CHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITY OF *GARCINIA PARVIFOLIA* MIQ. STEM BARK

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Abstract

The chemical constituents of *Garciniaparvifolia*Miq.stem bark have been studied. The dried samples have been extracted using Soxhlet apparatus to give the crude products. The constituents were separated and purified by using vacuum column chromatography, gravity column chromatography and recrystallisation. The chemical compounds obtained were elucidated by infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. Two compounds namely as 1,6,7-trihydroxy-3-methoxyxanthone and 3,8''-binaringenin were isolated from the ethyl acetate crude extracts of *G. parvifolia*Miq. The free-radical scavenging activity of the crude extracts were determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The ethyl acetate extract was found to be the most active free radical scavenger with IC_{50} value of 4.2 ppm, followed by methanol extract (IC_{50} 96 ppm) and petroleum ether extract (IC_{50} 200 ppm).

Keywords: *GarciniaparvifoliaMiq.,stem bark, 1,6,7-trihydroxy-3-methoxyxanthone, 3,8''-binaringenin.*

INTRODUCTION

*Garciniaparvifolia*Miq.is one of the species from Clusiaceae (Guttiferae**)** family distributed widely in the tropical region of the world and has high potential as spice and value medicinal plants. Clusiaceae family have 36 genera and 1,600 species with a pan tropical distribution. Four genera and 16 species are native to Australia and three genera and five species are found in the Northern Territory, Australia. Three genera were reported as *Calophyllum*L., *Garcinia*L., and *Hypericum*L. The Clusiaceae (Guttiferae**)** family is part of Malpighiales and Clusioideae as the subfamily [1]. In peninsular Malaysia there are 49 Garcinia species out of 350 species estimated, which include *Garciniaatroviridis*, *G. cowa, G. morella, G. lanceaefolia, G. hombroniana, G. prainiana*and *G. mangostana* [2,3].

GarciniaparvifoliaMiq is known as "asamkandis" and it has white colour with taste like mangosteen which is the most famous fruit in Malaysia [4]. Previous study on *G. parvifolia*Miq.reportedthe presence of xanthones as the major chemical constituents together with triterpenes, bioflavonoids and benzophenones. Moreover, *G. parvifoliaMiqshowed several* biological activities *included antioxidant, antibacterial, cytotoxicand antiplasmodial activities [5]. In this paper, we report the isolation of two chemical constituents from the stem bark of GarciniaparvifoliaMiq. and the antioxidant assay on the crude extracts.*

EXPERIMENTAL

General Experimental Procedures

Thin layer chromatography (TLC) analysis was done using Merck 60 F_{254} pre-coated silica gel aluminium sheet with thickness of 0.20 mm with different polarity of solvent system. The spots on the TLC plate was visualized by UV lamp (254 nm) and sprayed with vanillin-sulphuric acid spraying reagent. Fractionation and purification process was carried out by using vacuum liquid chromatography (VLC) and gravity column chromatography (CC) with different size of silica gel as stationary phase. Silica gel 230-400 mesh was used for VLC, while silica gel 70-230 mesh was used for gravity column chromatography (CC) with different polarity of solvent as the mobile phase. Elucidation of pure compounds was obtained by infrared (IR) and Nuclear Magnetic Resonance (NMR) spectroscopies. The IR spectra were measured using Perkin-Elmer Spectrum Two (ATR), while ^{1}H NMR spectra were recorded by BrukerAvance Spectrometer 400 MHz with deuterated chloroform ($CDCl_3$) and acetone (acetone-d_6) as solvents.

Plant Material

The stem barks of *G. parvifolia*Miq. with voucher specimen number SK 1961/11 was obtained from Sekayu Forest Reserved in Kuala Berang, Terengganu, Malaysia in June 2011.

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Extraction and Isolation

Soxhlet extraction method was performed on the dried stem bark (800 g) of *G. parvifolia*with three different solvent starting with petroleum ether, ethyl acetate and methanol for 8 hours each. The extracts were filtered out and concentrated using rotary evaporator to afford the petroleum ether, ethyl acetate, and methanol crude extracts.

The ethyl acetate crude extract (7.29 g) was fractionated by using VLC to give ten fractions (1-10). The crude extract was eluted with *n*-hexane, ethyl acetate and methanol. Fraction 6 (1.41 g) was further purified using CC and eluted with *n*-hexane and chloroform to give seventeen subfractions (1-17). Subfraction 14 give yellow solid of 1,6,7-trihydroxy-3-methoxyxanthone **(1)** (1.3 mg, 0.02%). While, subfraction 17 was then further purified using Sephadex LH-20 which resulted the isolation of 3,8''-binaringenin **(2)** (30 mg, 0.41%) as light brown amorphous powder.

1,6,7-Trihydroxy-3-methoxyxanthone (1)

Yellow solid, m.p. 212-214°C (Ref: 213-216°C), R_f= 0.58 (*n*-hexane:acetone = 1:1): IR (neat) ν_{max} cm^{-1}: 3332.3 (O–H), 2922.1 (sp^3 C–H), 1646.4 (C=O), 1608.3 and 1485.0 (C=C aromatic), 1159.5 (C–O); ^{1}H NMR (acetone-d_6, 400 MHz) : 13.18 (1H, br s, 1-OH), 6.25 (1H, d, *J*= 2.0 Hz, H-2), 6.41 (1H, d, *J* = 2.0 Hz H-4), 6.95 (1H, s, H-5), 7.56 (1H, s, H-8), 3.99 (3H, s, OCH_3), 9.85 (1H, br, s, 6-OH, 7-OH).

3,8''-Binaringenin (2)

Light brown amorphous powder, m.p. 220-225°C (Ref: 220-225°C), R_f= 0.5 (*n*-hexane:acetone = 2:3); IR (neat) ν_{max} cm^{-1}: 3180.2 (O–H), 2924.7 (sp^3 C–H), 1630.6 (C=O), 1596.4 and 1442.8 (C=C aromatic), 1158.7 (C–O), ^{1}H NMR (acetone-d_6, 400 MHz): 5.87 (1H, m, H-2), 4.72 (1H, m, H-3), 12.20 (1H, m, 5-OH), 5.98 (1H, br s, H-6, H-8), 7.23 (1H, br s, H-2', H-6'), 6.75 (1H, br s, H-3', H-5'), 5.33 (1H, m, H-2''), 2.69 (1H, m, H-3''a), 2.78 (1H, m, H-3''b), 12.31 (1H, m, 5''-OH), 5.93 (1H, br s, H-6''), 6.82 (1H, br s, H-2''', H-6'''), 6.91 (1H, m, H-3''', H-5''').

Free Radical Scavenging Activity (DPPH) Assay

The free radical scavenging activity with 1,1-diphenyl-2-picrylhydrazyl (DPPH) was tested on petroleum ether, ethyl acetate and methanol crude extracts and butylated hydroxytoluene (BHT) which used as a positive control. A volume 170 µL sample solution (1000 ppm) of all crude extract and BHT were diluted into 1000, 500, 250, 125, 62.5 and 31.8 µg/mL solution using methanol. A volume 30 µL of DPPH in methanol solution (300 µM) was added to different concentrations of sample solutions and was reacted until 30 min. at room temperature.

The absorbance of the reaction mixtures was recorded at wavelength 517 nm. Measurements of all samples were performed in triplicates and the average values were calculated. The percentage of antioxidant activity was calculated using the equation below:

$$\%\,\text{Scavenging} = \frac{[\text{Abs（control)} - \text{Abs（DPPH} + \text{sample)}]}{\text{Abs(control)}} \times 100\%$$

The significant radical scavenging activities of extracts can be determined when a reduction greater than that produced by 10 µM BHT and were evaluated for IC_{50} (the concentration of the sample at 50% inhibition). The concentration of the sample at 50% inhibition (IC_{50}) was obtained from the graph percentage inhibition against concentration in µg/mL.

RESULTS AND DISCUSSION

Soxhlet extraction method using non-polar to polar solvents was carried out on the stem bark of *Garciniaparvifolia*. The extraction process was started with petroleum ether, ethyl acetate and methanol for 8 hours each. Methanol was used to ensure that all compounds were extracted from the stem bark of *G. parvifolia.* The extracts were filtered out and concentrated using rotary evaporator to afford yellow petroleum ether, brown ethyl acetate and dark brown methanol of crude extracts with yield of 2.85 g (0.36%), 8.62 g (1.08%) and 13.26 g (1.66%), respectively.

1,6,7-Trihydroxy-3-methoxyxanthone **(1)** (1.3 mg, 0.02%) was yielded as yellow solid and the TLC spot shows green after sprayed with vanillin-sulphuric acid spraying reagent and this compound was predictable to have xanthone moiety. The compound had R_f= 0.30 (*n*-hexane:Et_2O = 3:2) and melting point value 212-214°C (Ref: 213-216°C). Based on IR and ^{1}H NMR, the compound was identified as 1,6,7-trihydroxy-3-methoxyxanthone **(1)**.The absorption at 2922.1 cm^{-1} and 1646.4 cm^{-1} were attributed to sp^3 C–H and conjugated C=O ketone, respectively. The absorption at 1608.3 cm^{-1} and 1485.0 cm^{-1} indicated to C=C aromatic and 1159.5 cm^{-1} for C-O band. The ^{1}H NMR spectrum showed characteristics of xanthone pattern based on a chelated hydroxyl group presence at the downfield region. A singlet signal observed at δ 3.99 was assigned to a methoxy group, 3-OCH_3 while singlet at δ 13.18 was due to a chelated hydroxyl group, 1-OH. A doublet at the downfield region of δ 9.85 was assigned to two hydroxyl groups attached to the aromatic ring, 6-OH and 7-OH. While, two doublet peaks at δ 6.25 and δ 6.41 with *J* value of 2.0 Hz corresponded to the presence of two *meta*-coupled aromatic protons, H-2 and H-4. The comparison with literature data show that 1,6,7-trihydroxy-3-methoxyxanthone **(2)** provide similar pattern of ^{1}H NMR spectrum with norathyriol**(3)** which was isolated previously from the twigs of *G. hombroniana*and *G. parvifolia*[6]. ^{1}H NMR spectra of 1,6,7-trihydroxy-3-methoxyxanthone**(1)** and norathyriol**(3)** were very similar except for the different substituents (hydroxyl and methoxy) bonded to the aromatic ring. Norathyriol**(3)** had four hydroxy groups whereas 1,6,7-trihydroxy-3-methoxyxanthone**(1)** had three hydroxy groups and one methoxy groups. Table 1 shows comparison of ^{1}H NMR data between 1,6,7-trihydroxy-3-methoxyxanthone **(1)** andnorathyriol**(3)**.

Table 1: Comparison of the ^{1}H NMR Data 1,6,7-trihydroxy-3-methoxyxanthone**(2)** with Norathyriol**(3)**

Carbon	1,6,7-Trihydroxy-3-methoxyxanthone**(2)** δ_H (ppm)	Norathyriol**(3)** δ_H (ppm)
1	13.18 (1H, br s, OH)	13.26 (1H, br s, OH)
2	6.25 (1H, d, *J* = 2.0 Hz)	6.18 (1H, d, *J* = 1.7 Hz)
3	3.99 (3H, s, OCH_3)	-
4	6.41 (1H, d, *J* = 2.0 Hz)	6.37 (1H, d, *J* = 1.7 Hz)
4a	-	-
5	6.95 (1H, s)	6.85 (1H, s)
6	9.85 (1 H, br s, OH)	-
7	9.85 (1 H, br s, OH)	-
8	7.56 (1H, s)	7.39 (1H, s)
8a	-	-
9	-	-
9a	-	-
10a	-	-

3,8''-Binaringenin **(3)**, a light brown amorphous powder which gave an orange spot on the TLC after sprayed with vanillin-sulphuric acid spraying reagent was recognised having a biflavonoid skeleton. The compound had R_f= 0.50 (*n*-hexane:Et_2O = 3:2) and melting point value m.p 220-225°C (Ref: 220-225°C). The compound was identified as 3,8''-binaringenin **(4)** based on previous study on *Garciniagriffithi*T.Anderson [7]. The IR spectrum showed a broad absorption band of OH group at 3180.2 cm^{-1}. The absorption of C-H sp^3 and conjugated C=O ketone were observed at 2924.7 cm^{-1} and 1630.6 cm^{-1}. Strong absorption band at 1596.4 and 1442.8 cm^{-1} was corresponded to the C=C aromatic while, absorption at 1158.7 cm^{-1} attributed to the presence of C-O bond. Analysis of the ^{1}H NMR spectrum showed characteristics of bilavonoids pattern based on a chelated hydroxyl group presence at the downfield region. The ^{1}H NMR spectrum showed the most deshielded peaks resonated at δ 12.20 and 12.31 corresponded to two chelated hydroxyl groups, 5-OH and 5''-OH, respectively which indicative a biflavonoid compound. Six sets of multiplets resonated at δ 2.69, 2.78, 4.72, 5.33, 6.91 were assigned for H-3'', H-3, H-2'', H-5''', H-2, respectively. Five singlet peaks were observed at δ 5.98, 7.23, 6.75, 5.93, 6.82 were assigned to protons bonded to the aromatic rings H-6, H-8, H-2', H-6', H-3', H-5', H-6'', H-2''', H-6''', respectively.

Antioxidant activities of the crude extracts were tested using radical scavenging DPPH method. The ethyl acetate extract was found to be the most active free radical scavenger with IC_{50} value of 4.2 ± 0.61 ppm, followed by methanol extract (IC_{50} 96 ± 4.93ppm) and petroleum ether extract (IC_{50} 200 ± 11.55 ppm). The ethyl acetate extract display the most active antioxidant property due to the existence of bulk polar compounds in the extract such as xanthones and bioflavonoids which contribute to the antioxidant activity [8].

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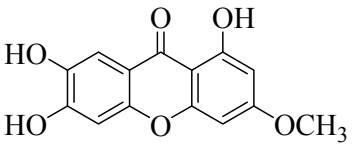

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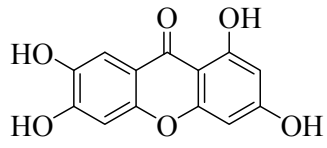

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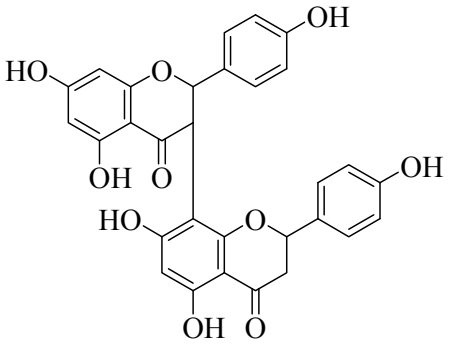

(**2**)

CONCLUSIONS

Purification of the crude extracts from the stem bark of *G. parvifolia*were successfully isolated two chemical compounds, identified as 1,6,7-trihydroxy-3-methoxyxanthone (**1**) and 3,8"-binaringenin (**2**)from the ethyl acetate crude extract.

Antioxidant activity was tested by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay on the petroleum ether, ethyl acetate and methanol crude extracts. The ethyl acetate extract was found to be the most active free radical scavenger with IC_{50} value of 4.2 ± 0.61 ppm due to the bulk amount of phenolic compounds present in the extract, followed by methanol extract (IC_{50} 96 ± 4.93 ppm) and petroleum ether extract (IC_{50} 200 ± 11.55 ppm).

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