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Antiplatelet Aggregation Activity of 5-Hydroxyflavone, 2'-Hydroxyflavanone, Paeonol and Bergenin Isolated from Stem Bark of *Garcinia malaccensis* in Human Whole Blood

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ABSTRACT

Four known compounds, 5-Hydroxyflavone (1), 2'-Hydroxyflavanone (2), Paeonol (3) and bergenin (4), were isolated from the methanolic extract of the stem bark of *Garcinia malaccensis* for the first time through combination of vacuum and radial chromatography techniques. The structures of the secondary metabolites (1-4) were elucidated on the basis of their spectroscopic evidence and comparison with the published data. All isolated compounds exhibited inhibition of platelet aggregation in human whole blood which induced by arachidonic acid (AA), adenosine diphosphate (ADP) and collagen. 2'-Hydroxyflavanone (2) showed inhibitory effect on platelet aggregation caused by two inducers with IC_{50} 47.8± 2.1 AA and 147.2 ± 4.1 ADP.

Key words: *Garcinia malaccensis*, Platelet aggregation, Impedance Method, Vacuum liquid chromatography (VLC), NMR spectroscopy.

INTRODUCTION

The plant kingdom has served as one of man's oldest sources of useful drugs. They are remarkable in their ability to produce a vast number of diverse secondary metabolites ranging in chemical complexity and biological activities. Natural products (NPs) have historically served as templates for the modern drug development. The Garcinia species are known to be rich in phytochemical contents such as flavonoids, phenolic acids and xanthones. *G. Malaccensis* Hk.f (Guttiferae), locally known as "manggis burung" is a member of the Guttiferae family. Its fruit much resembles that of *G. mangostana* and *G. hombroniana*. Its mature fruit is similar in size to *G. hombroniana* but smaller than *G. Mangostana* [1]. Mangosteen fruits from *Garcinia malaccensis* (*G. Mangostana* Hook. f.) Linn also known as the queen of fruit is a most popular xanthone-rich and edible

fruit that has been widely used and commercialized for its medicinal properties and is considered to be one of the best of all tropical fruits in Southeast Asia [2-3]. Some cytotoxic activities have been reported on the compounds isolated from Garcinia. a-Mangostin from *G. mangostana* has shown to protect mitochondria from peroxidative damage, apoptotic effects and reducing oxidative damages [4-5].

This paper describes the isolation of four known compounds, 5-Hydroxyflavone (1), 2'-Hydroxyflavanone (2), Paeonol (3) and bergenin (4) from the methanolic extract of the stem bark of *Garcinia malaccensis* for the very first time using vacuum liquid chromatography (VLC) technique. The identified compounds were exhibited inhibition of platelet aggregation in human whole blood which induced by arachidonic acid (AA), adenosine diphosphate (ADP) and collagen.

MATERIALS AND METHODS

General experimental procedures

Melting points were determined on a Yanaco MP-S3 apparatus. UV spectra were measured on a Shimadzu UV 240 spectrophotometer. JASCO DIP–360 Digital polarimeter was used to measure the optical rotations in chloroform by using 10 cm cell tube. FTIR-8900 Spectrophotometer was used to record IR spectra in $CHCl_3$. The ^{1}H-NMR and 2D NMR spectra were recorded on a Bruker Avance III 500 Ascend spectrometer using BBO probe, while ^{13}C-NMR spectra were recorded on Bruker Avance III 500 Ascend spectrometer operating at 125 MHz using $CDCl_3$ as solvent. Chemical shifts were reported in δ (ppm), relative to $SiMe_4$ as internal standard, and coupling constants (*J*) were measured in Hz. The EI-MS and HREIMS were measured on Jeol HX 110 mass spectrometer. TLC was performed on Si gel precoated plates (PF_{254}, 20 × 20, 0.25 mm, Merck, Germany). Ceric sulphate in 10% H_2SO_4 spraying reagent was used for the staining of compounds on TLC. All reagents used were of analytical grades.

Plant material

The stembarks of *G. malaccensis* were collected from Pasoh, Negeri Sembilan, Malaysia in September 2010. A voucher specimen (MT27) was deposited in the herbarium of the Herbarium of the Forest Research Institute Malaysia (FRIM), Kepong, Malaysia.

Extraction

Dried and powdered stembark of *G. malaccensis* (500 g) was extracted under reflux with MeOH for 6-8 hrs and filter it. Obtained MeOH extract was fractionate through vacuum liquid chromatography (VLC) technique using different polarity. Evaporation of the respective solvents and purified the compounds through Preparative thin layer chromatography.

Impedance Method

Whole blood (1ml) diluted with buffer saline (1:1) was incubated with samples 5µl,in DMSO)at 37° C for 2 min. After which collagen (2µg/ml), ADP (10µM), AA(o.5 mM) was added to initiate aggregation. The platelet aggregation was measured by whole blood Lumi-Aggregometer (Chrono-Log Corp.Haver-town,PA). The results of aggregation in whole blood was determined after 5 min. as the increase in impedance across a pair of electrodes placed in the blood sample by comparison to that of control group impedance. To eliminate the effect of the solvent on the aggregation, blood with 0.5% DMSO was used as the control. The percentage inhibition of platelet aggregation was calculated as:

$$\% \text{ Inhibition} = 1 - \frac{(\text{aggregation of sample})}{(\text{aggregation of control})} \times 100$$

RESULTS AND DISCUSSION

Phytochemical investigation

The dried and powdered stembark of *G. malaccensis* (500 g) were was extracted under reflux with MeOH for 6-8 hrs and filter it. Obtained MeOH extract was fractionate through vacuum liquid chromatography (VLC) technique using different polarity. Evaporation of the respective solvents and purified the four compounds (1-4) through Preparative thin layer chromatography (Figure 1). All spectroscopic data described below were showed excellent agreement with the previously published results [6-9].

5-Hydroxyflavone (1) was obtained as a

yellow crystal (8.2 mg); mp. 175-176° C. R_f 0.23 in MeOH; UV (MeOH) λ_{max} (log ε) 240 (2.3) nm; IR ($CHCl_3$) υ_{max} 3432, 2152, 1690 and 1670 cm^{-1}; EIMS for $C_{15}H_{10}O_3$ *m/z* (rel. int.): 238[M]; ^1H NMR ($CDCl_3$, 500 MHz): δ 6.71, 6.79, 6.97, 7.54, 7.88, 7.89, 12.57. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 135.6(C-1), 126.6(C-2', 6'), 129.3(C-3', 4', 5'), 77.3(C-2), 183.7(C-4), 161.0(C-4a), 164.7 (C-5), 156.6(C-6).

2'-Hydroxyflavanone (**2**) was obtained as a white yellowish powder (7.3 mg); mp. 178-180° C. R_f 0.30 in MeOH; UV (MeOH) λ_{max} (log ε) 235 (2.41) nm; IR ($CHCl_3$) υ_{max} 3412, 2152, 1680 and 1660 cm^{-1}; EIMS for $C_{15}H_{12}O_3$ *m/z* (rel. int.): 240[M]; ^1H NMR (Acetone-d_6, 500 MHz): δ 2.93, 3.09, 5.85, 6.98, 7.11, 7.23, 7.56, 7.88, 8.80. ^{13}C NMR (Acetone-d_6, 125 MHz): δ 29.2, 43.2, 75.1, 115.6,

Table 1: Percentage inhibition of isolated compounds (1-4) from Guttiferae species on platelet aggregation in human whole blood induced by arachidonic acid (AA) (0.5mM), collagen (2µg/mL), and adenosine diphosphate (ADP) (10µM)

Compounds name	Concentration (µg/ml)	AA	COLLAGEN	ADP
5-Hydroxyflavone (**1**)	100	85.1± 0.7[b]	55.1± 0.6	91.1±0.2b
	50	65.1 ±0.4	32.1± 0.42	67.6±0.58
	25	34.1± 0.2	22.1± 0.1	48.1±0.32
	12.5	23.4± 0.8	8.4± 0.28	18.3± 0.18
2'-hydroxyflavanone (**2**)	100	100±00a	35.2±0.71	57.8± 0.7
	50	75.1±0.71		42.6 ±0.9
	25	54.1±0.68		35.1± 0.63
	12.5	30.6± 0.64		17.2± 0.71
Paeonol (**3**)	100	100± 00a	57.3± 00	100±00a
	50	75.3 ±0.48	42.2± 0.9	76.6±0.15
	25	30.1± 1.2	28.7± 0.42	50.4±0.71
	12.5	13.2± 0.58	22.2± 0.48	15.1± 0.4
Bergenin (**4**)	100	75.3± 0.7c	82± 0.071b	65.1±0.48
	50	55.6 ±0.48	74.1± 0.7	56.5±0.7
	25	43.1± 1.0	55.3± 0.61	43.2±0.42
	12.5	27.2± 0.56	35.2± 0.48	24.6± 0.58
Aspirin	100	100±0.0	35.2±0.71	44.8±0.9
	50	75± 0.71		
	25	55.4±0.58		
	12.5	37.1±0.45		

Aspirin was used as a positive control. Concentration of aspirin in reaction mixture: 25µg/mL. Values are presented as mean ± SE (n=3); [a]p<0.05, [b]p<0.01, and [c]p<0.001 as compared with the respective control.

Table 2: IC_{50} values (µM) of isolated compounds (1-4) on platelet aggregation induced by arachidonic acid (AA) (0.5mM), Collagen (2µg/ml), and adenosine diphosphate (ADP) (10µM)

Compounds	AA	Collagen	ADP
5-Hydroxyflavane (**1**)	56.1±0.89	183.2 ± 3.2	69.7±0.76[c]
2'-Hydroxyflavanone (**2**)	47.8± 2.1	-	147.2 ± 4.1
Paeonol (**3**)	47.4 ± 2.7	148.7 ± 4.9	61.9 ± 1.1
Bergenin (**4**)	240.6 ± 8.1	60.9 ± 2.3	120.3 ± 5.2
Aspirin	27.5±2.8	-	-

118.2, 120.0, 121.2, 121.5, 126.0, 127.0, 129.5, 136.0, 154.1, 162.2, 191.7.

Paeonol (**3**) was obtained as a white powder (5.5 mg); mp. 175-179° C. R_f 0.42 in MeOH; UV (MeOH) λ_{max} (log ε) 233 (2.32) nm; IR ($CHCl_3$) υ_{max} 3443, 2121, 1680 and 1663 cm^{-1}; EIMS for $C_9H_{10}O_3$ m/z (rel. int.): 166[M]; ^1H NMR ($CDCl_3$, 500 MHz): δ 2.54 (3H, s), 3.82 (3H, s), 6.40 (1H, d), 6.42 (1H, d), 7.60 (1H, d), 12.75 (1H, s). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 114.0 (C-1), 165.4 (C-2), 100.9 (C-3), 166.2 (C-4), 107.7 (C-5), 132.5 (C-6), 202.7 ($COCH_3$), 26.3 ($COCH_3$), 55.7 (OCH_3).

Bergenin (4) was obtained as a brownish powder (6.2 mg); mp. 183-185° C. R_f 0.40 in MeOH; UV (MeOH) λ_{max} (log ε) 243 (2.67) nm; IR ($CHCl_3$) υ_{max} 3452, 2120, 1684 and 1665 cm^{-1}; EIMS for $C_{14}H_{16}O_9$ *m/z* (rel.int.): 328[M]; ^1H NMR (DMSO-d_6, 500 MHz): δ 3.56 (1H, dd), 3.44 (1H, dd), 3.64 (1H, dd), 3.98 (1H, dd), 6.95 (1H, s), 4.98 (1H, d), 3.96 (1H, d), 3.76 (3H,s). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 81.7(C-2), 70.7(C-3), 73.7(C-4), 79.8(C-4a), 163.4(C-6), 118.1(C-6a), 109.5(C-7), 151.0(C-8), 140.6(C-9), 148.1(C-10), 116.0(C-10a) , 72.1(C-10b), 61.1(C-11), 59.9(C-12).

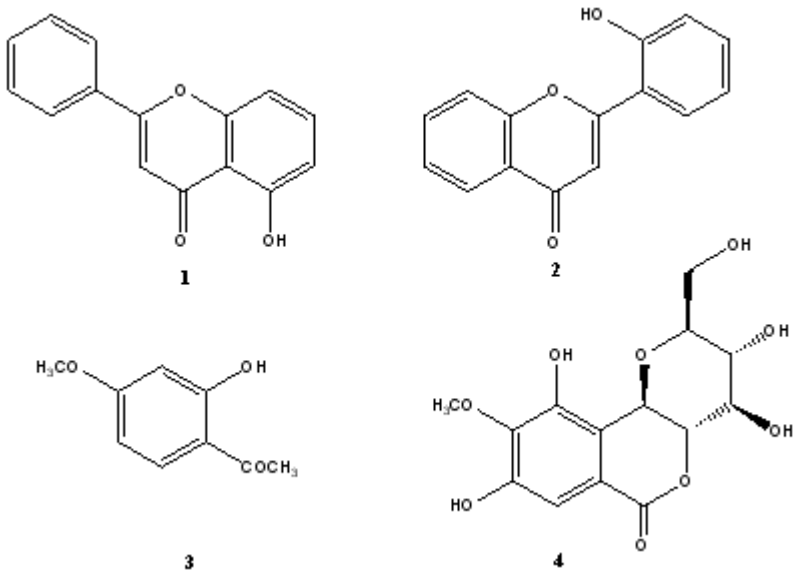

Fig. 1: Chemical structures of secondary metabolites 1-4 isolated from bark of *Garcinia malaccensis*

Antiplatelet aggregation activity

The effect of the isolated compounds (**1**–**4**) have been investigate against platelet aggregation test. Each sample was measured in triplicate one-way analysis of variance (ANOVA) was used for multiple comparison, the IC_{50} values, that is, the concentration of the compounds required to inhibit aggregation by 50% ,were obtained from at least three determinations[7]. Methanol extract of *Garcinia malaccensis* showed strong antiplatelet aggregation activity at 100µg/ml in human whole blood *in vitro.* Both 5-Hydroxyflavone (1), Paeonol (3) and Bergenin (4) showed marked inhibitory effect on platelet aggregation caused by the three inducers. The IC_{50} value for 5-Hydroxyflavone (1) was 56.1±0.89AA, 183.2 ± 3.2 Collagen and 69.7±0.76^c ADP, the IC_{50} value for Paeonol (3) was 47.4 ± 2.7 AA, 148.7 ± 4.9 Collagen and 61.9 ± 1.1 ADP and the IC_{50} value for Bergenin (4) was 240.6 ± 8.1 AA, 60.9 ± 2.3 Collagen and 120.3 ± 5.2 ADP, 2'-Hydroxyflavanone (2) showed inhibitory effect on platelet aggregation caused by two inducers with IC_{50} 47.8± 2.1 AA and 147.2 ± 4.1 ADP (table 1 & table 2).

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REFERENCES

1. Y. Nakagawa, M. Iinuma, T. Naoe, Y. Nozawa and Y. Akao, *Bioorg. Med. Chem*. **15**(16): 5620-5628 (2007).
2. B. Márquez-Valadez, R. Lugo-Huitrón, V. Valdivia-Cerda, L. R. Miranda-Ramírez, V, Pérez-De La Cruz, O, González-Cuahutencos, *Nutr. Neurosci.* **12**(1): 35-42 (2009).
3. J. Pedraza-Chaverri, N. Cárdenas-Rodríguez, M. Orozco-Ibarra and J. M. Pérez-Rojas, *Food Chem. Toxicol*. **46**(10): 3227-3239 (2008).
4. M. Taher, D. Susanti, M. F. Rezali, F. S. A. Zohri, S. J. A. Ichwan, S. I. A, F. Ahmad, Asian Pacific *Journal of Tropical Medicine*. 136-141 (2012).
5. E. Martínez-Abundis, N. García, F. Correa, S. Hernández-Reséndiz, J. Pedraza-Chaverri and C. Zazueta, *Mitochondrion*. **10**(2): 151-157 (2010).
6. A. Wibowo, N. Ahmat, A.S. Hamzah, A.S. Sufian, N.H. Ismail, R. Ahmad, F.M. Jaafar and H. Takayama, *Fitoterapia*. **82**: 676–681 (2011).
7. P. N. Kim Tuyen, L. H. Duy and K. P. P. Nguyen, Tap Chi Hoa Hoc. **48**(5): 546-550 (2010).
8. J. F. Stevens, M. Ivancic, M. L. Deinzer and Eckhard Wollenweber, *J. Nat. Prod.* ***62*** (2): 392–394 (1999).
9. F. A. Toma´s-Barbera´n, E. Wollenweber, *Plant Syst. Evol.* **173**: 109-118 (1990).
10. N, Kabaku, B, Yilmaz, U, Calikan, *Haematologia* (Budap). **30**(2): 107-115 (2000).