SHORT REPORT

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Phytochemical Studies and Antioxidant Activities of *Artocarpus scortechinii* King

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Abstract: Six known compounds identified as 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-γ,γ-dimethylallyflavone (**1**), cudraflavone A (**2**), artocarpin (**3**), cycloartobiloxanthone (**4**), artonin E (**5**) and oxyresveratrol (**6**) were isolated from the stem barks of *Artocapus scortechinii* King. Another four compounds namely macakurzin C (**7**), flemichapparin A (**8**), luteolin (**9**) and apigenin (**10**) were isolated from the leaves. Structures of all pure compounds were elucidated spectroscopically using 1D NMR (^{1}H, ^{13}C, DEPT), 2D NMR (COSY, HMQC, HMBC), MS and FTIR, as well as by comparison with literature data. All isolated compounds were evaluated for their antioxidant capacities using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) and ferric reducing antioxidant potential (FRAP) assays. Luteolin (**9**) showed the best ability to act as DPPH free radical scavenger with IC_{50} value of 9.51 μM. Luteolin (**9**) also exhibited the strongest scavenger and potent ferric ion reducer compared to other isolated compounds in the ABTS and FRAP assays with IC_{50} value of 124.4μM and 4.07 ± 0.180 mM FRAP equivalent respectively.

Keywords: *Artocarpus scortechinii*; isolation; antioxidant; DPPH; ABTS; FRAP.

1. Plant Source

Artocarpus scortechinii King (Moraceae) is known as "terap hitam" in Malaysia. It can easily be found scattered throughout Malaysia in lowland forest and Sumatra, Indonesia [1]. This species had been identified to have very close similarity with *A. elasticus* (terap nasi). The difference with *A. elasticus* is on the stem which is darker while the leaf is not too large and wide. Previous phytochemical studies on *Artocarpus* plants had revealed numerous phenolic compounds especially chalcones, flavones, xanthones and stilbenes [2-6]. The leaves and stem barks of *A. scortechinii* were collected from forest of Bukit Fraser, Pahang, Malaysia in December 2013 with voucher number SK2327/14. This plant samples were authenticated by Dr. Shamsul bin Khamis, botanist from Universiti Putra Malaysia.

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2. Previous Studies

Jamil *et al.* [7] had studied the barks of *A. scortechinnii* which were collected from Endau-Rompin Rain Forest, Johore and managed to isolate compounds (**4**) and (**5**). To date, there is no published report regarding study of the leaves of this species.

3. Present Study

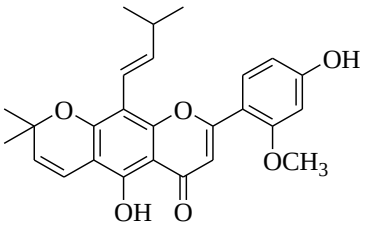
The dried stem barks (2.5 kg) and leaves (4.0 kg) of *A. scortechinnii* were extracted using *n*-hexane, CH_2Cl_2, EtOAc and MeOH at room temperature for three days each. The extraction was repeated three times by adding fresh solvent to ensure exhaustive extraction. The solvents were removed using rotary evaporator to give dark gummy crude extracts of *n*-hexane (4.88 g, 0.20%), CH_2Cl_2 (19.38 g, 0.78%), EtOAc (5.52 g, 0.22%) and MeOH (39.57 g, 1.58%) of the stem barks. Crude extracts obtained from the leaves were *n*-hexane (36.67 g, 0.92%), CH_2Cl_2 (43.75 g, 1.09%), EtOAc (45.27 g, 1.13%) and MeOH (127.14 g, 3.18%).

Purification of the *n*-hexane stem barks extract (4.0 g) using gravity column chromatography (CC) with mixture of *n*-hexane and EtOAc in increasing polarity had afforded 440 fractions. Fractions 146-240 were combined to give 4',5-dihydroxy-6,7-(2,2-dimethylpyrano)-2'-methoxy-8-γ,γ-dimethylallyflavone (**1**) as pale yellow solid (66.7 mg, 1.67%). Compound (**1**) was first isolated from *A. anisophyllus* by our research group [2].

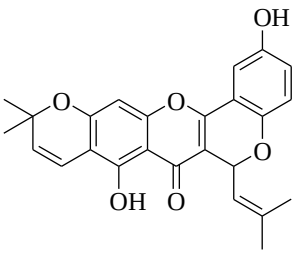
Fractionation of the CH_2Cl_2 stem barks extract (18.0 g) by vacuum liquid chromatography (VLC) using *n*-hexane and EtOAc in increasing polarity by 10% gave six major fractions (AScSD 1-6). The purification of AScLD 5 (0.88 g) by CC (*n*-hexane: EtOAc, 9:1) gave more of compound (**1**) (62 mg, 0.34%), cudraflavone A (**2**) (8.5 mg, 0.05%) as yellow solid, artocarpin (**3**) (41.2 mg, 0.23%) as orange powder and cycloartobiloxanthone (**4**) (7 mg, 0.04%) as orange solid. Cudraflavone A (**2**) and artocarpin (**3**) were previously identified from *A. altilis* [8-9]. Cycloartobiloxanthone (**4**) was previously isolated from *A. lanceifolius*, *A. elasticus*, *A. nobilis* and *A. kemando* [10-13].

Fractionation of the EtOAc stem barks extract (5.5 g) by VLC gave six major fractions (AScSE 1-6). Purification of AScSE 4 (0.6 g) by CC gave artonin E (**5**) (5.3 mg, 0.10%) as orange solid. Purification of AScSE 5 by CC yielded oxyresveratrol (**6**) (24.6 mg, 0.45%). To the best of our knowledge, this is the first report on isolation of compounds (**1**-**3**) and (**6**) from *A. scortechinii* while compounds (**4**) and (**5**) had been reported earlier by Jamil *et al.* [7].

Purification of the *n*-hexane leaves extract (5.0 g) by CC with mixture of *n*-hexane and EtOAc in increasing polarity had afforded macakurzin C (**7**) as yellow needle (3.3 mg, 0.07%). This is the first report on the isolation of macakurzin C (**7**) from *Artocarpus* species. Previously, macakurzin C (**7**) had been isolated from *Macaranga kurzii* by Thanh *et al.* [14].



(**1**)



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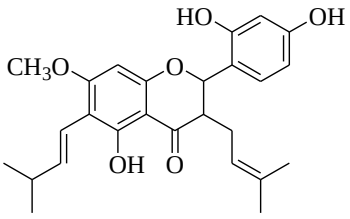

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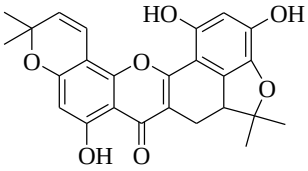
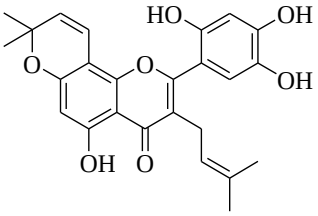
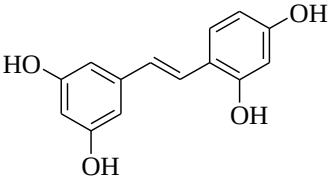


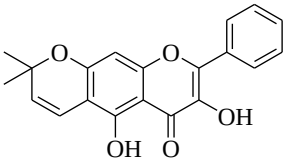
(**4**)



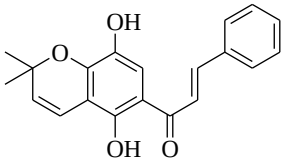
(**5**)



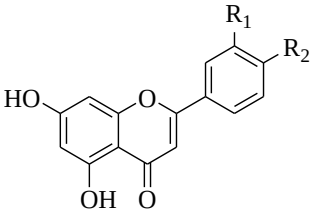
(**6**)



(**7**)



(**8**)



(**9**) $R_1 = R_2 = OH$
(**10**) $R_1 = H$, $R_2 = OH$

Fractionation of the CH_2Cl_2 leaves extract (20.0 g) by VLC (200 g, column size: 10.0 × 5.0 cm) using solvent system *n*-hexane, *n*-hexane-EtOAc and EtOAc in the order of increasing polarity gave nine fractions. Each fraction was subjected to TLC analysis and the same TLC patterns were combined to give four major fractions labeled as AScLD 1 (0.74 g), AScLD 2 (5.19 g), AScLD 3 (6.48 g) and AScLD 4 (7.41 g). Further purification of fraction AScLD 3 had yielded flemichapparin A (**8**) as red needle (17.5 mg, 0.09%).

Fractionation of the EtOAc leaves extract (10.0 g) by VLC gave six major fractions (AScLE 1-6). Purification of fraction AScLE 5 using CC followed by purification through Sephadex LH-20 had produced luteolin (**9**) as yellow solid (13 mg, 0.13%) and apigenin (**10**) as yellow solid (5 mg, 0.05%).This is the first report on isolation of compounds (**8 - 10**) from *A. scortechinii*.

All isolated compounds were tested for their antioxidant capacities using DPPH [15], ABTS [16] and FRAP [16] assays. Trolox, BHA and BHT were used as the standard controls. Luteolin (**9**) showed the best radical scavenger compared to other isolated compounds with IC_{50} values 9.51 µM followed by oxyresveratrol (**6**) with IC_{50} value of 91.21 µM. Cycloartobiloxanthone (**4**) and artonin E (**5**) also had significant DPPH scavenging activities with IC_{50} values of 196.0 µM and 151.6 µM respectively. Luteolin (**9**) also showed the most potent scavenger in the ABTS assay with IC_{50} value of 124.4 µM. The strongest antioxidant activities in ABTS assay were achieved by compounds with many hydroxyl groups [17]. FRAP assay also exhibited luteolin (**9**) as the most potent ferric ion reducer among all isolated compounds with reducing value of 4.07 ± 0.180 mM FRAP equivalent, followed by cycloartobiloxanthone (**4**) with reducing value of 2.79 ± 0.190 mM FRAP equivalent (Table 1). Compounds (**1**-**3**) were observed as inactive antioxidant agents [2]. Compounds with the presence of 2,3-double bond in conjugation with 4-carbonyl group in the ring C will increase the potential of the scavenging activities. The presence of 3-hydroxy group in ring C will also contribute to the scavenging activities in the compounds [17].

Table 1.Antioxidant activities results.

Pure compounds	DPPH IC_{50} (µM)	ABTS IC_{50} (µM)	mM FRAP equivalent to $FeSO_4.7H_2O$ (1.0 mM)
1	N.D	N.D	N.D
2	N.D	N.D	N.D
3	N.D	N.D	0.19 ± 0.19
4	196.0	269.0	2.79 ± 0.19
5	151.6	145.0	1.32 ± 1.17
6	91.21	248.0	1.61 ± 0.19
7	N.D	N.D	N.D
8	131.0	199.7	N.D
9	9.51	124.4	4.07 ± 0.18
10	408.8	156.4	0.07 ± 0.03
Standards			
Trolox	N.T	N.T	2.43 ± 0.40
BHA	49.87	91.01	0.60 ± 0.06
BHT	231.6	154.3	1.89 ± 0.02

IC_{50} is defined as the concentration sufficient to obtain 50% of the maximum scavenging capacity; N.D = Not Determined, N.T =Not tested

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Supporting Information

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

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