

Article

Inhibitory Effects of Acetylmelodorinol, Chrysin and Polycarpol from *Mitrella kentii* on Prostaglandin E₂ and Thromboxane B₂ Production and Platelet Activating Factor Receptor Binding

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Abstract: Acetylmelodorinol, chrysin and polycarpol, together with benzoic acid, benzoquinone and stigmasterol were isolated from the leaves of *Mitrella kentii* (Bl.) Miq. The compounds were evaluated for their ability to inhibit prostaglandin E₂ (PGE₂) and thromboxane B₂ (TXB₂) production in human whole blood using a radioimmunoassay technique. Their inhibitory effect on platelet activating factor (PAF) receptor binding to rabbit platelet was determined using ³H-PAF as a ligand. Among the compounds tested, chrysin showed a strong dose-dependent inhibitory activity on PGE₂ production (IC₅₀ value of 25.5 μM), which might be due to direct inhibition of cyclooxygenase-2 (COX-2) enzymatic activity. Polycarpol, acetylmelodorinol and stigmasterol exhibited significant and concentration-dependent inhibitory effects on TXB₂ production with IC₅₀ values of 15.6, 19.1 and 19.4 μM, respectively, suggesting that they strongly inhibited COX-1 activity. Polycarpol and acetylmelodorinol showed strong dose-dependent inhibitory effects on PAF receptor binding with IC₅₀ values of 24.3 and 24.5 μM, respectively.

Keywords: *Mitrella kentii*; chrysin; acetylmelodorinol; polycarpol; prostaglandin E₂ (PGE₂); thromboxane B₂ (TXB₂); platelet activating factor (PAF) antagonist

1. Introduction

The hydrolysis of the sn-2 position of membrane glycerophospholipids to liberate arachidonic acid (AA), a precursor of eicosanoids including prostaglandins (PGs) and leukotrienes (LTs), is catalyzed by phospholipase A₂ (PLA₂). Prostaglandin E₂ (PGE₂), a metabolite of AA through the cyclooxygenase-2 (COX-2) pathway, has received great attention because of its role and contribution to inflammation. It is also involved in a variety of other functions, such as vasodilation, febrile responses, and altered microvascular permeability [1]. Thromboxane A₂ (TXA₂) is an oxidation product derived from AA in cyclooxygenase and thromboxane synthase dependent reactions. TXA₂ is rather unstable and is rapidly hydrolyzed into the almost inactive, stable and more measurable metabolite thromboxane B₂ (TXB₂) [2]. The determination of serum TXB₂ production by platelets following blood coagulation is a specific and most common method for evaluation of COX-1 activity in human and other species [3]. TXA₂ is a vasoconstrictor and a promoter of platelet aggregation and plays an important role in the maintenance of vascular homeostasis. TXA₂ has been postulated to be a mediator contributing to the pathophysiology of several of disease processes such as thrombosis, atherosclerosis and myocardial ischemia [4]. The radioimmunoassay technique (RIA) is the most common way to quantify PGE₂ and TXB₂ in plasma as it is able to detect and quantify the antigen-antibody interaction [5].

PAF is a potent glycerophospholipid mediator, involved in several pathophysiological conditions such as inflammation [6], allergy [7], asthma [8] and thrombosis [9]. Specific receptors for PAF have been reported in a variety of cell membranes including those from platelets [10]. Therefore, compounds which inhibit the specific binding between PAF and receptors may be used as leads in the development of therapeutic agents in a variety of inflammation, respiratory, immunological and cardiovascular disorders [11]. It has been reported that the synthesis of both PAF and prostanoids shares common metabolic pathways, but the relationships between them have not been defined extensively [12]. Some authors have suggested that TXA₂ can be the mediator of the PAF effects in some pathophysiologic conditions [13]. In addition, PAF induce an increased PGE₂ synthesis in cultured rat mesangial cells. Moreover, they suggested an intermediate PAF role in the prostanoids secondary production [14].

Mitrella kentii (Bl.) Miq. is a tree-climbing liana belonging to the custard apple family called Annonaceae. Its synonyms are *Melodorum pisocarpum* and *Me. elegans*, while the common (Indonesian) name is 'kiawi'. The plant is found in the tropics, especially in the Asia-Pacific regions. It is used traditionally in Malaysia as a drink in the form of a root decoction to treat fever [15]. Earlier phytochemical investigation on the bark of the plant had reported the isolation of four alkaloids, namely liriodenine, anonaine, and asimilobin and aequaline [16]. Terpenylated dihydrochalcones: (–)-neolinderatin, (–)-linderatin and 2',6'-dihydroxy-4' methoxydihydrochalcone, and (+)-catechin have also been identified in the stem bark of *M. kentii* [17].

In our screening study to identify compounds from tropical plants as potential anti-inflammatory agents, we observed that the methanol extract of the leaves of *M. kentii* showed strong inhibitory effects on PGE₂ and TXB₂ production in human whole blood (>50.0% inhibition) and PAF receptor binding to rabbit platelets (>60% inhibition). In this paper, we report on the isolation of acetylmelodorinol, chrysin and polycarpol, together with benzoic acid, benzoquinone and stigmasterol,

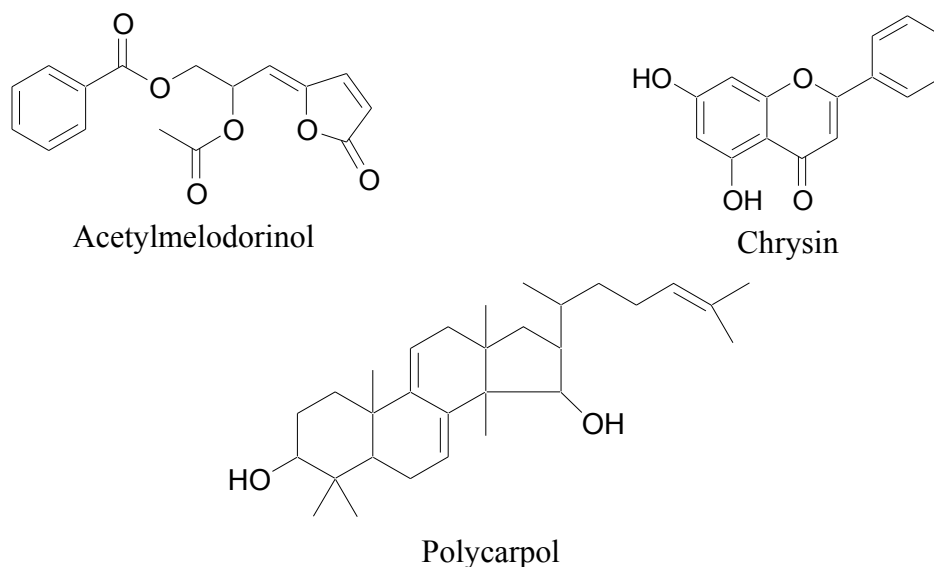
from this plant and their ability to inhibit COX-1 and COX-2 activities through inhibition of the production of TXB₂ and PGE₂ in human whole blood and displacement of ³H-PAF-specific binding in washed rabbit platelets.

2. Results and Discussion

2.1. Isolation and Identification of Compounds

In this study, six compounds have been isolated from the leaves of *Mitrella kentii* by chromatographic techniques. The compounds were identified as benzoic acid, acetylmelodorinol [18], chrysin [19], benzoquinone [20], stigmasterol [21] and polycarpol [18] by comparing their physicochemical and spectroscopic properties with literature values, in addition to their 2D NMR spectra and ESI-MS spectra (See Figure 1 for the structures of acetylmelodorinol, chrysin and polycarpol). Alkaloids and terpenylated dihydrochalcones reportedly identified in the bark of the plant in previous studies were not found in the leaves of the plant [16,17].

Figure 1. Chemical structures of acetylmelodorinol, chrysin and polycarpol.



2.2. Inhibition of Production of PGE₂ and TXB₂

The cell viability test carried out to evaluate the cytotoxicity of the compounds on the blood cells at 1.25 and 10.0 µg/mL indicated that the blood cells were viable (>95%) after 24 h incubation. The compounds were investigated for their inhibitory effects on production of PGE₂ and TXB₂ in whole blood at 10 µg/mL. Compounds which showed strong inhibitory activity (>50% inhibition) were subsequently tested at serial concentrations for determination of their IC₅₀ values. Amongst the compounds tested, chrysin exhibited the strongest inhibitory effect on the production of PGE₂ (63.6% inhibition) induced by lipopolysaccharide (LPS) in whole blood (Table 1). Chrysin was further investigated at various concentrations and it showed dose-dependent response, *i.e.*, as the concentration of the compound increased, the percentage inhibition increased (Table 2). However, the IC₅₀ value of chrysin (25.5 µM) was higher than that of indomethacin (9.5 µM), a potent cyclooxygenase inhibitor [22].

The inhibition of PGE₂ production might be due to direct inhibition of cyclooxygenase-2 (COX-2) enzymatic activity, possibly by the anti-oxidant effects of the compound or related to its capacity to bind COX-2 active site. However, phospholipase A₂ (PLA₂) may also be as a potential target for the compound.

Table 1. Percentage inhibition (%) of compounds isolated from *Mitrella kentii* at 10 µg/mL on PGE₂ production in human whole blood induced by LPS.

Compound	% Inhibition
Benzoic acid	41.5 ± 7.4 *
Acetylmelodorinol	23.1 ± 6.0 *
Chrysin	63.6 ± 6.0 *
Benzoquinone	29.9 ± 14.3 *
Stigmasterol	36.4 ± 5.6 *
Polycarpol	34.7 ± 10.9 *
Indomethacin	83.8 ± 4.9 *

Values are presented as mean ± SD (n = 3); * *P* < 0.05 as compared with control.

Table 2. Percentage inhibition (%) and IC₅₀ values of chrysin from *Mitrella kentii* on PGE₂ production in human whole blood induced by LPS.

Compounds	Concentrations (µg/mL)				IC ₅₀ µg/mL (µM)
	10	5	2.5	1.25	
Chrysin	63.6	38	14.2	5.8	6.5 ± 0.3 (25.5)
Indomethacin	83.8	63.1	32.4	12.35	3.4 ± 0.5 (9.5)

Values are presented as mean ± SD (n = 3); IC₅₀ values in µM are presented in parentheses.

In the TXB₂ assay, acetylmelodorinol displayed the highest percentage inhibition (61.5%), followed by polycarpol (60.6%), stigmasterol (54.3%) and chrysin (51.2%) (Table 3). The percentage inhibition of the compounds at different concentrations is shown in Table 4. The results showed that the compounds inhibited TXB₂ production in human whole blood in a dose-dependent manner, *i.e.*, as the concentrations of the compounds increased the percentages of inhibition increased (Table 4). The IC₅₀ values for polycarpol, acetylmelodorinol, stigmasterol and chrysin were 15.6, 19.2, 19.4 and 39.3 µM, respectively. The IC₅₀ value of polycarpol was comparable to that of indomethacin (12.8 µM). Since the measurement of serum TXB₂ production by the platelets following blood coagulation is a specific test for assessment of COX-1 activity, the results suggest that the inhibitory effect of the compounds could be due to direct inhibition of COX-1 enzymatic activity.

Chrysin, a flavone devoid of carboxylic acid functional group, which showed strong inhibitory effects on PGE₂ and TXB₂ production in human whole blood could be used as a lead compound in the development of COX inhibitors. Chrysin has been reported to have several biological activities including antioxidant, anti-allergic, anti-inflammatory, anticancer, antiestrogenic, hypoglycemic and anxiolytic activities [23]. Of all the compounds tested, polycarpol showed the strongest inhibitory activity on TXB₂ production, reflecting its specific inhibition of COX-1 activity. The butenolide, acetylmelodorinol, which was the major compound in *M. kentii*, also showed significant inhibitory activity on TXB₂ production in human whole blood. Stigmasterol which showed moderate effect on

TXB₂ production has been reported to possess strong anti-inflammatory activity [24]. In a study conducted by Gomez *et al.* [25], stigmasterol was found to be more effective as topical anti-inflammatory agent in acute than in chronic processes.

Table 3. Percentage inhibition (%) of compounds from *Mitrella kentii* at 10 µg/mL on TXB₂ production in human whole blood.

Compound	% Inhibition
Benzoic acid	42.1 ± 6.4 *
Acetylmelodorinol	61.5 ± 7.0 *
Chrysin	51.2 ± 4.0 *
Benzoquinone	33.1 ± 10.6 *
Stigmasterol	54.3 ± 10.4 *
Polycarpol	60.6 ± 7.8 *
Indomethacin	73.9 ± 6.5 *

Values are presented as mean ± SD (n = 3); * *P* < 0.05 as compared with control.

Table 4. Percentage inhibition (%) and IC₅₀ values of compounds isolated from *Mitrella kentii* on TXB₂ production in human whole blood.

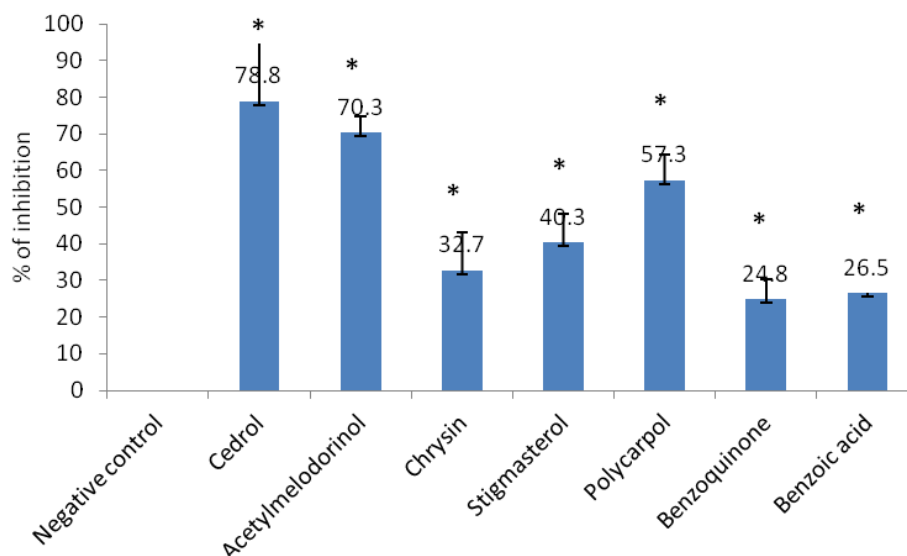
Compound	Concentrations (µg/mL)				IC ₅₀ µg/mL (µM)
	10	5	2.5	1.25	
Acetylmelodorinol	61.5	47.3	18.5	7.4	5.8 ± 0.1 (19.2)
Stigmasterol	54.3	30.2	10.4	5.8	8.0 ± 1.1 (19.4)
Polycarpol	60.6	30.8	18.3	3.2	6.9 ± 0.6 (15.6)
Chrysin	51.2	23	16.7	7.5	10.1 ± 0.8 (39.3)
Indomethacin	73.9	50.7	29.2	10.5	4.6 ± 0.1 (12.8)

Values are presented as mean ± SD (n = 3); IC₅₀ values in µM are presented in parentheses.

2.3. Effect of the Compounds on ³H-PAF Receptor Binding

The inhibitory effect of the compounds on PAF receptor binding to rabbit platelets at the concentration of 18.2 µg/mL is shown in Figure 2. Among the compounds studied, acetylmelodorinol and polycarpol showed significant inhibitory effects on the PAF receptor binding with percentages of inhibition of 70.3 and 57.3%, respectively. The percentage inhibition of acetylmelodorinol was comparable to that of the positive control, cedrol (78.8%), a known PAF antagonist from natural sources [26]. The inhibitory effects of the compounds were then evaluated at various concentrations and the IC₅₀ values of acetylmelodorinol and polycarpol were determined by probit analysis as 24.5 and 24.3 µM, respectively (Table 5). Acetylmelodorinol and polycarpol showed relatively strong PAF antagonistic activity and their dose-dependent responses are shown in Figure 3. The compounds inhibited the specific binding between PAF and receptors and thus they may be used as leads in the development of therapeutic agents in a variety of inflammation, respiratory, immunological and cardiovascular disorders [27]. Polycarpol has been shown to exhibit interesting antitrypanosomal activity but its anti-inflammatory effect has not been reported [28]. Acetylmelodorinol has been reported to exhibit general antiproliferative activity toward tumor cells [18].

Figure 2. Percentage inhibition (%) of the compounds isolated from *Mitrella kentii* on PAF receptor binding to rabbit platelets at 18.2 µg/mL.



Values are presented as mean $\pm$ SD (n = 3); Standard deviation of the mean are indicated as vertical bars; * P < 0.05 as compared with control.

Figure 3. Inhibitory effects of acetylmelodorinol, polycarpol and cedrol on PAF receptor binding to rabbit platelets at different concentrations.

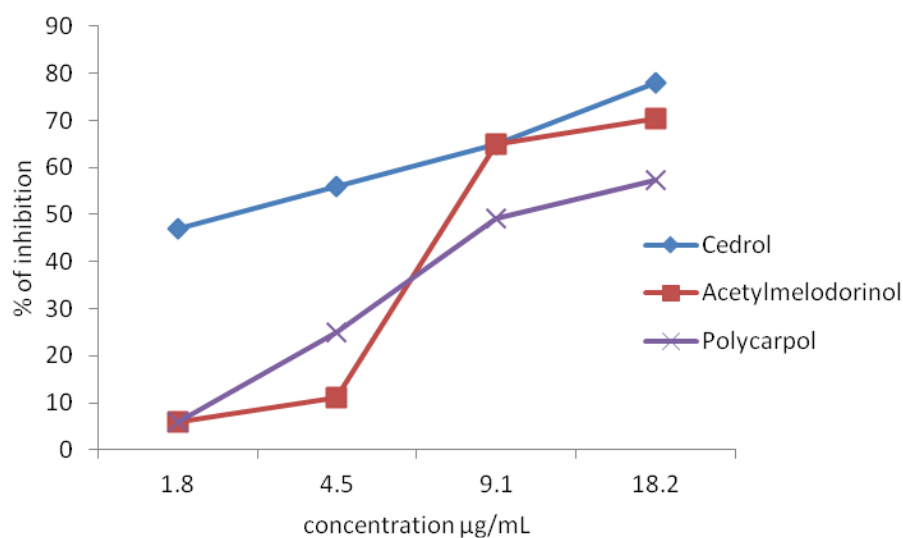


Table 5. IC₅₀ values of the active compounds from *Mitrella kentii* on PAF receptor binding to rabbit platelets.

Compound	IC ₅₀ µg/mL (µM)
Acetylmelodorinol	7.4 $\pm$ 0.4 (24.5)
Polycarpol	10.7 $\pm$ 0.3 (24.3)
Cedrol	2.9 $\pm$ 0.5 (13.0)

Values are presented as mean $\pm$ SD (n = 3). IC₅₀ values in µM are presented in parentheses.

3. Experimental

3.1. General

Hexane, ethyl acetate and methanol used were analytical grades. Radiolabelled PGE₂ ([³H]-PGE₂, 50 µCi mmol^{−1}) and TXB₂ ([³H]-TXB₂, 25 µCi mmol^{−1}) were purchased from Amersham (Buckinghamshire, UK). Unlabelled PGE₂, unlabelled TXB₂, anti-PGE₂ and anti-TXB₂ were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Radiolabeled PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, 125 Ci/mmol) was purchased from Amersham. Unlabeled PAF and cedrol were obtained from Sigma Chemical Co. Bovine Serum Albumin (BSA) was purchased from Boehringer Mannheim Co. (Mannheim, West Germany). Other chemicals were purchased from Merck Co. (Darmstadt, Germany) and BDH Laboratory Supplies (Poole, UK). Ethylenediamine tetraacetic acid (EDTA) 2% was used as anticoagulant. Lipopolysaccharide (LPS) 1 mg/mL was used as prostaglandin endoperoxide synthesis induction in whole blood. Phosphate buffer solutions (PBS) 0.01 M, pH 7.4 was used for assays buffer. Dextran charcoal (0.4% dextran, 2% charcoal) was used to separate the free and bound ligand. Scintillation cocktail was made up of 2,5-diphenyloxazole (PPO, 0.26%), 2,2'-*p*-phenylene-bis(5-phenyloxazole (POPOP, 0.006%), toluene (500 mL) and Triton X (250 mL). Radioactivity was measured by a liquid scintillation counter (LSC) (Packard Tri-Carb, models 2100TR/2300TR, Hamburg, Germany). Melting points were determined using a XSP-12 model 500X hot stage melting point apparatus equipped with a microscope, and were uncorrected. Electrospray ionization mass spectrometry (ESI-MS) was performed on MicroTOF-Q mass spectrometer (Bruker, Coventry, UK). The ¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz) spectra were recorded on a JEOL NMR spectrometer (JNM-GX, Echting, Germany) in CDCl₃ or CD₃OD with TMS as internal standard.

3.2. Plant Material

Fresh leaves of *Mitrella kentii* were collected from the Angsi mountain forest in Negeri Sembilan, Malaysia in July 2008 and identified by Dr Kamarudin Mat Salleh, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM). A voucher specimen (AZ 69) was deposited at the Herbarium of Faculty of Science and Technology, UKM.

3.3. Extraction and Isolation of Compounds

Dried ground leaves of *M. kentii* (1,000 g) were extracted successively with hexane (3 × 2.5 L, 24 h each), ethyl acetate (3 × 2.5 L, 24 h each) and methanol (3 × 2.5 L, 24 h each) using maceration technique. The solvents were then evaporated using a rotatory evaporator to yield hexane (14.9 g, 1.5%), ethyl acetate (29.5 g, 3.0%) and methanol (67.8 g, 6.8%) extracts, respectively. The extracts were subjected to column chromatography (CC) on silica gel 60 (230–400 mesh), eluted with a gradient system of *n*-hexane, ethyl acetate and methanol to afford six known compounds. The hexane extract yielded stigmasterol (a white amorphous solid, 52.0 mg) and polycarpol (a white amorphous solid, 43.0 mg), the ethyl acetate extract yielded benzoic acid (6.1 mg), acetylmelodorinol (colorless needles, 1.0 g), chrysin (a light yellow powder, 500.0 mg) and benzoquinone (colorless crystals, 2.0 mg), while acetylmelodorinol (54.0 mg) and chrysin (35.2 mg) were isolated from the methanol

extract. The compounds were identified by comparison of their physicochemical and spectroscopic properties with literature values. Purity of the compounds was >95%, based on their physicochemical properties, NMR and ESI-MS data.

3.4. Cell Viability

Cell viability was determined by the standard trypan blue exclusion method. The blood cells ($1 \times 10^6/\text{mL}$) were incubated with 1.25 and 10.0 $\mu\text{g/mL}$ of compounds, each in triplicate at room temperature for 24 h. The blue dye uptake was an indication of cell death. The percentage viability was calculated from the total cell counts.

3.5. Radioimmunoassay for Prostaglandin E_2 (PGE_2) and Thromboxane B_2 (TXB_2)

Radioimmunoassay was carried out to determine the levels of PGE_2 and TXB_2 productions by blood cells following incubation with compounds and coagulation according to the modified method of Patrignani *et al.* [29]. The use of human blood was approved by the Ethics Committee of Universiti Kebangsaan Malaysia (UKM) (approval no. FF-168-2007). Radioimmunoassay procedures were carried out in triplicate for each compound.

3.5.1. Preparation of Standards

A series of concentrations of PGE_2 and TXB_2 standards were prepared, ranging from 2.45–240 and 2.05–500 $\text{pg}/0.1 \text{ mL}$, respectively. One hundred μL of PGE_2 standard solution was added to 100 μL of anti- PGE_2 and 100 μL of [^3H]- PGE_2 . Meanwhile, 100 μL of TXB_2 standard solution was added to 100 μL of anti- TXB_2 and 100 μL of [^3H]- TXB_2 . The mixtures were incubated at 4 °C for 18–24 h. After incubation, the mixtures were added with 200 μL of dextran charcoal and were incubated again for 10 min. After centrifugation at $2000 \times g$ for 15 min at 4 °C, 3 mL of liquid scintillation cocktail was added to 300 μL of supernatant. The radioactivity was measured by a liquid scintillation counter.

3.5.2. Prostaglandin E_2 Radioimmunoassay

Briefly, venous blood was obtained in polypropylene tube containing 10% (v/v) of 2% EDTA by aseptic vein puncture from healthy human volunteers who fulfilled the following inclusion criteria: non-smoker, fasted overnight and did not take any medicine or supplements within the last two weeks. One mL of blood was incubated at 37 °C for 24 h with 10 μL of LPS and 10 μL of serial dilutions of each compound in DMSO and ethanol (1:1 ratio) (1.25–10 $\mu\text{g/mL}$) or control. DMSO and ethanol (1:1 ratio) was used as a negative control and indomethacin, a known cyclooxygenase inhibitor was used as a positive control. After incubation, the blood was centrifuged at $2,000 \times g$ for 10 min at 4 °C to separate the plasma. The reaction mixtures consisted of 100 μL of plasma, 100 μL of anti- PGE_2 and 100 μL of [^3H]- PGE_2 were incubated at 4 °C for 18–24 h. After incubation, the mixtures were added with 200 μL of dextran charcoal and were incubated again for 10 min. The final concentrations of the samples in the mixture were 10.0, 5.0, 2.5 and 1.25 $\mu\text{g/mL}$. After centrifugation at $3,000 \times g$ for 15 min at 4 °C, 3 mL of liquid scintillation cocktail was added to 300 μL of supernatant. The radioactivity was measured by a liquid scintillation counter.

3.5.3. Thromboxane B₂ Radioimmunoassay

Thromboxane B₂ assay was carried out similarly to the PGE₂ assay. In this assay, 1 mL of blood mixed with 10 µL of serial dilutions of each compound in DMSO and ethanol (1:1 ratio) (1.25–10 µg/mL) or control was allowed to clot for 60 min at 37 °C. DMSO and ethanol (1:1 ratio) was used as a negative control and indomethacin, a known cyclooxygenase inhibitor was used as a positive control. The blood was centrifuged at 2,000 × g for 10 min at 4 °C to separate the serum as supernatant. The reaction mixtures consisted of 100 µL of serum, 100 µL of anti-TXB₂ and 100 µL of [³H]-TXB₂ were incubated at 4 °C for 18–24 h. After incubation, the mixtures were added with 200 µL of dextran charcoal and were incubated again for 10 min. The final concentrations of the samples in the mixture were 10.0, 5.0, 2.5 and 1.25 µg/mL. After centrifugation at 3,000 × g for 15 min at 4 °C, 3 mL of liquid scintillation cocktail was added to 300 µL of supernatant. The radioactivity was measured by liquid scintillation counter.

3.5.4. Calculation of PGE₂ and TXB₂ Concentrations

The readings obtained for each set of triplicate were averaged. The net counts for all standards and samples were calculated by subtracting the value of the antibody binding to the antigen in the sample (Bx) with non specific binding (Nc). The normalized percentage bound (% B/Bo) for each standard and sample (Bx) were calculated as follows:

$$\% B/Bo = \frac{Bx - Nc}{Bo - Nc} \times 100$$

The % B/Bo for each standard *versus* the corresponding picogram (pg) concentration of PGE₂ and TXB₂ were plotted using semi-logarithmic graph. The concentrations of PGE₂ and TXB₂ in each sample were determined by interpolation from the standard curve. Percentage inhibition of samples was obtained as follows:

$$\% Inhibition = \left\{ 100 - \frac{[PGE2 \text{ or } TXB2 \text{ in sample}]}{[PGE2 \text{ or } TXB2 \text{ in control}]} \right\} \times 100$$

3.6. PAF Receptor Binding Inhibitory Assay (PAF Assay)

The assay was carried out according to the method as described previously by Jantan *et al.* [30]. The procedure was approved by the Animal Ethics Committee of UKM (approval no. FSKB/2007/Juriyati/10-July/192). The reaction mixtures consisted of 200 µL of washed rabbit platelet suspension, 25 µL of ³H-PAF (2.0nM) with or without unlabeled 25 µL of PAF (2.0 µM) and 25 µL of compound (200 µg/mL) or control solution. The final concentration of compounds in the reaction mixtures were 18.2, 9.1, 4.5, 2.3 µg/mL. Cedrol, a known PAF antagonist was used as a positive control 0.1% DMSO in saline was used as a control. The final concentration of DMSO in the reaction mixture was fixed at 0.1% to avoid interference with the receptor binding studies. The reaction mixture was incubated at room temperature for 1 h. The free and bound ligands were separated by a filtration technique using Whatman GF/C glass fiber filters. The radioactivity was measured by a scintillation counter. The difference between total amounts of bound ³H-PAF in the absence and the presence of excess unlabeled PAF is defined as specific binding of the radiolabeled ligand. The IC₅₀ values of the

compounds were obtained from at least three independent determinations. Percentage inhibition of the sample was obtained by the following equation:

$$\% \text{ Inhibition} = \frac{(Tc - Nc) - (Ts - Ns)}{Tc - Nc} \times 100$$

Tc = Total binding of control; Ts = Total binding of sample; Nc = Nonspecific binding of control; Ns = Nonspecific binding of sample

3.7. Statistical Analysis

All the data were analysed using the *Statistical Package for Social Sciences* (SPSS) software. Each sample was measured in triplicate and the data are presented as means $\pm$ standard deviation (SD). Probit programme was used to determine the IC₅₀ values for the active pure compounds. The values were obtained from at least three determinations. Data were analysed using one way ANOVA. $P < 0.05$ was considered to be statistically significant.

4. Conclusions

Among the compounds isolated from the leaves of *Mitrella kentii*, chrysin showed a potent dose-dependent inhibitory activity on PGE₂ production in human blood induced by LPS, indicating that it might directly inhibit COX-2 enzymatic activity. The results of this study indicate that the strong inhibitory activity of *M. kentii* on TXB₂ production was due to polycarpol, acetylmelodorinol and stigmasterol which exhibited significant and concentration-dependent inhibitory effect on COX-1 activity. The strong PAF antagonistic effect of the plant was contributed by polycarpol and acetylmelodorinol which showed strong and dose-dependent inhibitory effects on PAF receptor binding. Acetylmelodorinol, chrysin and polycarpol have the potential to be used as lead structures for the development of potent anti-inflammatory agents. However, further studies need to be carried out to study their mechanisms of anti-inflammatory actions such as the cell free assays for COX enzyme activity and to find derivatives with maximum inhibitory effects.

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Sample Availability: Samples of the compounds are available from the authors.