The bacteria stock concentration was referred to the McFarland standard solution cloudiness. The sample solution was pipetted (10 µL) onto the disc (Whatman paper disc size 6 mm) and left to dry. Every sample was duplicated while DMSO and streptomycin sulphate (SS) act as control. Bacteria stock (400 µL) was pipetted onto the surface of the agar nutrient. After that, the petri plate was kept in the incubator for 18 hours at 37°C. After 18 hours, the zone where there was no bacterial infection was measured. Clear inhibition zones around the discs indicated the presence of antimicrobial activity.

Minimum inhibition concentration (MIC) was determined by the broth micro dilution method. The test was performed in sterile 96-well microplates. Each sample (3.6 mg) were dissolved in DMSO (2 mL) to get 1800 µg/mL stock solution. A number of wells were reserved in each plate for positive and negative controls. Sterile broth nutrient (100 µL) was added to each microplate well from row B to H. The stock solution of samples (100 µL) was added to microplate wells at row A and B. Then, the mixture of samples and sterile broth (100 µL) at row B was transferred to each microplate well in order to obtain a two-fold serial dilution of the stock samples from A to H (concentration of 1800-14.06 µg/mL), respectively. Each bacteria (100 µL) was added into well from A to H according their types, and each bacteria was duplicated. The sealed 96-well plates were incubated at 37°C for 16-20 hours. Microbial growth was indicated by the presence of turbidity and a pellet at the bottom of the well. The MIC value was determined as the lowest concentrations that did not show any microbial growth (Mbaveng et al., 2008).

Aligians et al. (2001) proposed a classification for the antimicrobial activity of plant products, based on MIC results as follows: strong inhibitors-MIC below 500 µg/mL, moderate inhibitors-MIC between 600 and 1,500 µg/mL, weak inhibitors-MIC above 1,600 µg/mL. The samples from the MIC study which did not show any growth of bacteria was pipetted (10 µL) from each well and subcultured on the surface of the freshly prepared nutrient agar. The nutrient agar solution (5 mL) was placed in 50 mm × 15 mm disposable petri plate. Then, the sealed petri plates were incubated for 16-20 hours at 37°C. After 16-20 hours, the number of survived organisms was determined. MBC was defined as the lowest exact concentration at which 99.9 % of the bacteria were killed (Mbaveng et al., 2008).

RESULTS AND DISCUSSION

Phytochemical study on stem barks of *G.parvifolia* was successfully isolated three known xanthenes, named as rubraxanthone (**1**), parvixanthone G (**2**) and cowanin (**3**) on the basis of spectral analysis and data comparison with literature (Ee et al., 2009; Iinuma et al., 1996; Xu

et al., 2001; Pattalung et al., 1994). Two triterpenoids identified as garcihombronane B (**4**) and garcihombronane C (**5**) together with a plant sterol, stigmasterol (**6**) was isolated from pericarps of *G. hombroniana* which the structure was confirmed by GC-MS analysis and comparison with previous reported data (Vieriraa et al., 2004; Najmuldeen et al., 2011). The chemical structures of the isolated phytochemicals are shown in Figure 1.

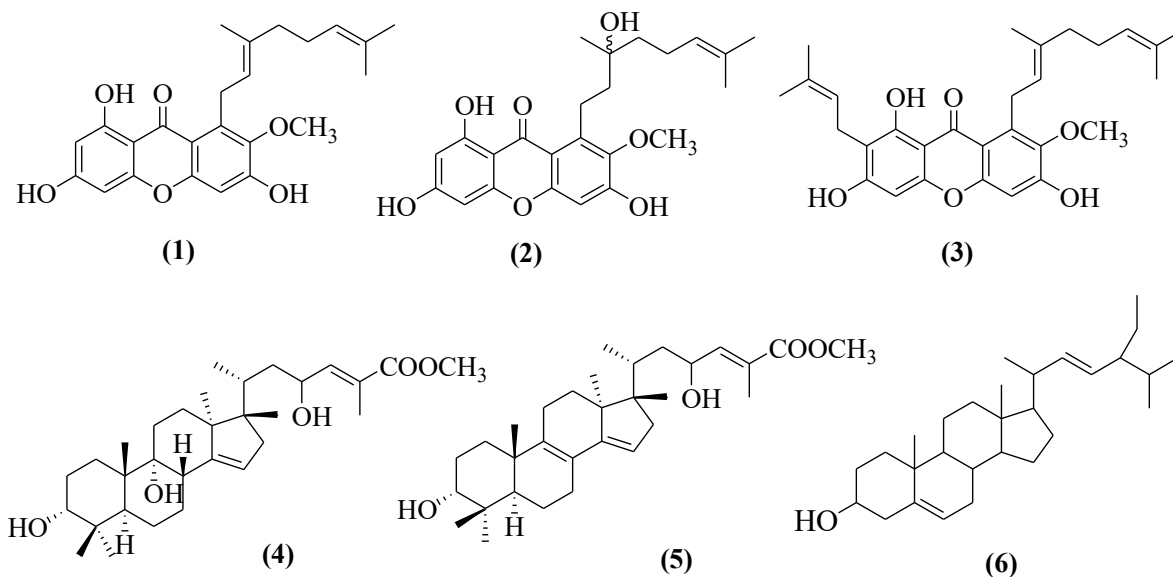


Figure 1. The chemical structures of compounds (1) – (6)

Rubraxanthone (**1**) (1.06 g, 0.13%) as a yellow crystal, m.p. 203-206°C; IR (neat) ν_{\max} cm^{-1} : 3298, 2924, 1645, 1587, 1442, 1276; ^1H NMR (acetone- d_6 , 400 MHz): 1.53 (3H, s, H-19), 1.56 (3H, s, H-18), 1.83 (3H, s, H-20), 1.98 (2H, m, H-14), 2.06 (2H, m, H-15), 3.81 (3H, s, OCH₃), 4.12 (2H, d, $J = 6.2$ Hz, H-11), 5.04 (1H, t, $J = 1.2$ Hz, H-16), 5.29 (1H, t, $J = 6.2$ Hz, H-12), 6.20 (1H, d, $J = 2.0$ Hz, H-2), 6.30 (1H, d, $J = 2.0$ Hz, H-4), 6.83 (1H, s, H-5), 9.60 (2H, br s, 3-OH, 6-OH), 13.50 (1H, s, 1-OH); ^{13}C NMR (acetone- d_6 , 100 MHz): 15.7 (C-20), 16.8 (C-18), 24.9 (C-19), 25.9 (C-11), 26.4 (C-15), 39.6 (C-14), 60.5 (7-OCH₃), 92.9 (C-4), 97.8 (C-2), 101.9 (C-5), 102.9 (C-9a), 111.1 (C-8a), 123.9 (C-12), 124.3 (C-16), 130.6 (C-17), 134.2 (C-13), 137.4 (C-8), 143.7 (C-7), 155.4 (C-10a), 156.6 (C-6), 157.1 (C-4a), 164.0 (C-1), 164.5 (C-3), 181.8 (C-9); EIMS m/z (rel. int.): 410 [M^+ , C₂₄H₂₆O₆] (24), 341 (100); HREIMS m/z [M^+] calcd. for C₂₄H₂₆O₆: 410.17239; found 410.17264.

Parvixanthone G (**2**) (0.01 g, 0.001%) as yellow powder, m.p. 204-207°C; IR (neat) ν_{\max} cm^{-1} : 3219, 2932, 1645, 1600, 1454, 1272; ^1H NMR (acetone- d_6 , 400 MHz): δ 1.32 (3H, s, H-19), 1.58 (2H, m, H-14), 1.65 (3H, s, H-20), 1.68 (3H, s, H-18), 1.78 (2H, m, H-12), 2.21 (2H, m, H-15), 3.43 (2H, dd, $J = 6.4, 10.0$ Hz, H-11), 3.86 (3H, s, 7-OCH₃), 5.18 (1H, t, $J = 7.2$ Hz, H-16), 6.19 (1H, d, $J = 1.4$ Hz, H-2), 6.30 (1H, d, $J = 1.4$ Hz, H-4), 6.83 (1H, s, H-5), 9.70 (2H, br s, 3-OH, 6-OH), 13.50 (1H, s, 1-OH); ^{13}C NMR (acetone- d_6 , 100 MHz): δ 16.9 (C-20), 22.0 (C-11), 22.5 (C-15), 25.0 (C-18), 26.5 (C-19), 41.6 (C-14), 42.5 (C-12), 60.6 (7-OCH₃), 71.6 (C-13), 92.6 (C-4), 97.9 (C-2), 101.8 (C-5), 102.8 (C-9a), 111.0 (C-8a), 125.3 (C-16), 130.3 (C-17), 139.1 (C-8), 143.7 (C-7), 155.4 (C-10a), 156.7 (C-6), 157.1 (C-4a), 163.9 (C-1), 164.6 (C-3), 181.9 (C-9); HREIMS m/z [$\text{M}-1$]⁺ found for C₂₄H₂₇O₇: 427.1720.

Cowanin (**3**) (0.005 g, $6.0 \times 10^{-4}\%$) as yellow powder, m.p. 132-134°C; IR (neat) ν_{\max} cm^{-1} : 3372, 2923, 1645, 1601, 1437, 1274; ^1H NMR (acetone- d_6 , 400 MHz): 1.51 (3H, s, H-24), 1.53 (3H, s, H-23), 1.66 (3H, s, H-15), 1.83 (3H, s, H-14), 1.90 (3H, s, H-25), 1.99 (2H, m, H-19), 2.06 (2H, m, H-20), 3.48 (2H, d, $J = 6.8$ Hz, H-11), 3.81 (3H, s, 7-OCH₃), 4.13 (2H, d, $J = 5.2$ Hz, H-16), 5.03 (1H, m, H-21), 5.28 (2H, m, H-12, H-17), 6.30 (1H, s, H-4), 6.93 (1H, s, H-5), 9.70 (2H, br s, 3-OH, 6-OH), 13.43 (1H, s, 1-OH); ^{13}C NMR (acetone- d_6 , 100 MHz): 15.5 (C-25), 16.7 (C-23), 17.4 (C-14), 21.1 (C-11), 24.8 (C-24), 25.0 (C-15), 25.9 (C-16), 26.4 (C-20), 39.5 (C-19), 60.4 (7-OCH₃), 97.4 (C-4), 102.0 (C-5), 103.0 (C-9a), 105.5 (C-2), 110.8 (C-8a), 122.6 (C-12), 123.9 (C-17), 124.1 (C-21), 130.7 (C-18), 134.2 (C-22), 134.3 (C-13), 137.2 (C-8), 143.7 (C-7), 154.2 (C-4a), 155.5 (C-10a), 156.6 (C-6), 161.5 (C-1), 161.9 (C-3), 182.2 (C-9); EIMS m/z (rel. int.): 478 [M^+ , C₂₉H₃₄O₆] (6).

Garcihombronane B (**4**) (0.02 g, 0.0014%); m.p. 129-132°C; IR (neat) ν_{\max} cm^{-1} : 3425, 2945, 1697, 1648, 1245; ^1H NMR (CDCl₃, 400 MHz): δ 0.78 (3H, s, H-18), 0.87 (3H, s, H-28), 0.93 (3H, s, H-30), 0.94 (3H, d, $J = 6.8$ Hz, H-21), 0.98 (3H, s, H-29), 1.26 (3H, s, H-19), 1.89 (3H, d, $J = 1.2$ Hz, H-27), 2.30 (3H, m, H-16, H-8), 1.35-2.35 (19H, m, H-1, H-2, H-5, H-6, H-7, H-8, H-11, H-12, H-16, H-20, H-22), 3.41 (1H, brs, H-3), 3.77 (3H, s, OCH₃), 4.58 (1H, m, H-23), 5.35 (1H, br s, H-15), 6.73 (1H, qd, $J = 6.8, 1.2$ Hz, H-24), ^{13}C NMR (CDCl₃, 100 MHz): δ 12.7 (C-27), 15.1 (C-21), 15.3 (C-18), 16.4 (C-19), 19.4 (C-30), 20.8 (C-6), 22.0 (C-29), 23.6 (C-1), 25.1 (C-2), 25.6 (C-7), 28.5 (C-28), 29.0 (C-12), 29.6 (C-11), 33.0 (C-20), 37.5 (C-4), 39.0 (C-5), 39.2 (C-22), 39.2 (C-8), 42.2 (C-10), 44.7 (C-16), 49.1 (C-13), 51.9 (C-31), 54.0 (C-17), 66.7 (C-23), 75.6 (C-9), 76.1 (C-3), 120.4 (C-15), 126.9 (C-25), 144.7 (C-24), 153.6 (C-14), 168.6 (C-26); EIMS m/z (rel. int.): 502 [M^+ , C₃₁H₅₀O₅] (3), 484 (13).

Garcihombronane C (**5**) as white powder (0.01 g, 0.0005%); m.p. 109-111°C; IR (neat) ν_{\max} cm^{-1} : 3411, 2927, 1697, 1648, 1245; ^1H NMR (CDCl₃, 400 MHz): δ 0.78 (3H, s, H-18), 0.91 (3H, s, H-28), 0.93 (3H, s, H-30), 0.96 (3H, s, H-21), 1.01 (3H, s, H-29), 1.04 (3H, s, H-19), 1.90 (3H, s, H-27), 1.25-2.50 (18H, m, H-1, H-2, H-5, H-6, H-7, H-11, H-12, H-16, H-20, H-22), 3.48 (1H, m, H-3), 3.78 (3H, s, OCH₃), 4.60 (1H, m, H-23), 5.30 (1H, s, H-15), 6.74 (1H, m, H-24); ^{13}C NMR (CDCl₃, 100 MHz): δ 12.7 (C-27), 15.3 (C-21), 15.6 (C-18), 17.1 (C-19), 18.2 (C-6), 19.0 (C-30), 22.2 (C-29), 22.7 (C-22), 25.6 (C-12), 26.7 (C-7), 28.0 (C-28), 29.3 (C-2), 30.1 (C-1), 33.4 (C-20), 37.6 (C-4), 37.8 (C-10), 39.5 (C-11), 44.5 (C-5), 45.6 (C-16), 48.0 (C-13), 50.1 (C-17), 51.9 (C-31), 66.9 (C-23), 75.9 (C-3), 115.8 (C-15), 122.9 (C-8), 127.2 (C-25), 142.4 (C-24), 144.3 (C-9), 148.8 (C-14), 168.4 (C-26); EIMS m/z (rel. int.): 484 [M^+ , C₃₁H₄₈O₄] (20).

Stigmasterol (**6**) (0.02 g, 0.01%) as white crystalline needles, m.p. 141-143°C; IR (neat) ν_{\max} cm^{-1} : 3411, 2934, 1666, 1042; ^1H NMR (CDCl₃, 400 MHz): δ 0.71 (3H, s, H-18), 0.87 (3H, s, H-29), 0.85-1.02 (9H, m, H-21, H-26, H-27), 1.02 (3H, s, H-19), 1.10-2.30 (26H, m, H-1, H-2, H-4, H-7, H-8, H-9, H-11, H-12, H-14, H-15, H-16, H-17, H-20, H-28), 3.54 (1H, m, H-3), 5.04 (1H, dd, $J = 15.2, 8.8$ Hz, H-22), 5.17 (1H, dd, $J = 15.2, 8.8$ Hz, H-23), 5.37 (1H, br s, H-6); ^{13}C NMR (CDCl₃, 100 MHz): δ 12.0 (C-18), 12.2 (C-29), 19.4 (C-27), 21.1 (C-11), 21.2 (C-19), 23.1 (C-4), 24.3 (C-26), 24.4 (C-15), 25.4 (C-28), 28.9 (C-16), 31.7 (C-2), 31.7 (C-7), 31.9 (C-8), 31.9 (C-25), 36.5 (C-10), 37.3 (C-1), 39.7 (C-12), 40.5 (C-20), 42.4 (C-4), 42.3 (C-13), 50.2 (C-24), 51.2 (C-9), 56.0 (C-17), 56.9 (C-14), 71.8 (C-3), 121.7 (C-6), 129.3 (C-23), 138.3 (C-22), 140.8 (C-5); GC-MS m/z : 412 [M^+ , C₂₉H₄₈O].

Most of the extracts and isolated compounds of *G. parvifolia* and *G. hombroniana* were active against the tested bacteria. These extracts and phytochemicals inhibited the growth of almost

all tested microorganisms with inhibition zone diameter between 8-15 mm. The acetone extract of *G. hombroniana* (GHA) and cowanin (**3**) showed the most broad-spectrum antibacterial activity with the MIC value of 225 µg/mL. The minimum bactericidal concentration (MBC) was then further in order to reconfirm the lowest concentration which killed 99.9% bacteria. The results of MIC and MBC were tabulated in Table 1.

Table 1. Results of Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Samples	MIC (µg/mL)				MBC (µg/mL)			
	Gram-positive bacteria		Gram-negative bacteria		Gram-positive bacteria		Gram-negative bacteria	
	<i>B.s</i>	<i>E.f</i>	<i>K.p</i>	<i>E.c</i>	<i>B.s</i>	<i>E.f</i>	<i>K.p</i>	<i>E.c</i>
Crude Extracts								
GHH	900	900	900	900	900	1800	900	900
GHD	450	900	900	900	900	1800	1800	900
GHA	225	900	900	900	225	1800	1800	900
GPH	900	900	1800	1800	900	900	1800	1800
GPC	900	900	900	900	900	900	900	900
GPM	450	900	900	900	900	1800	1800	1800
Isolated Compounds								
(1)	450	450	450	450	450	450	900	900
(2)	450	900	900	900	900	900	900	900
(3)	225	450	450	450	225	450	450	900
(4)	450	900	900	450	450	1800	900	900
(5)	900	900	900	900	900	900	900	900
(6)	900	1800	900	450	900	1800	900	900
Standard								
SS	14.06	14.06	14.06	14.06	14.06	14.06	14.06	14.06

B.s = *Bacillus subtilis*; *E.f* = *Enterococcus faecalis*; *K.p* = *Klebsiella pneumoniae*; *E.c* = *Escherichia coli*; SS = Streptomycin Sulphate; GHH = *G. hombroniana* n-hexane extract, GHD = *G. hombroniana* dichloromethane extract, GHA = *G. hombroniana* acetone extract, GPH = *G. parvifolia* n-hexane extract, GPC = *G. parvifolia* chloroform extract, GPM = *G. parvifolia* methanol extract

The values obtained in the MBC studies were the same or higher than those of the MIC values listed in Table 1. Overall, the pure compounds give significant antibacterial activities towards all tested bacteria, specifically gram-negative bacteria with MBC value range 450 to 900 µg/mL. Among all the pure compounds tested, cowanin (**3**) exhibit the strongest antibacterial activity towards all the bacteria except for *E. coli* which showed moderate activity with MBC 900 µg/mL. The antibacterial activity of xanthenes are vary depends on the position and presence of hydroxyl groups and prenyl/geranyl substituents. From the viewpoint of structure-activity relationships, cowanin (**3**) was discovered as the strongest inhibitor might due to presence of double bonds in the geranyl substituent at C-8, which proved as an essential factor of antibacterial activity for xanthone with 1,3,7-trihydroxy-7-methoxy oxidation pattern (Inuma et al., 1996). Besides that, Dharmaratne *et al.* stated that presence of hydroxyl groups at C-3 and C-6 play a prominent role to inhibit bacterial activity, whereas prenyl side chain at C-2 was also found capable of enhance the antibacterial activities (Dharmaratne et al., 2012).

Rubraxanthone (**1**) was as a strong inhibitor against both gram-positive bacteria, and a moderate inhibitor for gram-negative bacteria, whereas parvixanthone G (**2**) were found moderate inhibitors towards all tested bacteria. This result might differs due to absence of prenyl substituent at C-2 in both xanthenes which lower the inhibition activity (Dharmaratne et al., 2012). In addition, loss of a double bond in geranyl substituent at C-2 was also a causative factor of low activity in (**2**) compared to (**1**) (Inuma et al., 1996). Garcihombronane B (**4**), garcihombronane C (**5**) and stigmasterol (**6**) which isolated from *G. hombroniana* showed potent antibacterial activity as a moderate inhibitor towards all the tested bacteria with MBC

value 900 µg/mL, except for *E. faecalis*. This might due to presence of hydroxyl group in the structure which possesses high affinity for proteins and therefore, acted as inhibitors of microbial enzymes (Mori et al., 1987). Among all the crude extracts, GHA exhibit the strongest inhibition activity with MBC value of 225 µg/mL compared to other extracts. Besides that, GHD and GPM were also found as strong inhibitor, with lower MBC value, 450 µg/mL. Overall, the other crude extracts were found as moderate and weak inhibitor, which might a result of synergistic effects in crude extracts. The antibacterial activity of (4) and (5) and the crude extracts of *G. hombroniana* was reported for the first time in this study.

CONCLUSION

This study investigated the isolated xanthenes and triterpenes from *G. parvifolia* and *G. hombroniana* and their antibacterial activity. The evaluation of phytochemicals provide valuable information regarding the prospective use of medicinal plants as sources of new drugs. Also, it emphasizes the rationale for using medicinal plants in folk medicine. The antibacterial of phytochemicals may validate the traditional use of the plant as an anticancer agent. Therefore, more directed research is needed to explore the ability of plants to enhance the discovery and development of new chemical entities.

ACKNOWLEDGEMENT

The authors acknowledge Ministry of Higher Education (MOHE) for financial support under Research University Grant (RUG) vote number Q.J130000.7126.02J63 and the Faculty of Science, Universiti Teknologi Malaysia for the NMR and bioactivity facilities.

REFERENCES

- Aligians, N., Kalpoutzakis, E., Mitaku, S., Chinou, I.B. (2001). Composition and antimicrobial activity of the essential oil from *Origanum* Species. *Journal of Agricultural and Food Chemistry*, 49, 4168-4170.
- Dharmaratne, H.R.W., Sakagami, Y., Piyasena, K.G.P., Thevanesam, V. (2013). Antibacterial activity of xanthenes from *Garcinia mangostana* (L.) and their structure-activity relationship studies. *Natural Product Research*, 27(10), 938-41.
- Ee, G.C.L., Ng, S.H., Goh, J.K., Sukari, M.A., Rahmania, M. (2009). Chemical constituents of *Garcinia parvifolia* (Guttiferae). *Malaysian Journal of Science*, 28 (1), 105-110.
- Iinuma, M., Tosa, H., Tanaka, T., Asai, F., Kobayashi, Y., Shimano, R., Miyauchi, K. (1996). Antibacterial activity of xanthenes from guttiferaceous plants against methicillin-resistant *Staphylococcus aureus*. *Journal of Pharmacy and Pharmacology*, 48(8), 861-865.
- John, K.J., Senthil Kumar, R., Suresh, C.P., George, J.K., Abraham, Z. (2008). Occurrence, distribution and economic potential of seashore mangosteen (*Garcinia hombroniana* Pierre) in India. *Genetic Resources and Crop Evolution*, 55, 183-186.
- Kardono, L.B.S., Hanafi, M., Sherley, G., Kosela, S., Harrison, L.J. (2006). Bioactive constituents of *Garcinia porrecta* and *G. parvifolia* Grown in Indonesia. *Pakistan Journal of Biological Sciences*, 9(3), 483-486.
- Mbaveng, A.T., Ngameni, B., Kuete, V., Simo, I.K., Ambassa, P., Roy, R., Bezabih, M., Etoa, F.X., Ngadjui, B.T., Abegaz, B.M., Meyer, J.J., Lall, N., Beng, V.P. (2008). Antimicrobial activity of the crude extracts and five flavanoids from the twigs of *Dorstenia barteri* (Moraceae). *Journal of Ethnopharmacology*, 116, 489-489.
- Mori, A., Nishino, C., Enoki, N., Tawata, S. (1987). Antibacterial activity and mode of plant flavonoids against *Proteus vulgaris* and *Staphylococcus aureus* (1987). *Phytochemistry*, 26, 223-224.
- Najmuldeen, I.A., Hadi, A.H., Mohamad, K., Awang, K., Nasab, M.F., Ketuly, K.A., Mukhtar, M.R., Morita, H. (2011). Steroids from *Chisocheton Tomentosus*. *Malaysian Journal of Science*, 30(2), 144-153.
- Nguyen, H.D., Trinh, B.T.D., Tran, Q.N., Pham, H.D., Hansen, P.E., Duus, F., Connolly, J.D., Nguyen, L.D. (2011).

- Friedolanostane, friedocycloartane and benzophenone constituents of the bark and leaves of *Garcinia benthami*. *Phytochemistry*, 72, 290-295.
- Niu, S., Li, Z., Ji, F., Liu, G., Zhao, N., Liu, X., Jing, Y., Hua, H. (2012). Xanthones from the stem bark of *Garcinia bracteata* with growth inhibitory effects against HL-60 cells. *Phytochemistry*, 77, 280-286.
- Pattalung, P., Thongtheeraparp, W., Wiriyachitra, P.C., Taylor, W. (1994). Xanthones of *Garcinia cowa*. *Planta Medica*, 60, 365-368.
- Rukachaisirikul, V., Saelim, S., Karnsomchoke, P., Phongpaichit, S. (2005). Friedolanostanes and lanostanes from the leaves of *Garcinia hombroniana*. *Journal of Natural Products*, 68, 1222-1225.
- Rukachaisirikul, V., Trisuwan, K., Sukpondma, Y., Phongpaichit, S. (2008). A new benzoquinone derivative from the leaves of *Garcinia parvifolia*. *Archives of Pharmacal Research*, 31(1), 17-20.
- Vieiraa, L.M., Kijjoaa, A., Silvad, A.M.S., Mondranondrae, I., Kengthonge, S., Galesa, L., Damasa, A. M., Herz, W. (2004). Lanostanes and friedolanostanes from the bark of *Garcinia speciosa*. *Phytochemistry*, 65, 393-398.
- Xu, Y., Lai, Y., Imiyabir, Z., Goh, S. (2001). Xanthones from *Garcinia parvifolia*. *Journal of Natural Products*, 64, 1191-1195.