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CHEMICAL AND PAF ACTIVITY OF SEVERAL MALAYSIAN *ARTOCARPUS*

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ABSTRACT

Phytochemical studies of *Artocarpus lowii* King, *A. scortechinii* King and *A. teysmanii* Miq. have resulted in the isolation of four new compounds and eight known compounds. Three new compounds have been successfully isolated from *A. lowii* King, i.e. 2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone, 2',3,4',4-tetrahydroxy-3'-prenylchalcone and 2-hydroxyparatocarpin C. Three known compounds were identified as cycloheterophyllin, 2',4',4-trihydroxy-3'-prenylchalcone and 4-hydroxylonchocarpin. A new compound was isolated from *A. scortechinii* King and was identified as 2',4',5',5'-tetrahydroxy-3-geranyl-7,8-(2,2-dimethyl-6H-pyrano)-6-prenylflavone together with three known compounds, i.e. artonin E, artobiloxanthone and lupeol 3-acetate. Four known compounds were isolated from *A. teysmanii* Miq., which were identified as artonin E, artobiloxanthone, artonol B and cycloartobiloxanthone. The structures of all compounds were established based on spectral studies using nuclear magnetic resonance spectroscopy, mass spectrometry, infrared spectroscopy and ultraviolet spectroscopy. The biological studies on the crude extracts and pure compounds of these three species showed that several pure compounds have significant biological activity especially in the platelet aggregation assays. Cycloheterophyllin, artonin E, isobavachalcone and 2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone totally inhibited ADP-induced platelet aggregation compared to standard aspirin which suppressed only 31.6% of the platelet aggregation.

ABSTRAK

Kajian fitokimia ke atas *Artocarpus lowii* King, *A. scortechinii* King dan *A. teysmanii* Miq. berjaya menemukan empat sebatian baru dan lapan sebatian yang diketahui. Tiga sebatian baru ditemui daripada *A. lowii* King dikenalpasti sebagai 2',4'-dihidroksi-4-metoksi-3'-prenildihidroksalkon, 2',3,4',4-tetrahidroksi-3'-prenil-kalkon dan 2-hidroksiparatokarpin C. Tiga sebatian lain dikenalpasti sebagai sikloheterofillin, 2',4',4'-trihidroksi-3'-prenilkalkon dan 4-hidroksilonchokarpin. Satu sebatian baru telah berjaya diasingkan daripada *A. scortechinii* King dan dikenalpasti sebagai 2',4',5',5-tetrahidroksi-3-geranil-7,8-(2,2-dimetil-6H-pirano)-6-prenilflavon bersama-sama tiga sebatian diketahui iaitu artonin E, artobiloxanton dan lupeol 3-asetat. Empat sebatian berjaya diasingkan daripada *A. teysmanii* Miq. dan dikenalpasti sebagai artonin E, artobiloxanton, artonol B dan sikloartobiloxanton. Struktur kesemua sebatian dikenalpasti berdasarkan kepada kajian spektra dengan menggunakan spektroskopi resonan magnetik nukleus, spektrometri jisim, spektroskopi infra merah dan spektroskopi ultralembayung. Kajian aktiviti biologi ke atas ekstrak mentah dan sebatian tulen daripada ketiga-tiga spesies *Artocarpus* ini mendapati beberapa sebatian menunjukkan aktiviti biologi yang signifikan terutamanya di dalam asai agregasi platelet. Sikloheterofillin, artonin E, isobavakalkon dan 2',4'-dihidroksi-4-metoksi-3'-prenildihidroksalkon merencat 100% agregasi platelet yang dirangsang oleh ADP, berbanding dengan aspirin yang hanya mampu merencat sebanyak 31.6% sahaja.

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1.1 INTRODUCTION

Artocarpus is the most commonly encountered genus, representatives of the Moraceae in the lowland forest of the tropical Southeast Asia, apart from *Ficus*. There are 47 species of *Artocarpus* in which only 20 species including the cultivated plants could be found in Malaysia. This genus is known world wide for its edible fruits like the jackfruit, *A. heterophyllus* locally known as ‘nangka’, bread fruit, *A. communis* (‘sukun’) and ‘cempedak’, *A. integer*. These species are widely cultivated in Malaysia as villagers and traders commercially sell their fruits in local market. The lightwood known locally as ‘terap’ and the medium hardwood known as ‘keledang’ constitute valuable timber resources [1, 2]. Some of Malaysian *Artocarpus* species are rare. Most of these species have never been chemically and biologically investigated including *A. anisophyllus*, *A. bracteata*, *A. fulvicortex*, *A. hispidus*, *A. kemando*, *A. lowii*, *A. nitidus*, and *A. odoratissima* [1].

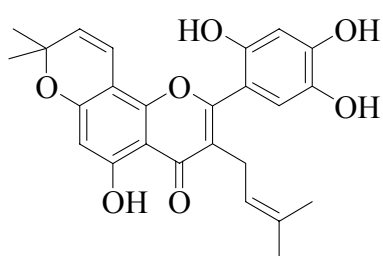
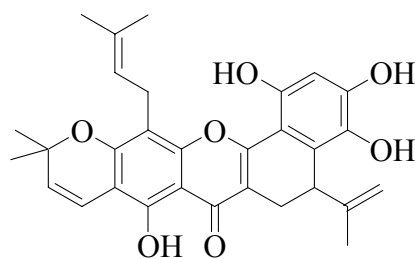
Artocarpus species have been studied quite thoroughly, chemically or biologically by few groups of researchers from Indonesia, Japan, and Taiwan. Most of the plants studied were collected from the rain forest of Indonesia. Some studies cover *Artocarpus* species of Taiwan, Caribbean and Thailand. Several new and interesting compounds have been isolated, characterized and evaluated for their biological activities. *Artocarpus* plants have also been used as traditional medicine especially in Indonesia against inflammation, malarial fever and stomachache.

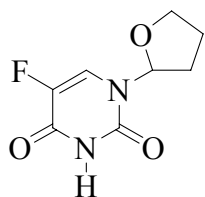
1.2 BIOLOGICALLY ACTIVE PHYTOCHEMICALS FROM SEVERAL *ARTOCARPUS* SPECIES

Artocarpus species are noted as an abundant source of phenolic constituents. These constituents can be classified into isoprenylflavonoids, stilbenoid and 2-arylbenzofuran derivatives, phenolic compounds with oxepine ring and natural Diels-Alder type adducts. Biological studies on the phytochemicals isolated from these plants

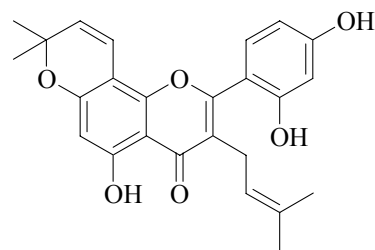
showed some interesting findings which some how justified the usage of *Artocarpus* plants in traditional practices.

Artonin E (**1**) and B (**2**) which were isolated from *A. communis* showed stronger activity against cancer cells, mouse L-1210 and colon 38 compared to the critical drug, theafulvin (**3**). Artonin E (**1**) also exhibited the most potent inhibition on arachidonate 5-lipoxygenase with IC₅₀ value of 0.36 μM [3, 4]. Artonin E (**1**) was also expected to be an anti-tumor promoter based on the similarity of the structure with morusin (**4**) which was isolated from the root bark of *Morus alba* L.. The latter compound had been reported as an anti-tumor promoter in a two-stage carcinogenesis experiment with teleocidin [5, 6]. Both compounds have an isoprenoid moiety at the C-3 position. The difference is in the B ring where morusin (**4**) has a structure bearing a 2',4'-dioxxygenated pattern while artonin E (**1**) has a structure bearing a 2',4',5'-trioxygenated pattern. Thus, the inhibitory effect of the *Artocarpus* flavonoids on tumor-necrosis factor-α (TNF-α) release stimulated by okadaic acid using BALB/3T3 cells has been examined [7, 8]. All the tested compounds inhibited the TNF-α release stimulated by okadaic acid at suitable lower concentration. Artonin E (**1**) was found to be the most potent inhibitor. Heterophyllin (**5**) and artobiloxanthone (**6**) inhibited stronger than cycloheterophyllin (**7**), cycloartobiloxanthone (**8**), and morusin (**4**). The compounds which exhibited strong activity have three hydroxyl groups in the B ring. This characteristic feature might be one of the important factors for their biological activity.

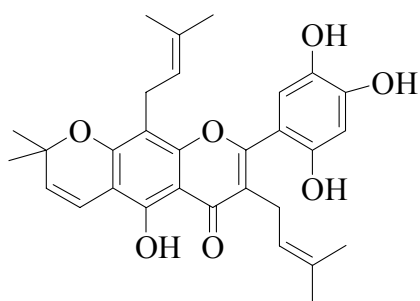
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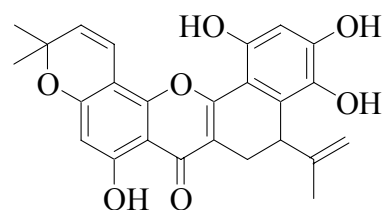
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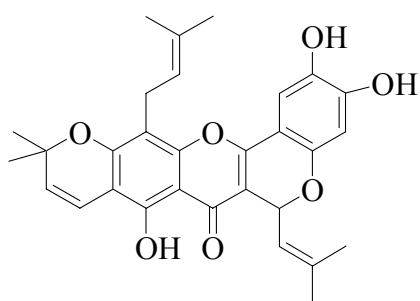
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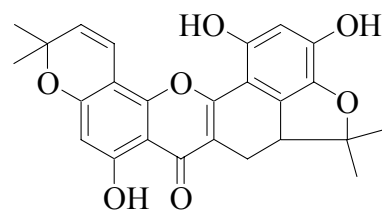
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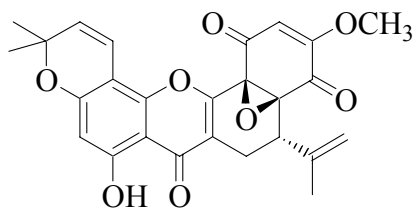
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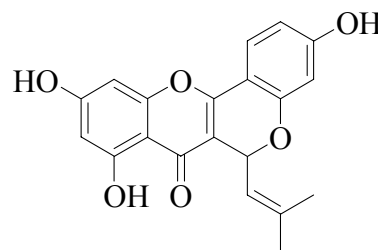
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The cytotoxicity of prenylflavonoids isolated from the Formosan *A. communis* against human hepatoma PLC/PRF/5 and KB cells *in vitro* had also been reported [9]. Artomunoxanthotrione epoxide (9), cyclocommunol (10), and cyclocommunin (11) showed cytotoxic activities against both cells with ED₅₀ values not more than 2.5 μg/mL. Cyclocommunol (10), and cyclocommunin (11) were also reported to have antiplatelet actions on collagen-induced and arachidonic acid-induced platelet aggregation with little or no effect on PAF-induced platelet aggregation [10]. The antiplatelet activities of

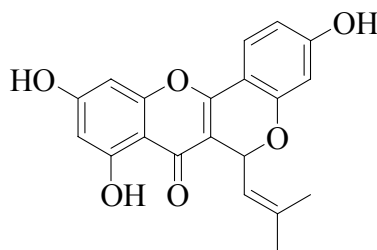
prenylated flavonoids isolated from *A. heterophyllus* was also reported where cycloheterophyllin (**7**) showed inhibition of arachidonic acid-induced platelet aggregation with IC_{50} value of $10.9 \mu\text{M}$ [11]. The antibacterial activity of *A. heterophyllus* against Gram-positive and Gram-negative bacteria has also been studied. The extract of *A. heterophyllus* showed a broad spectrum of antibacterial activity [12]. The extract also showed activity against cariogenic bacteria. The extract inhibited the growth of primary cariogenic bacteria at concentration of $3.11\text{-}12.5 \mu\text{g/mL}$ and also exhibited the growth inhibitory effects on plaque-forming *Streptococci*. This finding showed that phytochemicals from *A. heterophyllus* would be potent compounds for the prevention of dental caries [13].



(9)



(10)



(11)

In this study, the crude extracts and phytochemicals from Malaysian species i.e. *Artocarpus lowii*, *A. scortechinii* and *A. teysmanii* were tested for their platelet activating factor (PAF) receptor binding inhibitory and antiplatelet aggregation activity.

1.3 EXPERIMENTAL

1.3.1 Platelet Activating Factor (PAF) Receptor Binding Activity

The assay was carried out according to the modified method of Valone *et al.* [14]. The pure compounds were each dissolved in DMSO and ethanol (1:1). The stock solutions were diluted with normal saline to give final concentrations of 200, 100, 50, 20 and 10 $\mu\text{g}/\text{mL}$. The final concentrations of the sample in the reaction mixtures were either 18.2, 9.1, 4.5, 1.8 or 0.9 $\mu\text{g}/\text{mL}$. The final concentration of DMSO in the reaction mixture was fixed at 0.2% to avoid interference with the receptor binding studies. The reaction mixture with saline and 0.2% DMSO in saline was used as a control.

Six volumes of blood were collected from the rabbit (New Zealand White) marginal ear vein directly into 1 volume of ACD solution (0.15 M trisodium citrate, 0.075 M citric acid, pH 5.2). The blood was centrifuged at $270 \times g$ for 10 min at room temperature, and the top platelet-rich plasma was removed carefully. The latter was further centrifuged at $500 \times g$ for 15 min. The platelet pellets were washed twice by centrifugation at $500 \times g$ (15 min) in buffer A (20% ACD solution, 60% K_2HPO_4 buffer, 20% sodium citrate, pH 6.8) followed once at $150 \times g$ (10 min) in buffer B (50 mL K_2HPO_4 , 0.1 g bovine serum albumin, pH 7.0). The final platelet concentration was adjusted to 3×10^8 platelets/mL.

The reaction mixture consisted of 200 μL of washed rabbit platelet suspension, 25 μL of ^3H -PAF (2.0 nM) with or without unlabelled PAF (2.0 nM) and 25 μL of sample or control solution. Cedrol, a known PAF receptor antagonist, was used as a standard in the bioassay. The reaction mixture was incubated at room temperature for 1 hour. The free and bound ligands were separated by filtration technique using Whatman GF/C glass fibre filters. The radioactivity was measured by scintillation counting. The difference between the total radioactivities of bound ^3H -PAF in the absence and in the presence of excess unlabelled PAF is defined as the specific binding of the radiolabelled ligand. The IC_{50} values of the samples were obtained from at least three determinations. Percentage inhibition effect of the sample was obtained by the following equation:

$$\begin{aligned} \text{Inhibitory effect, \%} &= \left[\frac{S_c - S_s}{S_c} \right] \times 100 \\ &= \left[\frac{(T_c - N_c) - (T_s - N_s)}{(T_c - N_c)} \right] \times 100 \end{aligned}$$

where S_c = specific binding of control; S_s = specific binding of sample; T_c = total binding of control; T_s = total binding of sample; N_c = nonspecific binding of control; N_s = nonspecific binding of sample

1.3.2 Platelet Aggregation Assay

The platelet aggregation assay was conducted using electrical impedance method [21]. Whole blood (1 mL) diluted with phosphate-buffered saline (1:1 ratio) was incubated with samples (5 μ L, in DMSO) at 37°C for 2 min, after which collagen (2 μ g/mL), ADP (10 μ M), and AA (0.5 mM) was added to initiate aggregation. The platelet aggregation was measured by a Whole Blood Lumi-Aggregometer using an electrical impedance method. The mean percentages of aggregation in whole blood was determined at 5 min as the increase in impedance across a pair of electrodes placed in the blood sample by comparison to that of a control group impedance. To eliminate the effect of the solvent on the aggregation, blood with 0.5% DMSO was used as the control. Each sample was measured in triplicate. The percentage inhibition of platelet aggregation was calculated as follows:

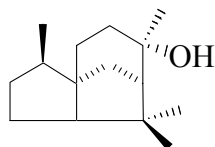
$$\% \text{ Inhibition} = [1 - (\text{aggregation of sample} / \text{aggregation of control})] \times 100$$

1.4 RESULTS AND DISCUSSION

1.4.1 Platelet Activating Factor (PAF) Receptor Binding Activity

The PAF assay was conducted based on Valone *et al.* [14]. Platelet activating factor (PAF) or 1-alkyl-2-acetyl-glycero-3-phosphocholine is a pair of phospholipids produced by proinflammatory cells such as endothelia cells, platelets, macrophages, and monocytes [15]. It involves several pharmacological actions, such as the induction of platelet and neutrophil aggregation, bronchoconstriction, hypotension, and increased vascular permeability. PAF may also be involved in a variety of pathophysiological conditions, including arterial thrombosis, acute inflammation, endotoxic shock, acute allergic disease, and early pregnancy [16-18]. Therefore, specific antagonists of PAF that inhibit the specific binding to the receptors may be useful therapeutic agents and have been extensively sought from natural sources or produced synthetically. Several natural and synthetic antagonists with diverse chemical structures have been discovered [19]. This suggests that the receptor molecule may accommodate a wide variety of ligand structures, hence, there is still the possibility of finding new PAF antagonists especially from natural products [20].

Eleven compounds were investigated for their PAF receptor binding inhibitory effects using rabbit platelets. Cedrol (**12**), a known PAF receptor antagonist was used as a standard. **Table 1.1** shows the percentage inhibitory effects of the compounds at concentration of 18.2 $\mu\text{g/mL}$. Compound that gives more than 55% inhibitory effect is considered active. In this assay, all compounds exhibited low effects except for artonin E, cycloheterophyllin, and isobavachalcone which showed moderate inhibitory effects compared to cedrol with values of 42%, 51%, and 49% respectively. These results indicate that most of the tested compounds were weak PAF receptor antagonists.



(12)

Table 1.1: Inhibitory Effects of Compounds of *Artocarpus* Species on PAF Receptor Binding to Rabbit Platelets^a

Compounds	Inhibition (%)
Cycloheterophyllin	42
Artonin E	51
Artobiloxanthone	19
Cycloartobiloxanthone	13
Artonol B	3
Isobavachalcone	49
4-hydroxylonchocarpin	34
2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone	32
Cycloheterophyllin diacetate	30
Lupeol 3-acetate	5
Artonin E tetraacetate	29
Cedrol (12) ^b	70

^a Concentration of sample in reaction mixture = 18.2 µg/mL

^b Standard antagonist

1.4.2 Platelet Aggregation Activity

Seven compounds isolated from *Artocarpus lowii*, *A. scortechinii* and *A. teysmanii* and two of their derivatives were evaluated for their ability to inhibit platelet aggregation of human whole blood induced by collagen, adenosine diphosphate (ADP) and arachidonic acid (AA) *in vitro* using an electrical impedance method [21]. Acetylsalicylic acid (aspirin) was used as a reference. Aspirin is an antiplatelet agent that inhibits platelet cyclooxygenase-1 (COX-1) and, as a consequence, prevents the formation of the pro-aggregatory substance, thromboxane A₂ (TXA₂) [22]. The whole blood with 0.5% DMSO was used as the control. This is to eliminate the effect of the solvent on the aggregation. Collagen, ADP and AA was the agonists that triggered platelet aggregation in the pathogenesis of several diseases including atherosclerosis and

cardiovascular malfunction [23, 24]. In this study, the percentage of the inhibitory effects of each tested compound and aspirin on platelet aggregation induced by 2 $\mu\text{g/mL}$ collagen, 10 μM ADP and 0.5 mM AA were sought. The results were summarized in **Table 1.2**.

The results showed that cycloheterophyllin, artonin E, isobavachalcone, and 2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone totally inhibited ADP-induced platelet aggregation (100.0%) compared to aspirin which suppressed only 31.6% of the platelet aggregation. Cycloartobiloxanthone and artonin tetraacetate also showed promising results by 89.5% inhibition each. Further, cycloheterophyllin and artonin E showed moderate effect on AA-induced aggregation by 66.7% and 58.3%, respectively, while aspirin showed 100.0% inhibition. For collagen-induced aggregation, only artonin E and artonin tetraacetate gave significant results with inhibition values of 55.0% and 45.0% respectively compared to aspirin which gave 45.0% inhibition.

In conclusion, all compounds inhibited platelet aggregation induced by ADP more than the reference, aspirin except for artobiloxanthone and cycloheterophyllin diacetate. Only artonin E and its derivatives showed significant inhibition on platelet aggregation induced by collagen. All compounds showed weak activity on platelet aggregation induced by AA compared to aspirin.

Table 1.2 : Percentage Inhibition of Compounds (100 µg/mL) From *Artocarpus lowii*, *A. scortechinii* and *A. teysmanii*, Two Derivatives and Aspirin (25 µg/mL) on Platelet Aggregation of Human Whole Blood

Compound	Inhibition (%)		
	Collagen (2 µg/mL)	ADP (10 µM)	AA (0.5 mM)
Cycloheterophyllin	30.0 ± 1.41	100.0 ± 0.0	66.7 ± 0.0
Artonin E	55.0 ± 0.71	100.0 ± 0.0	19.1 ± 0.71
Artobiloxanthone	0.0 ± 0.0	15.8 ± 1.41	4.8 ± 0.0
Cycloartobiloxanthone	0.0 ± 0.0	89.5 ± 0.0	4.8 ± 0.0
Artonol B	30.0 ± 1.41	36.8 ± 0.0	14.3 ± 1.41
Isobavachalcone	40.0 ± 1.0	100.0 ± 0.0	38.1 ± 0.71
2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone	30.0 ± 0.0	100.0 ± 0.0	58.3 ± 0.71
Cycloheterophyllin diacetate	20.0 ± 0.0	0.0 ± 0.0	19.1 ± 0.71
Artonin E tetraacetate	45.0 ± 0.71	89.5 ± 1.41	14.3 ± 0.0
Aspirin	45.0 ± 0.71	31.6 ± 1.0	100.0 ± 0.0

1.5 CONCLUSION

The results, especially in the platelet aggregation study revealed that phytochemicals isolated from Malaysian *Artocarpus* plants can be further developed to be used as antiallergy, antiasthmatic and antiinflammatory drugs.

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