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Flavonoid constituents from the stem bark of *polyalthia cauliflora* var. *Cauliflora*

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Abstract: Phytochemical investigation of the methanolic crude extract of the stem bark of *Polyalthia cauliflora* var. *cauliflora* of Annonaceae yielded five flavones and were identified as 3,7-dimethoxy-5-hydroxyflavone (**1**), 5,8-dihydroxy-6,7-dimethoxyflavone (**2**), tectochrysin (**3**), 6,7-dimethoxy-5-hydroxyflavone (**4**) and alnustin (**5**). The structures of compounds were analyzed using various spectroscopic methods such as 1D and 2D NMR, GC-MS, UV-Vis and IR. The flavonoids were isolated for the first time in the genus *Polyalthia.*

Key word: Annonaceae, *Polyalthia cauliflora*, flavonoids, flavone, Nuclear Magnetic Resonance.

INTRODUCTION

The genus *Polyalthia* comprises about 120 species of shrubs and trees, which is widely distributed in tropical and subtropical region (Hooker, 1875). *Polyalthia cauliflora* var. *cauliflora* is a small tree, grow up to 14 m tall and 13cm diameter. Its leaves are simple, flat and alternate. The yellow-orange flowers have 32 mm long petals and 8 mm-purple fruit are places in apocarps. It can be found in undisturbed forest up to 1670 m altitude. Most of this plant can be found in dry forest as well as in fresh water swamp. This plant can also grow on clayey loam soils, on hillsides and ridges. In the secondary forest, it usually present as a pre-disturbance remnant. This plant can be found in Peninsular Malaysia, Thailand, Sumatra and Borneo and used by the 'Kalabit' community in Bario, Sarawak for birth control (Fasihuddin *et al*., 1995). Previous chemical study on *Polyalthia* reported it to contain alkaloids, flavonoids, acetogenin and triterpenoids (Paarakh and Khosa, 2009). This paper describes on the isolation of flavones from *Polyalthia cauliflora* var. *cauliflora*.

MATERIAL AND METHOD

General procedures:

The ^{1}H-NMR and ^{13}C-NMR were recorded in chloroform-D on Bruker 300 Ultrashield NMR spectrometer measured at 300 and 75 MHz. Chemical shifts are reported in ppm and δ and the coupling constants are given in Hz. Melting point was taken on a hot stage Gallen Kamp melting point apparatus with microscope, and was uncorrected. The infrared (IR) was recorded on the Perkin Elmer spectrum one FT-IR spectrometer. The ultraviolet (UV) spectra were recorded on Shimadzu UV-Vis 160i. The mass spectra were measured on Perkin Elmer Clarus 600T spectrometer 70 eV. Glass column used silica gel 60 230-400 mesh ASTM (Merck 1.09385), centrifugal thin layer chromatography used silica gel 60 PF_{254} (Merck 1.07749). Aluminum supported silica gel 60 F_{254} was used for analytical thin layer chromatography, while glass supported silica gel 60 F_{254} was used for preparative thin layer chromatography.

Plant material:

The stem bark of *Polyalthia cauliflora* var. *cauliflora* was collected from Kuala Keniam, Pahang, Malaysia in May 2008 and a voucher specimen (UiTM 64/2008) was deposited at the Herbarium of Faculty of Applied Sciences, Universiti Teknologi MARA, Malaysia.

Extraction and isolation:

The stem bark (350 g) of *Polyalthia cauliflora* var. *cauliflora* was air-dried, ground and soaked in methanol for 72 hours. The methanolic extract (16.25g) appeared as dark powder was subjected to acid-base extraction to yield two fractions which were alkaloidal and non-alkaloidal extracts. The non-alkaloidal extract was subjected to silica gel column chromatography, eluted with *n*-hex:DCM, DCM:EtOAc and EtOAc:MeOH with gradual increasing solvent polarity to afford fifty-two fractions. Fractions that show similar pattern on TLC were pooled and combined into twelve fractions. Fraction 6 (F6) was further chromatographed using radial chromatography (*n*-hex:DCM, 6:4,

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5:5) to yield amorphous crystal of compound **1** (4.0 mg, 0.02%). Fraction $F6_{7-10}$ was subjected to preparative thin layer chromatography (*n*-hex:EtOAc, 8.5:1.5) to obtain **2** (1.2 mg, 0.007%). Multiple developments of preparative thin layer chromatography of F8 and F11 gave **3** (13.0 mg, 0.08%) and **5** (0.9 mg, 0.006%), respectively. Recolumn-chromatography of F12 gave **4** (12.0 mg, 0.07%).

Spectral data of the flavones:

Compound **1**: 3,7-dimethoxy-5-hydroxyflavone, yellow needles, wt: 4.0 mg, mp: 148-150 ^{0}C. MS m/z: 298, $C_{17}H_{14}O_5$, UV λ_{max} nm MeOH: 276, 334. IR cm $^{-1}$: 3465, 3068, 1604. ^{1}H NMR (CDCl3, 300 MHz) δ: 12.60 (1H, *s*, H-5), 6.39 (1H, *d*, *J*=2.1 Hz, H-6), 6.49 (1H, *d*, *J*=2.1 Hz, H-8), 8.09 (2H, *m*, H-2', H-6') 7.55 (3H, *m*, H-3', H-4', H-5'), 3.89 (3H, *s*, OCH_3-7), 3.90 (3H, *s*, OCH_3-3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 55.8 (OCH_3-3), 60.4 (OCH_3-7), 155.9 (C-2), 139.7 (C-3), 179.0 (C-4), 162.1 (C-10), 98.0 (C-6), 165.6 (C-5), 92.2 (C-8), 156.9 (C-9), 106.2 (C-7), 130.5 (C-1'), 128.4 (C-2', C-6'), 128.6 (C-3', C-5'), 131.0 (C-4').

Compound **2**: 5, 8-dihydroxy-6,7-dimethoxyflavone, yellow powder. wt: 1.2 mg, mp: 180-182 ^{0}C. MS *m/z*: 314, $C_{17}H_{14}O_6$, UV λ_{max} nm MeOH : 274, 358. IR cm^{-1}: 3434, 2834, 1637, 1266. ^{1}H NMR ($CDCl_3$, 300MHz) δ ppm: 6.60 (1H, *s*, H-3), 8.23 (2H, *dd, J*= 8.7, 1.8 Hz, H-2', H-6'), 7.52 (3H, *m*, H-3', H-4', H-5'), 3.96 (3H, *s*, OCH_3-C-6), 4.00 (3H, *s*, OCH_3-C-7), 11.65 (*s*, OH).

Compound **3**: Tectochrysin, white amorphous, wt: 13.0 mg, mp: 165-166 ^{0}C. MS *m/z*: 268 $C_{16}H_{12}O_4$. UV λ_{max} nm MeOH: 257, 306. IR cm $^{-1}$: 1660, 795, 759, 681, 632. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 6.70 (1H, *s*, H-3), 12.75 (1H, *s*, H-5), 6.41 (1H, *d*, *J*=2.1 Hz, H-6), 6.54 (1H, *d*, *J*=2.1 Hz, H-8), 7.92 (2H, *m*, H-2', H-6'), 7.56 (3H, *m*, H-3', H-4', H-5'), 3.91 (3H, *s*, OCH_3-7). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 55.8 (OCH_3-C-7), 165.2 (C-2), 105.9 (C-3), 182.5 (C-4), 162.2 (C-5), 98.2 (C-6),165.6 (C-7), 92.7 (C-8), 165.9 (C-9), 103.1 (C-10), 131.4 (C-1'), 126.3 (C-2',C-6'), 129.1 (C-3', C-5'), 131.9 (C-4').

Compound **4**: 6,7-dimethoxy-5-hydroxyflavone, white amorphous, wt. 12.0 mg, mp: 162-163 ^{0}C. MS m/z: 298 $C_{17}H_{14}O_5$. UV λ max nm MeOH: 245, 327. IR cm $^{-1}$: 3404, 1644. ^{1}H NMR ($CDCl_3$ 300 MHz) δ: 12.67 (1H, *s*, H-5), 6.68 (1H, *s*, H-3), 6.57 (1H, *s*, H-8), 7.89 (2H, *m*, H-2', H-6'), 7.53 (3H, *m*, H-3', H-4', H-5'), 3.95 (3H, *s*, OCH_3-6), 4.00 (3H, *s*, OCH_3-7). ^{13}C NMR ($CDCl_3$ 75 MHz) δ: 66.9 (OCH_3-6), 56.4 (OCH_3-7), 164.0 (C-2), 105.7 (C-3), 182.8 (C-4), 153.1 (C-5), 132.7 (C-6), 159.0 (C-7), 90.7 (C-8), 153.4 (C-9), 106.3 (C-10), 131.3 (C-1'), 126.3 (C-2', C-6'), 129.1 (C-3', C-5'), 131.9 (C-4').

Compound **5**: Alnustin, yellow amorphous, mp: 175-176 ^{0}C. MS *m/z*: 328 $C_{18}H_{16}O_6$ UV λ_{max} nm MeOH: 271, 317. IR cm^{-1}: 3434, 2987, 1638. ^{1}H NMR ($CDCl_3$ 300 MHz) δ: 12.45 (1H, *s*, H-5), 6.46 (1H, *s*, H-8), 7.56 (2H, *m*, H-2', H-6'), 8.18 (3H, *m*, H-3', H-4', H-5'), 3.90 (3H, *s*, OCH_3-3), 3.94 (3H, *s*, OCH_3-6), 3.97 (3H, *s*, OCH_3-7).

RESULT AND DISCUSSION

Phytochemical investigation of the stem bark of *Polyalthia cauliflora* var. *cauliflora* led to the isolation of five flavonoid constituents namely 3,7-dimethoxy-5-hydroxyflavone (**1**), 5,8-dihydroxy-6,7-dimethoxyflavone (**2**), tectochrysin (**3**), 6,7-dimethoxy-5-hydroxyflavone (**4**) and alnustin (**5**). All flavonoids have been identified to possess flavone skeleton. These flavones displayed similar characteristic in which the B ring in all compounds are monosubstituted. The presence of monosubstituted benzene ring was shown in ^{1}H NMR as two multiplets at δ 7.5-7.56 (3H, *m*, H-3', H-4' and H-5') and δ 7.92-8.23 (2H, *m*, H-2' and H-6'). The separation of aromatic signals into two multiplet groups indicated that no substituent attaches at B ring and the benzene ring is in symmetry with the *ortho* proton resonated at lower field and *meta*/*para* proton at upper field.

The signal of a far-down field singlet at δ 11.65-12.75 (1H, *s*, 5-OH) showed the presence of hydroxyl group at C-5 forming hydrogen bond with oxygen of carbonyl group C-4 (Zakaria *et al*., 2010). The presence of hydroxyl at C-5 cause the carbonyl carbon C-4 to resonate at δ 179.0-182.8 as compared to C-5 unsubstituted flavone (δ 178.0). Majority of the naturally occurring flavonoids have substitutions on ring A. In this species, most compounds possess methoxy group that are substituted at C-3, C-6 or C-7. UV-Vis spectral of the flavone was recorded in methanol. The value of absorption maxima recorded were in range 250-280 nm (band II, ring A absorption) and 304-350 nm (band I, ring B absorption).

Compound **1** was obtained as yellow needles (4.0 mg) with a melting point 148-150 ^{0}C, exhibited molecular ion peak at m/z 298 corresponding to molecular formula $C_{17}H_{14}O_5$. It's UV spectrum showed maxima at λ_{max} 276 and 334 which is characteristic of band II and band 1 of flavone moiety. IR showed absorption at 3465, 3068 and 1604 cm^{-1} which indicated the presence of OH, C-H aromatic and C=C, respectively. ^{1}H NMR spectrum of **1** illustrated the present of six groups of proton. Two multiplets at δ 7.55 and δ 8.09 intergrated for three and two protons

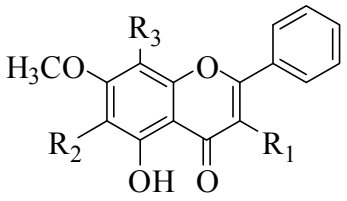
respectively are attributed to monosubstituted B ring. Two signals appeared as doublets at δ 6.39 (1H, *J*=2.1 Hz) and δ 6.49 (1H, *J*=2.1 Hz) indicated the protons as at *meta* position. Two singlets at δ 3.89 (3H, *s*) and δ 3.90 (3H, *s*) showed the presence of two methoxy groups and a singlet at a very downfield region (δ 12.60, 1H, *s*) belong to a chelated OH. The presence of OH at C-5 led to the placement of *meta* couple protons at H-6 and H-8. Methoxyl groups then have to be positioned at C-3 and C-7. The ^{13}C NMR shows the presence of fifteen carbon signals consisting of one carbonyl (δ 179.0), five oxyaryl (δ 139.7, 162.1, 165.6, 156.9, 155.9), two methoxy (δ 55.8, 60.4), two sp^2 quaternary (δ 106.2, 130.5) and five methine sp^2 carbons (δ 98.0, 90.2, 128.4, 128.6, 131.0) respectively. Spectroscopic data observed in **1** showed a very close similarity with 3,7-dimethoxy-5-hydroxyflavone (Buckingham, 1994) isolated from the rhizomes of *Kaempferia parviflora* (Zingiberaceae) (Sutthanut *et al*., 2007). This compound was examined for its inhibitory activity against nitric oxide (NO) production and the IC_{50} 41.6 μM which showed a moderate activity (Tewtrakul *et al*., 2009).

Compound **2** (1.2 mg) was obtained as yellow powder with melting point 180-182 ^{0}C. The MS spectrum shows molecular ion peak at m/z 314 corresponding to molecular formula $C_{17}H_{14}O_6$. The UV spectrum shows maximum absorbance at λ_{max} 274 and 358 which has characteristic of flavone skeleton and the IR spectrum shows absorbance at 3434, 2834, 1637 and 1266 cm^{-1}. The ^{1}H NMR spectrum showed a doublet of doublet with the coupling constant 8.7 Hz and 1.8 Hz observed at δ 8.23. This signal belong to proton H-2' and H-6' located at ring B. A multiplet with integration of three proton at δ 7.52 was assigned to the protons of H-3', H-4' and H-5'. A singlet at δ 6.60 was assigned as H-3. This signal appeared at upper field region due to anisotropic effect at ring C. Another peak resonated at a very low field region δ 11.65 was assigned as hydroxyl group located at H-5. The presence of two methoxyl groups could be observed at δ 3.96 (3H, *s*) and δ 4.00 (3H, *s*). Comparison with the literature values suggested flavonoid **2** as 5,8-dihydroxy-6,7-dimethoxyflavone (Horie *et al.*, 1995).

Compound **3** (13.0 mg) was isolated as white amorphous solid with a melting point 165-166 ^{0}C, and molecular ion peak M^+ at *m/z* 268, corresponding to the molecular formula $C_{16}H_{12}O_4$. The IR shows absorption at 3434, 2987, 1626 and 1262 cm^{-1}. Its UV spectrum showed the λ_{max} at 257 and 306 nm which are characteristic of flavone moiety. The ^{1}H NMR of **2** showed two doublet protons at δ 6.41(1H, *J*=2.1 Hz, H-6) and δ 6.54 (1H, *J*=2.1 Hz, H-8) located at *meta* positions. A singlet at δ 6.41 was assigned to H-3 while two multiplets at δ 7.56 (3H, *m*, H-3', H-4', H-5') and δ 7.92 (2H, *m*, H-2', H-6') belong to protons at monosubstituted ring B. A singlet indicated as a chelated OH was observed at δ 12.75 (1H, *s*, 5-OH) at a very low field region due to the formation of hydrogen bond between proton from the hydroxyl group with the carbonyl group (C=O) in the heterocyclic ring C. A singlet at δ 3.91 was due to the presence of a methoxy group. The DEPT spectrum showed a signal of methoxy carbon at δ 55.8 and six aromatic methine carbons at δ 105.9, 98.2, 92.7, 126.3, 129.1 and 131.9. The rests of signals that do not present in DEPT but observed in ^{13}C spectrum are of quaternary type of carbons. A signal at a very low region (δ 182.5) is definitive of carbonyl carbon of the flavone structure. Four oxyaryl carbons were observed at δ 162.2, 165.6, 165.9 and 165.2 and two quaternary carbons gave signals at δ 131.4 and δ 103.1. In the HMBC spectrum, correlation between δ 3.91 with δ 165.2 further confirmed the attachment of methoxy to C-7 and correlation between δ 6.70 with δ 105.9 confirmed the placement of δ 6.70 at H-3. The rests of the correlations observed were OH/C-5 and H-2', H-6'/C-1'. Comparison of the available value with the literature confirmed that flavonoid **3** is tectochrysin (Nagarajan and Parmar, 1977). This compound was also found in *Pranus cerasus* (McNulty *et al.*, 2009; Nagarajan and Parmar, 1977), *Pranus avium* (McNulty *et al.,* 2009), *Piper manaii* (Parmar *et al.*, 2003), propolis (Bankova *et al.,* 1983) and *Kaempferia parviflora* (Sutthanut *et al.*, 2007).

Compound **4** (12.0 mg) was isolated as white amorphous. The mass spectrum exhibited a molecular ion peak at *m/z* 298, suggesting a possible molecular formula of $C_{17}H_{14}O_5$. The IR spectrum showed the absorption at 3404 cm^{-1} indicating the presence of hydroxyl group, and also absorptions at 1644 cm^{-1} is characteristic of aromatic C=C stretching. The UV spectrum of **4** showed the λ_{max} absorption at 245 and 327 nm suggestive of the flavone moiety. The ^{1}H NMR of **4** illustrated a typical flavone skeleton with monosubstituted ring, chelated OH, two aromatic and two methoxy groups at δ 7.92 and δ 7.55, δ 12.68, δ 3.95 and δ 4.00 respectively. The presence of 15 carbon signals and two methoxyl groups in ^{13}C NMR spectrum showed that **4** is a flavone compound with two methoxyl as the substituents. In ^{13}C NMR spectrum, a very downfield signal at δ 182.8 belongs to carbonyl C-4. The presence of electron withdrawing group oxygen and resonance effect shifted it to downfield region. In addition, five oxyaryl carbons were observed at δ 164.0, 153.1, 132.7, 159.0 and 153.4. Two quaternary carbons δ 106.3 and δ 131.3 were assigned as C-10 and C-1', respectively. The rest of the signals at δ105.7, 90.7, 126.3, 129.1 and 131.9 belong to aromatic methine C-3, C-8, C-2'/C-6', C-3'/C-5' and C-4' respectively. Based on the spectroscopic data and comparison with literature, flavonoid **4** was deduced as 6,7-dimethoxy-5-hydroxyflavone which was previously found to occur in the liverwort of *Leptoscphus expanses* (Lehm.) Grolle (Lophocoleaceae) (Wu, 2008).

Compound **5** (3.0 mg) was isolated as yellow amorphous solid with a melting point 175-176 ^{0}C. The mass spectrum gave a molecular ion peak at *m/z* 328 corresponding to the molecular formula of $C_{18}H_{16}O_6$. The IR spectrum showed an absorption at 3434, 2987 and 1638 cm^{-1} indicating the presence of OH, C-H aromatic and C=C aromatic. The UV spectrum showed absorptions at λ_{max} 271 and 317 nm suggestive of flavone moiety. The ^{1}H NMR showed a singlet resonated at a slightly up field region (δ 6.46) and was assigned as H-8. The presence of monosubstituted ring B at δ 7.56 (3H,*m*, H-3', H-4', H-5') and at δ 8.17 (2H, *m,* H-2', H-6') was obviously observed. Three strong singlet at δ 3.5-4.0 region were due to three methoxy groups giving signals at δ 3.90, δ 3.94 and δ 3.97(9H, *s*, 3OMe). As usual, a signal of chelated OH was detected at low field region at δ 12.45(1H, *s*). Based on spectroscopic data and comparison with the literature, **5** was confirmed as 5-hydroxy-3,6,7-trimethoxyflavone (Alnustin) (Asakawa, 1971, (Mesquita *et al*., 1986; Souleles, 1990).



1. R_1= OCH_3, R_2, R_3=H
2. R_1=H, R_2=OCH_3, R_3=OH
3. R_1, R_2, R_3=H
4. R_1, R_3=H, R_2=OCH_3
5. R_1, R_2=OCH_3, R_3=H

Conclusion:

In summary, the phytochemical study of *P. cauliflora* var. *cauliflora* has led to the isolation of five flavones which are 3,7-dimethoxy-5-hydroxyflavone (**1**), 5,8-dihydroxy-6,7-dimethoxyflavone (**2**), tectochrysin (**3**), 6,7-dimethoxy-5-hydroxyflavone (**4)** and alnustin (**5**). *Polyalthia* was known to contain alkaloids but this phytochemical study revealed that this species contain flavonoids as well. This is the first report of compound **1-5** from *Polyalthia* genus.

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