Jurnal Sains Kesihatan Malaysia **19** (1) 2021: 151 - 159
DOI : http://dx.doi.org/10.17576/JSKM-2021-1901-17

Kertas Asli/*Original Article*s

Chemical Constituents and Biological Activities of Mitrella Kentii (Blume) Miq. Leaf Oil

(Komponen Kimia dan Aktiviti Biologi Minyak Daun Mitrella Kentii (Blume) Miq.)

JURIYATI JALIL, SAKINA SAADAWI, IBRAHIM JANTAN & MALINA JASAMAI*

ABSTRACT

Chemical constituents and biological activities of the Mitrella kentii leaf oil were investigated in this study. Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) were used to determine the chemical constituents of the oil. The oil was evaluated for its ability to inhibit prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2) productions in human whole blood using a radioimmunoassay technique. Its inhibitory effect on platelet-activating factor (PAF) receptor binding with rabbit platelets using ^{3}H-PAF as a ligand and its free radical scavenging effect on DPPH were also investigated. Caryophyllene oxide (33.8%w/w), E,Z-farnesol (6.9%), benzyl benzoate (6.5%w/w) and viridiflorol (6.5%w/w) were among the major components of the oil. Even though weak inhibitory activities were observed in both PGE_2 and TXB_2 assays, significant results were obtained in both PAF receptor binding inhibition and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging effect with IC_{50} value of 6.6 µg/mL and 155.6 µg/mL respectively. These promising activities warrant the development of the oil as an anti-inflammatory agent.

Keywords: Mitrella kentii; platelet-activating factor (PAF); prostaglandin E_2 (PGE_2); thromboxane B_2 (TXB_2); DPPH assay

ABSTRAK

Komponen kimia dan aktiviti biologi minyak daun Mitrella kentii dikaji. Kromatografi gas (GC) dan spektrometri jisim-kromatografi gas (GC-MS) digunakan untuk menentukan unsur-unsur kimia minyak. Minyak dinilai kerana keupayaannya untuk merencat penghasilan prostaglandin E_2 (PGE_2) dan tromboksan B_2 (TXB_2) dalam darah manusia menggunakan teknik radioimunoasai. Kesan perencatan pada reseptor faktor pengaktif platelet (PAF) yang mengikat dengan platelet arnab menggunakan 3H-PAF sebagai ligan dan kesan pengaruhnya radikal bebas terhadap DPPH juga dikaji. Kariofilena oksida (33.8%), E, Z-farnesol (6.9%), benzil benzoat (6.5%) dan viridiflorol (6.5%) adalah di antara komponen utama minyak. Walaupun aktiviti perencatan yang lemah diperhatikan dalam ujian PGE_2 dan TXB_2, hasil yang signifikan diperoleh dalam kedua-dua perencatan pengikat PAF dan kesan hapus-sisa 2,2-difenil-1-pikrihidazil (DPPH) dengan nilai IC_{50} 6.6 µg / mL dan 155.6 µg / mL masing-masing. Aktiviti yang menunjukkan bahawa minyak daun boleh dibangunkan sebagai agen anti-radang.

Kata kunci: Mitrella kentii; faktor pengaktif platelet (PAF); prostaglandin E_2 (PGE_2); thromboxane B_2 (TXB_2); Asai DPPH

INTRODUCTION

Mitrella kentii (Blume) Miq. which synonyms are *Melodorum pisocarpum* and *M. Elegans* belongs to the Annonaceae (custard-apple) family. It is widely distributed in the tropics and has been used traditionally as a remedy for fever (Wiart 2006). Among the chemical constituents that have been isolated from this species are liriodenine, anonaine, asimilobine, aequaline (Ellis et al. 1972), neolinderatin, linderatin, 2',6'-dihydroxy-4' methoxydihydrochalcone and catechin (Benosman et al. 1997), Despite the reported compounds of the extracts, very little is known about the oil of *M. kentii*. Nevertheless, essential oils of other Annonaceae species are well studied for instance Ylang-ylang oil (*Cananga odorata*) which has high content of linalool and benzyl acetate (Sacchetti et al. 2005), monoterpene hydrocarbons and sesquiterpene hydrocarbons in *Monodora myristica* (Onyenekwe et al. 1993), sesquiterpenes and cadinene derivatives in *Asimina triloba* (Farag 2009). Moreover, essential oil from *Dennettia tripetala* was reported to have analgesic and anti-inflammatory properties (Oyemitan et al. 2008) whereas

leaf oil of *Desmopsis bibracteata*, *Guatteria diospyroides*, *Guatteria oliviformis* and *Unonopsis costaricensis* possess an antibacterial activity (Palazzo et al. 2009). Antibacterial activity also was reported for the essential oil from *Miliusa tomentosa* (Badgujar et al. 2011).

Taking into consideration the anti-inflammatory activity reported for *Dennettia* tripetala (Oyemitan et al. 2008), it is worthwhile to investigate the action of *M. kentii* on inflammatory mediators such as prostaglandin E_2 (PGE_2), thromboxane A_2 (TXB_2) and platelet aggregating factor (PAF). PGE_2 is a metabolite of arachidonic acid (AA) through the cyclooxygenase-2 (COX-2) pathway and TXB_2 is a metabolite from the cyclooxygenase-1 (COX-1) pathway. PGE_2 is a key mediator in the inflammatory process (Yu et al. 2007) whereas TXB_2 is a potent vasoconstrictor and it also stimulates platelet aggregation (Fitzgerald et al. 1983). Moreover, PAF is a potent glycerophospholipid inflammatory mediator which involve in several pathophysiological conditions such as inflammation (Braquet et al. 1987), allergy (Krauze-Baranowska et al. 2002), asthma (Vargaftig et al. 1987) and thrombosis (Kloprogge et al. 1984). Specific receptors for PAF (PAFr) have been reported in a variety of cell membranes including those from platelets (Hwang et al. 1983). Compounds which can inhibit the productions of PGE_2 and TXB_2 and prevent the specific binding of PAF to PAFr may be used as lead structures in the development of potent anti-inflammatory and cardiovascular agents. Furthermore, PAF antagonists have also been reported to have some correlation with the production of free radicals (Arribas-Gomez et al. 1995) and hence, compounds which interfere with the biosynthesis of inflammatory mediators are likely to inhibit the production of free radicals. Free radicals are involve in various pathological conditions including the development of degenerative diseases, diabetes, hepatotoxicity, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurological disorders, and the aging process (Marx 1987).

In this work, the chemical constituents of *M. kentii* leaf oil were analysed using gas chromatography mass spectrometry (GC/MS). The inhibitory activities of the leaf oil on TXB_2 and PGE_2 productions were investigated using radioimmunoassay. The antagonistic effect of the leaf oil on PAFr and its free radical scavenging ability were also studied.

MATERIALS AND METHODS

MATERIAL

Hexane, ethyl acetate and methanol used were of analytical grades. Radiolabelled PGE_2 ($[^3H]$-PGE_2, 50 µCi/mmol) and TXB_2 ($[^3H]$-TXB_2, 25 µCi/mmol) were purchased from Amersham (Buckinghamshire, UK). Unlabelled PGE_2, unlabelled TXB_2, anti-PGE_2 and anti-TXB_2 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 an anticoagulant. Lipopolysaccharide (LPS) 1 mg/mL was used to induce prostaglandin endoperoxide synthesis in whole blood. Phosphate buffer solutions (PBS) 0.01M, pH 7.4 was used as buffer for assays. 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).

PLANT MATERIALS

Fresh leaves of *M. kentii* were collected from the Cameron Highlands mountain forest in Pahang, Malaysia in October 2006 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 the Faculty of Science and Technology, UKM.

Plant leaves were air-dried, ground to mesh size 40-60 (1 kg) and extracted with hexane in triplicate (2.5 L, 24 hours each) using cold maceration technique (Wu et al. 2015) at room temperature (29°C). The solvent was then evaporated using a rotary evaporator to give 0.9 ± 0.3 %w/w of the leaf oil (dried sample).

ANALYSIS OF THE LEAF OIL

The GC/MS analyses (in triplicate) were carried out using the Hewlett Packard GC-MSD 7890A/5975C gas chromatographs equipped with a fused silica capillary column HP-5MS (5% phenylmethylsiloxane, 30 m x 0.25 mm, film thickness of 0.25 µm) and the Shimadzu GC-2010 equipped with a flame ionizing detector (FID) and a CBP-5 (25 m x 0.22 mm, film thickness of 0.25 µm) capillary column. Temperature of injector and detector was maintained at 230ºC. The oven temperature

was raised from 60ºC to 230ºC at a heating rate of 3ºC/min and then held at 230ºC for 1 minute. Helium gas at a linear velocity of 50 cm/s was used as a carrier. The diluted leaf oil (1 µL) in ethyl acetate (1:5) was injected in a pulsed split mode (auto-injector Shimadzu AOC-20i).

Compositions of the leaf oil were determined by comparing their retention indices relative to C_9-C_{22} alkanes (van Den Dool H & Kratz 1963) on the CBP-5 column with literature values (McFadden 1973) and their mass spectra with the Wiley and Adams libraries. The percentage compositions of the leaf oil were calculated based on the peak areas obtained from the FID without any corrections.

CELL VIABILITY

Cell viability was determined by the standard trypan blue exclusion method. The blood cells (1×10^6/mL) were incubated with 1.25 and 10.0 µg/mL of extracts each in triplicate at room temperature (27°C) for 24 hours. The blue dye uptake was an indication of cell death. The percentage viability was calculated from the total cell counts.

RADIOIMMUNOASSAY FOR PGE_2 AND 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. (1994). The use of human blood was approved by the Ethics Committee of the Universiti Kebangsaan Malaysia (UKM) (approval no. FF-168-2007). Radioimmunoassay procedures were carried out in triplicate for each compound.

PREPARATION OF STANDARDS

A series of PGE_2 and TXB_2 standards concentrations were prepared, ranging from 2.45–240 and 2.05–500 pg/0.1 mL, respectively. PGE_2 standard solution (100 µL) was added to 100 µL of anti-PGE_2 and 100 µL of ^{3}H- PGE_2. Meanwhile, 100 µL of TXB_2 standard solution was added to 100 µL of anti-TXB_2 and 100 µL of ^{3}H - TXB_2. The mixtures were incubated at 4°C for 18–24 hours. After incubation, the mixtures were added with 200 µL of dextran charcoal and were incubated again for 10 minutes. After centrifugation at 2000 × g for 15 minutes 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.

PGE_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. Blood (1 mL) was incubated at 37°C for 24 hours with 10 µL of LPS and 10 µL of serial dilutions of leaf oil in dimethyl sulphoxide (DMSO) and ethanol (1:1 ratio) (1.25–10 µg/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 × 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 [^{3}H]-PGE_2 were incubated at 4°C for 18–24 hours. After incubation, the mixtures were added with 200 µL of dextran charcoal and were incubated again for 10 minutes. 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 minutes 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 (Saadawi et al. 2012).

TXB_2 *RADIOIMMUNOASSAY*

Thromboxane B_2 assay was carried out similarly to the PGE_2 assay. In this assay, 1 mL of blood was mixed with 10 µL of serial dilutions of leaf oil in DMSO and ethanol (1:1 ratio) (1.25–10 µg/mL) or control was allowed to clot for 60 minutes at 37°C. DMSO and ethanol (1:1 ratio) was used as a negative control and indomethacin was used as a positive control. The blood was centrifuged at 2,000 × g for 10 minutes at 4°C to separate the serum as supernatant. The reaction mixtures consisted of 100 µL of serum, 100 µL of anti-TXB_2 and 100 µL of [^{3}H]-TXB_2 were incubated at 4°C for 18–24 hours. After incubation, the mixtures were added with 200 µL of dextran charcoal and were incubated again for 10 minutes. The final concentrations of the sample in the mixture were 10.0, 5.0, 2.5 and 1.25 µg/mL. After centrifugation at 3,000 × g for 15 minutes at 4°C, 3 mL of liquid scintillation cocktail was added to 300 µL of supernatant. The radioactivity was measured by liquid scintillation counter (Saadawi et al. 2012).

CALCULATION OF PGE_2 AND TXB_2 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:

$$\% \text{ B/Bo} = \frac{\text{Bx} - \text{Nc}}{\text{Bo} - \text{Nc}} \times 100 \% \quad (1)$$

The % B/Bo for each standard versus the corresponding concentration of PGE_2 and TXB_2 were plotted using semi-logarithmic graph. The concentrations of PGE_2 and TXB_2 in each sample were determined by interpolation from the standard curve. Percentage inhibition of samples was obtained as follows:

$$\% \text{ inhibition} = \left\{ 100 - \frac{[PGE_2 \text{ or } TXB_2 \text{ in sample}]}{[PGE_2 \text{ or } TXB_2 \text{ in control}]} \right\} \times 100 \quad (2)$$

PAF RECEPTOR BINDING INHIBITORY ASSAY (PAF ASSAY)

The assay was carried out according to the method described by Jantan et al. (2001). 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 ^{3}H-PAF (2.0nM) with or without unlabeled 25 µL of PAF (2.0 µM) and 25 µL of leaf oil (200 µg/mL) or control solution. The final concentration of leaf oil 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 and 0.1% DMSO in saline was used as a negative 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 (29ºC) for 1 hour. 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 ^{3}H-PAF in the absence and the presence of excess unlabeled PAF is defined as specific binding of the radiolabeled ligand. The IC_{50} values of the extracts were obtained from at least three independent determinations. Percentage inhibition of the sample was obtained by the following equation:

$$\% \text{ Inhibition} = \frac{(\text{Tc-Nc}) - (\text{Ts-Ns})}{\text{Tc-Nc}} \times 100 \quad (3)$$

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

1,1-DIPHENYL -2-PICRYLHYDRAZYL (DPPH) RADICAL SCAVENGING ASSAY

Radical scavenging activity of the leaf oil was determined as described (Moharam et al. 2010) where 2 mL of methanol oil (0.8 mg/mL of methanol) was mixed with 2 mL of DPPH (0.1 mM in methanol). The mixture was shaken vigorously and stored in darkness at 37°C for 30 minutes. Then, the absorbance was measured at 517 nm (Shimadzu 1601 UV-visible spectrophotometer). The reaction was carried out in triplicate. The percentage of radical scavenging activity was calculated by comparing the results of the test with those of the control (not treated with oil):

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test}) \times 100}{\text{Absorbance of control}} \quad (4)$$

The leaf oil was subsequently tested at serial concentrations (0.19-400 µg/mL) to determine the IC_{50} values. The IC_{50} values of the sample were obtained by the Probit computer program.

RESULTS AND DISCUSSION

EXTRACTION AND IDENTIFICATION OF COMPOUNDS

Maceration and solvent extraction (hexane) of *M. Kentii* leaves yielded 0.9 $\pm$ 0.3% of oil (w/w based on dry plant material weight). The ion chromatograms of the studied oil are shown on Figure 1. Thirteen compounds were identified in the leaf oil (Table 1).

Solvent extraction is among a common method used to isolate oils from natural products (Jantan et al. 2004, Taylor 1993). To the best of the authors' knowledge, total constituents of the oil have not been reported elsewhere. The oil is rich in sesquiterpenes (hydrocarbon and oxygenated) represented the highest percentage in the leaf oil. The major compounds are caryophyllene oxide (33.8%), (*E,Z*) farnesol (6.9%), viridiflorol and benzyl benzoate (6.5%) whereas cubenol (0.9%), (*E*)-phytol acetate (1.0%) whereas methyl linoleate (1.2%) is the least component of the leaves. Annonacceae species are known to contain monoterpene and sesquiterpene hydrocarbons (Onyenekwe et al. 1993). Caryophyllene on the other hand was found in the essential oil derived from *Annona cherimola* (Bowman et al. 1997), *Annona senegalensis*

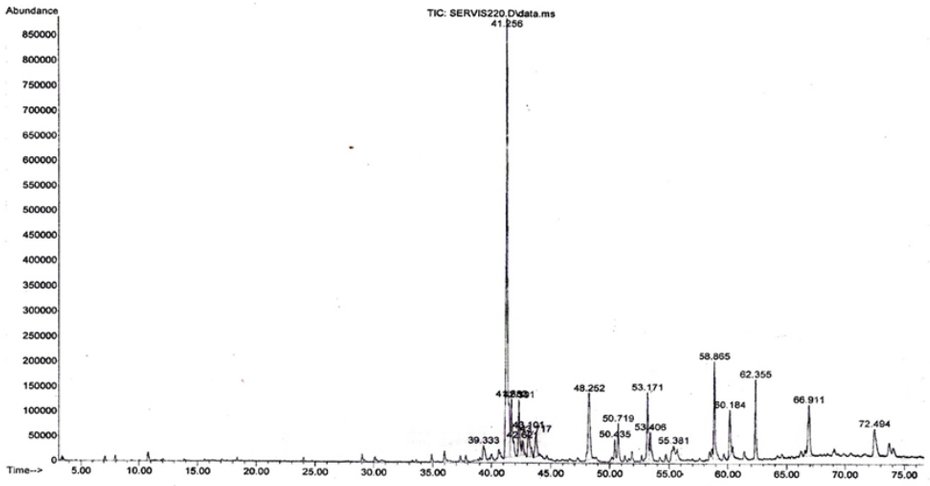

FIGURE 1. Gas chromatogram-mass spectrometry of the *Mitrella kentii* leaf oil.

TABLE 1. Percentage composition of the *Mitrella kentii* leaf oil

No	Components	Retention indices		Percentage (%)	ID methods
		RI found	RI literature		
1	Dauca-5,8-diene	1470	1477 (Radulović et al. 2011)	1.5	i,ii
2	Caryophyllene oxide	1594	1592 (Krauze-Baranowska et al. 2002)	33.8	i,ii
3	Viridiflorol	1602	1596 (Krauze-Baranowska et al. 2002)	6.5	i,ii
4	Humulene epoxide II	1608	1610 (Sajjadi 2006)	4.6	i,ii
5	1,10-di-*epi*-cubenol	1614	1616 (Sajjadi 2006)	1.6	i,ii
6	Cubenol	1645	1642 (Adams 2007)	0.9	i,ii
7	Dihydro-eudesmol	1660	1664 (Asgar etal. 2010)	1.8	i,ii
8	Benzyl benzoate	1731	1736 (Tudor 1997)	6.5	i,ii
9	(*E,Z*) farnesol	1752	1748 (Ahmad & Jantan 2003)	6.9	i,ii
10	(*E,E*) farnesyl acetate	1817	1816 (Ahmad & Jantan 2003)	4.6	i,ii
11	Methyl linoleate	2080	2076 (Jantan et al. 2004)	1.2	i,ii
12	(*E*)-phytol acetate	2214	2217 (Jantan et al. 2004)	1.0	i,ii
		Total		76.3	

ID methods = identification methods: i = retention index, ii = mass spectrum, RI_x = retention index: measured relative to n-alkane on CBP-5 column. RI_{Lit} = retention index on CBP-5 column obtained in literature.

(Ríos et al. 2003) and *Goniothalamus tapis* (Noudogbessi et al. 2011).

INHIBITION OF PGE_2 AND TXB_2 PRODUCTIONS

The inhibitory effects of the leaf oil on PGE_2 and TXB_2 productions are shown in Figure 2 and 3. The cell viability test was carried out to evaluate the cytotoxicity of the leaf oil on the blood cells and at 1.25 and 10.0 µg/mL indicated that the blood cells were viable (>95%) after 24 hour of incubation. The leaf oil was investigated for its inhibitory effects on the production of PGE_2 and TXB_2 in whole blood at 10 µg/mL. The leaf oil exhibited low inhibitory effect on the production of PGE_2 (35.4% inhibition) induced by LPS and TXB_2 (12.7% inhibition) in whole blood (Figure 3). Caryophyllene oxide might contribute to the activity observed (Han et al. 2014). To the best of the authors' knowledge, no similar study on Annonaceae family has been conducted.

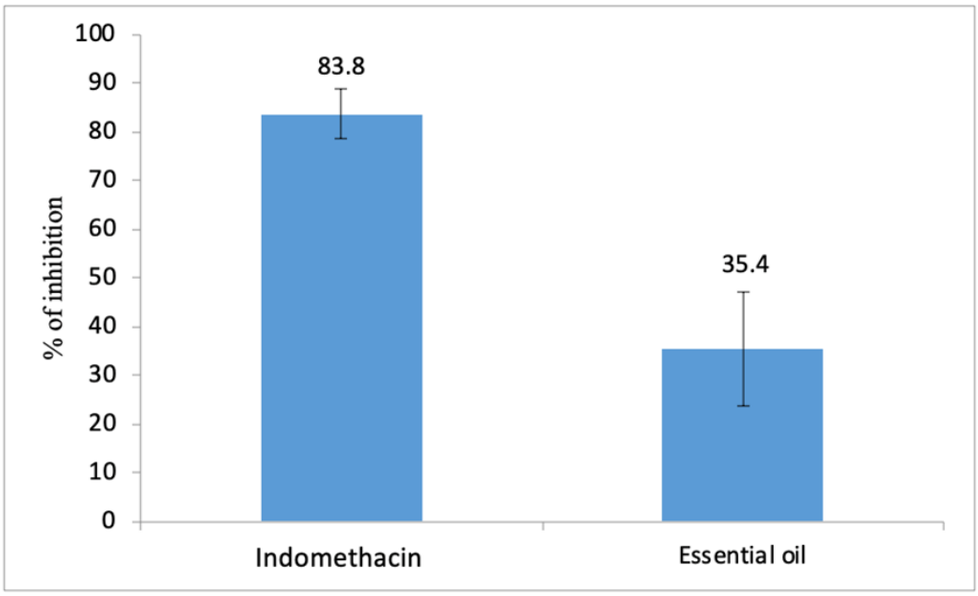

FIGURE 2. Percentage (%) inhibition of essential oil from leaves of *M. Kentii* at a concentration of 10 µg/mL on PGE_2 production in human blood plasma induced by LPS (10 µg/mL). *P > 0.05, mean + SD (n=3).

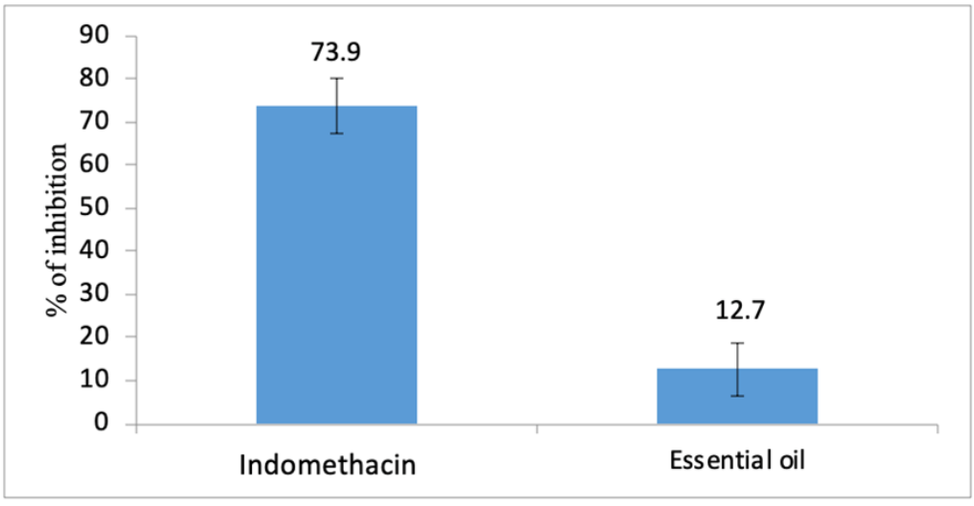

FIGURE 3. Percentage (%) inhibition of essential oil from leaves of *M. kentii* at a concentration of 10 µg/mL on TXB_2 production in human blood serum. *P > 0.05, mean + SD (n=3).

EFFECT OF THE LEAF OIL ON ^{3}H-PAF RECEPTOR BINDING

The leaf oil showed significant inhibitory effects on the PAF receptor binding with percentages of inhibition of 73.1% which is comparable to that of the positive control, cedrol (76.3%) (Figure 4), a known PAF antagonist from natural sources (Jantan et al. 2004) at concentration of 18.2 µg/mL. The inhibitory effect of the leaf oil was then evaluated at various concentrations (1.8 -18.2 µg/mL) and the IC_{50} values were determined by probit analysis as 6.6 µg/mL (Table 2).

The leaf oil showed relatively strong PAF antagonistic activity and its dose-dependent responses are shown in Figure 4. Caryophyllene oxide content of the leaf oil might contribute to this activity as similar activity was observed on the *Goniothalamus* species which contain the mentioned essential oil component (Moharam et al. 2010). The leaf oil inhibited the specific binding between PAF and receptors and thus can be investigated further in the development of therapeutic agents for a variety of inflammation, respiratory, immunological and cardiovascular disorders (Han et al. 2014).

RADICAL SCAVENGING ACTIVITY

Spectrophotometric measurement for color changes at 517 nm wavelength showed that the essential oil effect as DPPH scavenger is dose-dependent (Figure 5). At the concentration of 0.8 mg/mL, the essential oil has strong effect as DPPH scavenger (86.9% inhibition) when compared with the positive control vitamin C (91.3% inhibition). Nonetheless, the IC_{50} value of the oil (155.6 ± 2.8) is higher than that of vitamin C (4.2 ± 1.3) (Table 3). The radical scavenging activity observed might be due to the benzyl esters content of the leaf oil. *Cananga odorata* which belongs to the family of Annonaceae and has high content of benzyl esters showed similar activity (Sacchetti et al. 2005).

TABLE 2. IC_{50} of the *M. kentii* (µg/mL) leaf oil on PAF receptor binding to rabbit platelets

No	Compound	IC_{50} (µg/mL)
1	Essential oil	6.6 ± 0.7
2	Cedrol (positive control)	6.2 ± 0.3

Data are mean ± SD (n = 3).

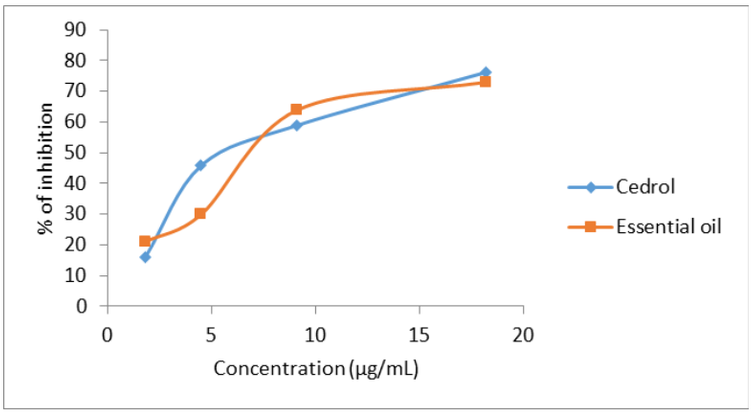

FIGURE 4. Percentage (%) inhibition of essential oil from the leaves of *M. kentii* on PAF receptor binding to rabbit platelets.

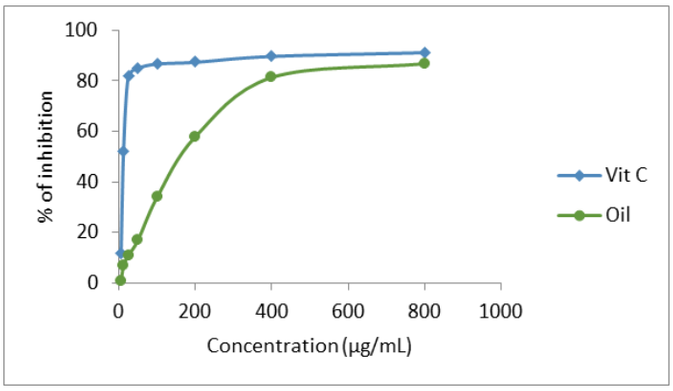

FIGURE 5. Percentage (%) of DPPH scavenging activity for essential oil from *M. kentii*.

TABLE 3. IC_{50} (µg/mL) for the *M. kentii* leaf oil (more than 50% inhibition) as a DPPH scavenger

No	Compound	IC_{50} (µg/mL)
1	Essential oil	155.6 ± 2.8
2	Vitamin C (positive control)	4.2 ± 1.3

Data are mean ± standard deviation (n = 3).

CONCLUSION

The leaf oil of *M. Kentii* was identified to contain thirteen compounds and some of the compounds are similar to those found in the Annonacea family. The leaf oil showed low inhibitory activities on PGE_2 production in human blood induced by LPS and TXB_2 production. Nonetheless, the promising PAF antagonistic effect of the leaf oil might be due to the presence of caryophyllene oxide. Futhermore, benzyl ester components of the leaf oil might contribute to the radical scavenging activity exhibited by the leaf oil. The leaf oil has potential to be developed further as an antiinflammatory and antioxidant agents. Detailed study on whether a single compound of the leaf oil is reponsible for the activities mentioned or the compounds work synergistically can be undertaken.

ACKNOWLEDGEMENT

The authors would like to thank the Ministry of Science, Technology and Innovation, Malaysia for the financial support (Grants no.: 02-01-02-SF0016 and 02-01-02-SF0510) and Dr. Azmath Jaleel for his help in editing the manuscript.

REFERENCES

Adams, R.P. 2007. Identification of essential oil components by gas chromatography/mass spectrometry, 4th ed. Allured Publishing Corporation United States.

Ahmad, F. & Jantan. I. 2003. Chemical constituents of the essential oils of *Goniothalamus uvariodes* King. *Flavour Frag J* 18(2): 128-130.

Arribas-Gomez, I., Duque-Marin, I., de Lema, G.P., Diez-Marqués, M.L., Lucio-Cazaiia, J., Rodriguez-Puyol, M. et al. 1995. A possible role for platelet-activating factor in the hydrogen peroxide-induced TXB_2 and PGE_2 glomerular synthesis. *J Lipid Res* 36: 260-265.

Asgar, E., Mohammad Hassan, S., Asghar, P.A. & Seyyed Ali, G 2010. Toxicity of essential oil of *Agastache foeniculum* (Pursh) Kuntze to *Oryzaephilus surinamensis* L. and *Lasioderma serricorne* F. *J Plant Pro Res* 50(2): 215–219.

Badgujar, V.B. & Surana, S.J. 2011. Comparative investigation on antimicrobial property of Miliusa tomentosa leaf oil and leaf extract. *Iranian J Pharmacol Thera* 10: 7-10.

Benosman, A., Oger, J.M., Richomme, P. & Bruneton, J. 1997. New terpenylated dihydrochalcone derivatives isolated from *Mitrella kentii. J Nat Pro* 60: 921–924.

Bowman, J.M., Braxton, M.S., Churchill, M.A., Hellie, J.D., Starrett, S.J., Causby, G.Y., et al. 1997. Isolation of terpenes from plant tissue and subsequent determination by gas chromatography. *Microchem J* 56: 10-18.

Braquet, P., Tougui, L., Shen, T.Y. & Vargaftig, B.B. 1987. Perspectives in platelet- activating factor research. *Pharmacol Rev* 39: 97-145.

Ellis, J., Gellert, E. & Sumons, R.E. 1972. The alkaloids of *Mitrella kentii* (Annonaceae). *Aust J Chem* 25: 2735-2736.

Farag, M.A. 2009. Chemical composition and biological activities of *Asimina triloba* leaf essential oil. *Phar Biol* 47: 982-986.

Fitzgerald, G.A., Brash, A.R., Oates, J.A. & Pedersen, A.K. 1983. Endogenous prostacyclin biosynthesis and platelet function during selective inhibition of thromboxane synthase in man. *J Clin Invest* 71: 1336-1343.

Han, L., Yang, L., Liu, B. & Cheng, X. 2014. Trans-caryophyllene suppresses tumor necrosis factor (TNFα)-induced inflammation in human chondrocytes. *Eur Food Res Tech* 239(6): 1061–1066.

Hwang, S.B., Lee, C.S.C., Cheah, M.J. & Shen, T.Y. 1983. Specific receptor sites for 1-*O*- alkyl-2-*O* acetyl-*sn*-glycero-3-phosphocholine (platelet activating factor) on rabbit platelet and guinea pig smooth muscle membranes. *Biochemistry* 22: 4756-4763.

Jantan, I., Jalil, J. & Warif, N.M.A. 2001. Platelet activating factor (PAF) antagonistic activities of compounds isolated from *Guttiferae* species. *Pharm Biol* 39: 243-245.

Jantan, I., Pisar, M., Sirat, H.M., Basar, N., Jamil, S., Ali, R.M. & Jalil, J. 2004. Inhibitory effects of compounds from *Zingiberaceae* species on platelet activating factor receptor binding. *Phytother Res* 18: 1005–1007.

Koltai, M., Hosford, D., Guinot, P., Esanu, A. & Braquet, P. 1991. Platelet activating factor (PAF). A review of its effects, antagonists and possible future clinical implications (Part I). *Drugs* 42: 9-29.

Kloprogge, E. & Akkerman, N. 1984. Binding kinetics of PAF-acether (1-*O*-alkyl-2-acetyl-*sn* glycero-3-phosphocholine) to intact human platelets. *Biochem J* 223: 901-909.

Krauze-Baranowska, M., Mardarowicz, M. & Wiart, M. 2002. The chemical composition of *Microbiota decussate. Z Naturforsch C* 57: 998-1003.

Marx, J.L. 1987. Oxygen free radicals linked to many diseases. *Science* 235: 529-531.

McFadden, W. 1973.Techniques of Combined Gas Chromatography/Mass Spectrometry (Application in organic analysis). John Wiley and Sons New York.

Moharam, B.A., Jantan, I., Ahmad, F. & Jalil, J. 2010. Antiplatelet aggregation and platelet activating factor (PAF) receptor antagonistic activities of the essential oils of five *Goniothalamus* species. *Molecules* 15: 5124-5138.

Noudogbessi, J.P., Natta, A., Avlessi, F., Sohounhloué, D.K.C., Figueredo, G. & Chalchat, J.C. 2011. Chemical composition of the essential oils extracted from two Annonaceae required in Beninese Pharmacopeia. *Aust J Basic App Sci* 5(2): 34-40.

Onyenekwe, P.C., Ogbadu, G.H., Deslauriers, H., Gagnon, M. & Collin, G.J. 1993. Volatile constituents of the essential oil of *Monodora myristica* (Gaertn) dunal. *J Sci Food Agric* 61: 379-381.

Oyemitan, I.A., Iwalewa, E.O., Akanmu, M.A. & Olugbade, T.A. 2008. Antinoceptive and anti-inflammatory effects of essential oil of *Dennettia tripetala* G. Baker (Annonaceae) in rodents. *Afr J Tradit Complem* 18: 355-362.

Palazzo, M., Wright, H.L., Agius, B., Wright, B.S., Moriarity, D.M., Haber, W.A., et al. 2009. Chemical compositions and biological activities of leaf essential oils of six species of Annonaceae from Monteverde, Costa Rica. *Rec Nat Prod* 3: 153-160.

Patrignani, P., Panara, M.R., Greco, A., Fusco, O., Natoli, C., Iacobelli, S. et al. 1994. Biochemical and pharmacological characterization of the cyclooxygenase activity of human blood prostaglandin endoperoxide synthases. *J Pharmacol Exp Thera* 271: 1705.

Radulović, N., Đorđević, N. & Stojanović-Radić, Z. 2011. Volatiles of the Balkan endemic *Daucus guttatus ssp. zahariadii* and cultivated and wild-growing *D. carota* – A comparison study. *Food Chem* 125(1): 35-43.

Ríos, M.Y., Castrejón, F., Robledo, N., León, I., Rojas, G. & Navarro, V. 2003. Chemical composition and antimicrobial activity of the essential oils from *Annona cherimola* (Annonaceae). *Rev Soc Quím México* 47(2): 139-142.

Saadawi, S., Jalil, J., Jasamai, M. & Jantan, I. 2012. Inhibitory effects of acetylmelodorinol, chrysin and polycarpol from *Mitrella kentii* on Prostaglandin E_2 and Thromboxane B_2 Production and Platelet Activating Factor Receptor Binding. *Molecules* 17: 4824- 4835.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M., et al. 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chem* 91: 621-632.

Sajjadi, SE. 2006. Analysis of the essential oil of two cultivated Basil (*Ocimum basilicum* L.) from Iran. *DARU J Fac Pharm* 14(3): 128-130.

Taylor, S. 1993. Extraction method for the improved determination of vanillin and related phenolic components in vanilla (*Vanilla fragrans* (salisb.) ames) by high-performance liquid chromatography. *Flavour Frag J* 8: 281-287.

Tudor, E. 1997. Temperature dependence of the retention index for perfumery compounds on a SE-30 glass capillary column I. Linear equations. *J Chromatogr A* 779(1-2): 287- 297.

van Den Dool, H. & Kratz, P.D.J.A. 1963. Generalisation of the retention index system incuding linear temperature programme gas-liquid partition chromatography. *J Chromatogr A* 11: 463-471.

Vargaftig, B.B. & Braquet, P. 1987. PAF-acether today-Relevance for acute experimental anaphylaxis. *Brit Med Bull* 43: 312-335.

Wiart, C. 2006. Medicinal Plants of the Asia-Pasific: Drugs for the Future? World Scientific Publishing Co. Pte. Ltd Singapore.

Wu, C., Wang, F., Liu, J., Zou, Y. & Chen, X. 2015. A comparison of volatile fractions obtained from Lonicera macranthoides via different extraction processes: ultrasound, microwave, Soxhlet extraction, hydrodistillation, and cold maceration. *Integr Med Res* 4(3): 171-177.

Yu, Y., Peng, C., Chun, L.Z., Yong, Y., Wei, G.D., Lei, W., et al. 2007. Copper-aspirin complex inhibits cyclooxygenase-2 more selectively than aspirin. *Yakugaku Zasshi* 127: 1869-1875.

Juriyati Jalil
Sakina Saadawi
Ibrahim Jantan
Malina Jasamai
Drugs & Herbal Research Centre, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300, Kuala Lumpur, Malaysia

Ibrahim Jantan
School of Pharmacy, Taylor's University, Lakeside campus, Subang Jaya, Selangor 47500, Malaysia

Corresponding author: Malina Jasamai
E-mail: malina@ukm.edu.my