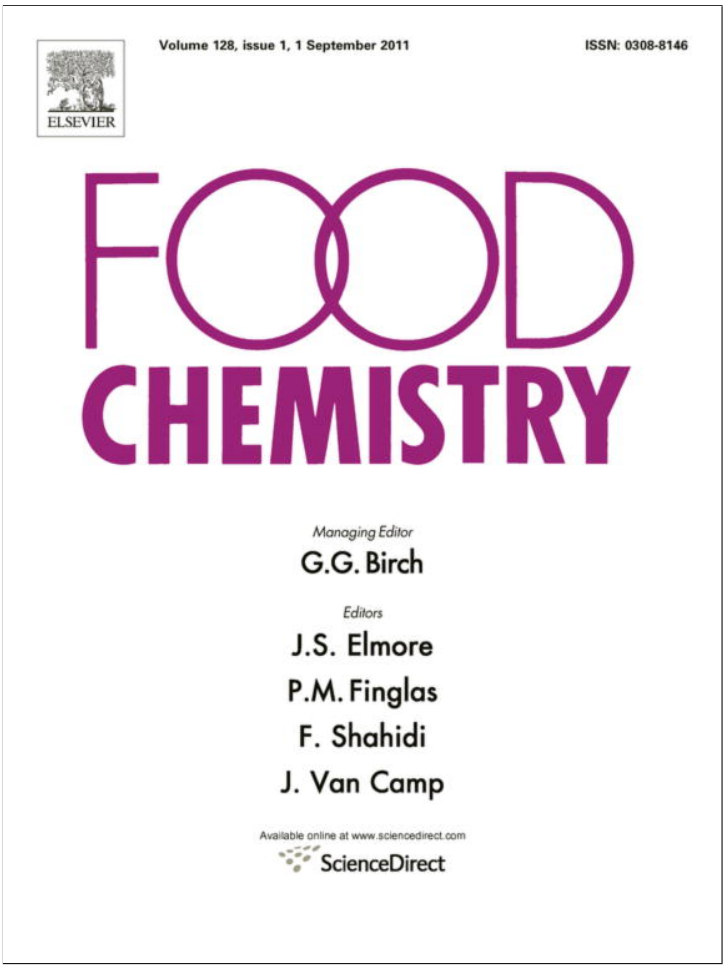

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Flavonoids from acai (*Euterpe oleracea* Mart.) pulp and their antioxidant and anti-inflammatory activities ☆

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ABSTRACT

Five flavonoids, (2S,3S)-dihyrokaempferol 3-*O*-β-D-glucoside (**1**) and its isomer (2R,3R)-dihydrokaempferol 3-*O*-β-D-glucoside (**2**) , isovitexin (**3**), velutin (**4**) and 5,4′-dihydroxy-7,3′,5′-trimethoxyflavone (**5**), were isolated from acai (*Euterpe oleracea* Mart.) pulp. The structures of these compounds were elucidated based upon spectroscopic and chemical analyses. To our knowledge, compounds **1**, **2**, **4** and **5** were identified from acai pulp for the first time. The *in vitro* antioxidant activities of these compounds were evaluated by the oxygen radial absorbance capacity (ORAC) assay. The ORAC values varied distinctly (4458.0–22404.5 μmol Trolox equivalent (TE)/g) from 5,4′-dihydroxy-7,3′,5′-trimethoxyflavone (**5**) to isovitexin (**3**) and were affected by the numbers/positions of hydroxyl groups, substitute groups, as well as stereo configuration. The anti-inflammatory effects of these compounds were screened by the secreted embryonic alkaline phosphatase (SEAP) reporter assay, which is designed to measure NF-κB activation. Velutin (**4**) was found to dose-dependently inhibit SEAP secretion in RAW-blue cells induced by LPS, with an IC_{50} value of 2.0 μM. Velutin (**4**) also inhibited SEAP secretion induced by oxidised LDL, indicating potential athero-protective effects.

1. Introduction

Acai fruits gained popularity in North America and in the European countries lately as a new "super fruit" largely due to its extremely high antioxidant capacity and potential anti-inflammatory activities (Schauss, Wu, Prior, Ou, Huang, et al., 2006). Antioxidant and anti-inflammatory activities of acai pulp or acai juice have been studied in human, animal and cell culture models (Del Pozo-Insfran, Percival, & Talcott, 2006; Jensen et al., 2008; Spada et al., 2009). In a recent study of the effects of acai pulp supplementation on the lifespan of oxidative stressed female flies, molecular analysis revealed that supplementation with acai pulp restored the transcript level of lethal (2) essential for life (l(2)efl), a downstream event of the oxidative stress response pathway Jun-*N*-terminal kinase (JNK), in loss-of-function and reduction-of-function sod1mutants. This resulted in a significant life span extension, suggesting that the compounds in acai may play a role in cell signalling and changes in gene expression (Sun et al., 2010).

Major polyphenolic components in acai pulp include anthocyanins, proanthocyanidins, other flavonoids and lignans (Chin, Chai, Keller, & Kinghorn, 2008; Gallori, Bilia, Bergonzi, Barbosa, & Vincieri, 2004; Schauss, Wu, Prior, Ou, Patel, et al., 2006). Among them, the flavonoids were found to be the major polyphenols. Flavonoids are ubiquitously present in fruits and vegetables. As a group, flavonoids have been shown to exhibit strong antioxidant capacities. The mechanism of antioxidant activity of flavonoids involves the direct scavenging or quenching of oxygen free radicals or excited oxygen species, as well as the inhibition of oxidative enzymes that generate these reactive oxygen species (Pietta, 2000; Terao, 2009). Flavonoids have also shown anti-inflammatory activity in both the proliferative and exudative phases of inflammation (Rathee et al., 2009).

In our previous paper (Kang et al., 2010), seven known flavonoids were isolated and their antioxidant activities were evaluated using three assays. The first objective of this study was to isolate and identify additional flavonoids from acai pulp. Furthermore, antioxidant and anti-inflammatory activities were evaluated by the oxygen radial absorbance capacity (ORAC) assay and the secreted embryonic alkaline phosphatase (SEAP) reporter assay.

☆ Mentioning of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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The SEAP reporter assay was designed to measure the nuclear factor-kappa B (NF-κB) activation (Berger, Hauber, Hauber, Geiger, & Cullen, 1988; Moon, Hahn, Lee, & Kim, 2001). As a major transcription factor, NF-κB plays a key role in regulating the genes responsible for innate and adaptive immune responses (Brasier, 2006; Hoffmann, Natoli, & Ghosh, 2006). The activation of NF-κB has been shown to mediate inflammation by increasing the expression of pro-inflammatory cytokines, chemokines and enzymes (Pahl, 1999). In the SEAP reporter assay, lipopolysaccharide (LPS) or oxidised LDL (oxLDL) was used as stimuli; the later one may particularly implicate potential protective actions of test compounds against atherosclerosis.

2. Materials and methods

2.1. Instrumentation

Optical rotations were measured on a Perkin-Elmer 341 digital polarimeter (Waltham, USA). The UV spectra were obtained on a Shimadzu-2500PC spectrophotometer (Kyoto, Japan). The circular dichroism (CD) spectra were recorded in MeOH using a JASCO J-810 spectropolarimeter (Easton, USA). The infrared (IR) spectra were obtained with a NEXUS 670-FTIR spectrophotometer (San Jose, USA). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova 500 MHz NMR spectrometer (Palo Alto, USA). Electrospray ionisation mass spectrometry (ESIMS) was carried out using Micromass Q-Tof mass spectrometer (Milford, USA). High performance liquid chromatography (HPLC) was performed in a Agilent 1100 HPLC equipped with a UV detector (Palo Alto, USA); a C-18 Dikma Diamonsil analytical column (250 × 4.6 mm, 5 μm) (Scarborough, Canada) and a C-18 Waters uBondapak column (3000 × 7.8 mm, 10 μm) (Milford, USA) were employed for analysis and preparation respectively.

2.2. Plant material

The fruits of *Euterpe oleracea* Mart. were harvested in Para state, Brazil, in September, 2009. A voucher specimen was deposited at Embrapa Amazônia Oriental (Belém, Brazil). The fruits were processed within hours of harvesting to pure pulp and stored at −20 °C until transferred for freeze drying. The frozen pulp was lyophilised at Liotecnica Tecnologia em Alimentos (Sao Paolo, Brazil) and the freeze-dried pulps were transported into the US and supplied by Earth Fruits (South Jordan, USA).

2.3. Chemicals and reagents

Methanol (MeOH), 95% ethanol (EtOH), *n*-butyl alcohol (n-BuOH), petrol ether, and chloroform ($CHCl_3$) were purchased from Shanghai Zhengxing Chemical (Shanghai, China). Ethyl acetate (EtOAc) and acetone were obtained from Sinopharm Chemical Reagent (Shanghai, China). Silica gel (100–200 mesh) and Sephadex LH-20 were supplied by the Branch of Qingdao Marine Chemical (Qingdao, China) and Shanghai Juyuan Biotechnology (Shanghai, China), respectively. Diatomite was obtained from Sinopharm Chemical Reagent (Shanghai, China). 2,2′-Azobis(2-amidinopropane)dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, USA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and fluorescein (sodium salt) (FL) were obtained from Aldrich (Milwaukee, USA). Potassium phosphate dibasic (K_2HPO_4), potassium phosphate monobasic (KH_2PO_4) and Microplates (48-well, Falcon 3230) were obtained from VWR (West Chester, USA).

2.4. Extraction and isolation procedure

The freeze-dried acai pulp powder (3000 g) was mixed with diatomite, percolated with 95% alcohol for two weeks. After evaporation of the solvents under the vacuum, the residue (500 g) was then extracted with petroleum ether, $CHCl_3$, EtOAC, and *n*-BuOH, in succession. The EtOAc and *n*-BuOH fractions were further separated by open column with silica gel, Sephadex LH-20, preparative thin layer chromatography (PTLC) and preparative high performance liquid chromatography (HPLC) respectively. The EtOAC extract (50 g) were loaded into an open silica gel column and eluted with $CHCl_3$–MeOH mixtures with increasing polarity. Fraction 27–34 ($CHCl_3$:MeOH = 90:1) was subjected to a Sephadex LH-20 column, eluted with MeOH. Sub-fraction 12–13 was then purified by PTLC (petroleum ether:EtOAc = 1:3) to get compounds **4** (R_f = 0.75, 4 mg) and **5** (R_f = 0.81, 3 mg).

Fraction 137–159 ($CHCl_3$:MeOH = 10:1) was submitted to a Sephadex LH-20 column and eluted with MeOH. Sub-fraction 12–13 was further purified using HPLC with a Waters uBondapak column. The mobile phase was CH_3CN–H_2O (containing 0.1% formic acid) (20:80, v/v), at a flow rate of 1 ml/min to yield compound **3** (3 mg). The chromatographic profile was detected at 270 nm.*n*-BuOH extract (80 g) was separated with a Sephadex LH-20 column eluted with MeOH, followed by further purification using HPLC on a Waters uBondapak column (flow rate, 1 ml/min; detection wave length, 270 nm), with CH_3CN–H_2O (containing 0.1% formic acid) (30:70, v/v) as the mobile phase, to yield compounds **1** (15 mg) and **2** (7 mg).

2.5. ORAC assay

The ORAC assay was conducted based on the method reported previously (Wu et al., 2004). Briefly, the assay was carried out on a FLUOstar Galaxy plate reader (BMG Labtech, Durham, USA) used with fluorescence filters for an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The temperature of the incubator was set to 37 °C. Fluorescein was used as fluorescence probe; 2,2'-azobis (2-methylpropionamide) dihydrochloride (AAPH) was used as peroxyl generator; Trolox was used as standard. The results were expressed as μmol TE per gram.

2.6. SEAP reporter assay

RAW-Blue cells (Invitrogen, San Diego, USA) are derived from RAW264.7 macrophages with chromosomal integration of a SEAP reporter construct inducible by NF-κB and AP-1. RAW-Blue mouse macrophage cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) (Hyclone, Logan, USA) and zeocineosin (200 μg/ml). All cell culture reagents were purchased from Invitrogen (San Diego, USA).

RAW-Blue cells (1×10^5 cells/well) were pretreated with compounds for 3 h, stimulated by LPS (100 ng/ml, Invitrogen, San Diego, USA) or oxLDL (100 ng/ml, Academy Biomedical, Houston, USA) for 18 h. Luteolin was isolated from the acai pulp described in our previous study (Kang et al., 2010). The supernatants were collected for the SEAP secretion assay. The QUANTI-Blue™ powder was dissolved in endotoxin-free water and sterile filtered (0.22 μm) (QuantiQuanta-blue substrate). RAW-Blue cell supernatant (40 μL/well) was added to QuantiQuanta-blue substrate (160 μL/well) and incubated at 37 °C for 0.5–1 h. The absorbance was measured at 620 nm in a Polarstar microplate reader (BMG Labtech, Durham, USA).

2.7. Statistical analysis

The results for anti-inflammatory assays were expressed as mean ± SD (n = 3). Data were subjected to one-way ANOVA for statistical analyses and the Student–Newman–Keuls Method for multiple comparison procedures. A value of $P < 0.05$ was considered as significant difference. Statistical analyses were performed using SigmaStat statistical software (SigmaStat 3.5).

3. Results and discussion

3.1. Identification of isolated compounds

Five flavonoids were obtained (Fig. 1) from acai (*Euterpe oleracea* Mart.) pulp. Their structures were elucidated by ESIMS, UV, IR, ^{1}H and ^{13}C NMR, CD spectra and by comparison with the literature.

(2S,3S)-dihyrokaempferol 3-*O*-β-D-glucoside (**1**): yellow amorphous powder; $[\alpha]_D^{25}$-29.6 (c 0.2, EtOH); UV (MeOH) γ_{max} 292, 340 (sh) nm; IR (KBr) υ_{max} 3405 (OH), 2919 (CH), 1642 (C=O), 1164, 1078 (C–O) cm^{-1}; ESIMS m/z 473 $[M + Na]^+$; for the ^{1}H and ^{13}C NMR data see Table 1 and for CD spectral data see Table 2. The data were consistent with the known compound (Kato, Li, Koike, Wang, & Koike, 2010).

(2R,3R)-dihydrokaempferol 3-*O*-β-D-glucoside (**2**): yellow amorphous powder; $[\alpha]_D^{25}$+35.8 (c 0.2, EtOH); UV (MeOH) γ_{max} 292, 340 (sh) nm; IR (KBr) υ_{max} 3405 (OH), 2919 (CH), 1642 (C=O), 1164, 1078 (C–O) cm^{-1}; ESIMS m/z 473 $[M + Na]^+$; for ^{1}H and ^{13}C NMR data see Table 1 and for CD spectral data see Table 2. The data were consistent with the known compound (Yu, Li, Chen, & Yang, 1992).

Isovitexin (**3**): yellowish amorphous powder; ESIMS: m/z = 431 $[M–H]^-$; ^{1}H NMR (DMSO-d_6) ppm: δ 13.50 (1H, s, 5-OH), 7.89 (2 H, d, J = 8.4 Hz, H-2, 6), 6.92 (2 H, d, J = 8.4 Hz, H-3, 5), 6.70 (1 H, s, H-3), 6.44 (1 H, s, H-8), 4.60 (1 H, d, J = 10.0 Hz, H-glu-1). The data were consistent with the known compound (Leong et al., 2010).

Table 1
^{1}H and ^{13}C NMR spectral data (500 and 125 MHz, DMSO-d_6) of compounds **1** and **2.**

Position	**1** (2S,3S) δ_H (J in Hz)	δ_C	**2** (2R, 3R) δ_H (J in Hz)	δ_C
Aglycone moiety				
2	5.49 (d, 7.0)	81.2	5.44 (d, 8.0)	81.0
3	4.93 (d, 7.0)	74.7	4.92 (d, 7.0)	75.2
4		193.2		193.0
5		163.6		163.6
6	5.86 (s)	96.3	5.86 (s)	96.3
7		168.6		168.0
8	5.85 (s)	95.4	5.86 (s)	95.3
8a		161.8		162.4
4a		100.5		100.9
1′		126.1		126.5
2′	7.23 (d, 8.0)	129.1	7.26 (d, 8.4)	129.0
3′	6.72 (d, 8.0)	115.0	6.75 (d, 8.4)	115.2
4′		157.6		157.8
5′	6.72 (d, 8.0)	115.0	6.75 (d, 8.4)	115.2
6′	7.23 (d, 8.0)	129.1	7.26 (d, 8.4)	129.0
Glucose moiety				
1″	4.60 (d, 8.0)	102.8	3.97 (d, 6.4)	101.0
2″	2.85 (m)	73.7	2.90 (m)	73.6
3″	3.09 (m)	76.7	3.01–3.62 (5H, m)	76.7
4″	2.94 (m)	70.2		70.1
5″	3.09 (m)	77.2		77.3
6″	3.28 (m)	61.4		61.3
	3.66 (m)			

Velutin (**4**): yellowish amorphous powder; ESIMS: m/z = 313 $[M–H]^-$; ^{1}H NMR (DMSO-d_6) ppm: δ 12.97 (1H, s, 5-OH), 7.60 (1 H, d, J = 8.5 Hz, H-6), 7.58 (1 H, brs, H-2), 6.94 (1 H, d, J = 8.5 Hz, H-5), 6.95 (1 H, s, H-3), 6.80 (1 H, d, J = 2.0 Hz, H-8), 6.37 (1 H, d, J = 2.0 Hz, H-6), 3.89 (3 H, s, CH_3O-3), 3.87 (3 H, s, CH_3O-7). The data were consistent with the known compound (Çitoğlu, Sever, Antus, Baitz-Gács, & Altanlar, 2003).5,4′-Dihydroxy-7,3′,5′-trimethoxyflavone (**5**): yellowish amorphous powder; ESIMS: m/z = 343 $[M–H]^-$; ^{1}H NMR (DMSO-d_6) ppm: δ 12.97 (1H, s, 5-OH), 8.34 (1 H, s, 4-OH), 7.37 (2 H, s, H-2, 6), 7.06 (1 H, s, H-3), 6.87 (1 H, brs, H-8), 6.38 (1 H, brs, H-6), 3.89 (6 H, s, CH_3O-3, 5), 3.88

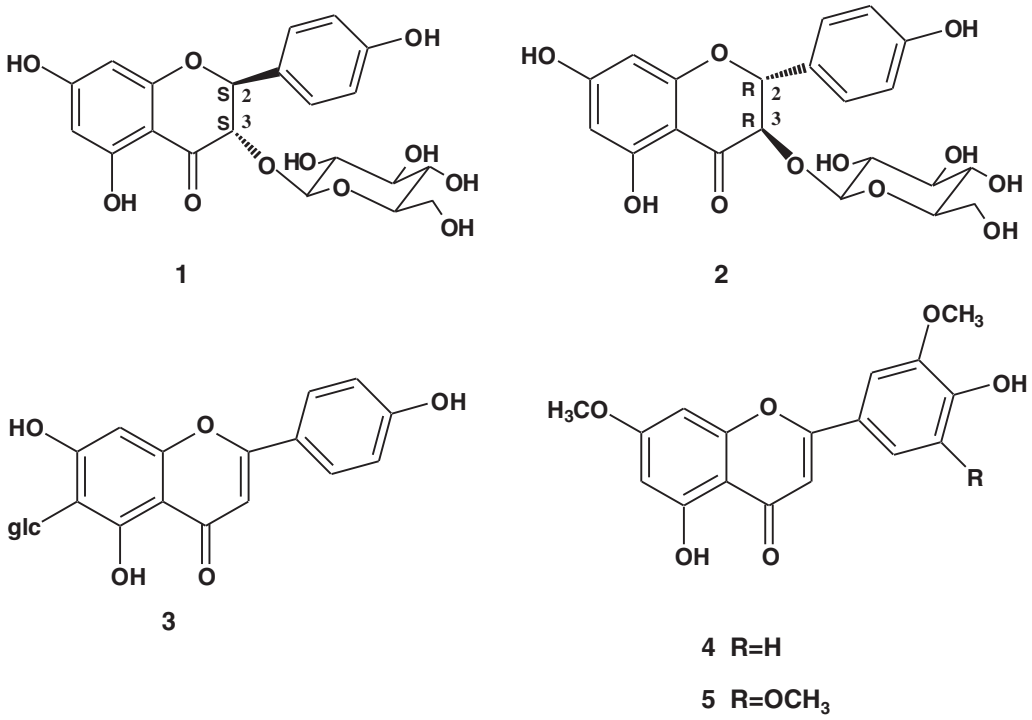

Fig. 1. Chemical structures of five flavonoids, (2S,3S)-dihyrokaempferol 3-*O*-β-D-glucoside (**1**), (2R,3R)-dihydrokaempferol 3-*O*-β-D-glucoside (**2**), isovitexin (**3**), velutin (**4**) and 5,4′-dihydroxy-7,3′,5′-trimethoxyflavone (**5**) isolated from acai (*Euterpe oleracea* Mart.) pulp.

Table 2
CD spectra of compounds **1** and **2**.

Compounds	$n\rightarrow\pi*$	$n\rightarrow\pi*$
(2S,3S)-Dihydrokaempferol 3*O*-β-D-glucoside (**1**)	$[\theta]_{324}$−4951	$[\theta]_{290}$ + 5195
(2R,3R)-Dihydrokaempferol 3*O*-β-D-glucoside (**2**)	$[\theta]_{324}$ + 9993	$[\theta]_{290}$−27,329

(3 H, s, CH_3O-7). The data were consistent with the known compound (Zahir et al., 1996).

Among these five flavonoids, compounds **1** and **2** were found to be isomers, according to their spectroscopic data. Slight differences in the spectra between **1** and **2** suggested that these compounds have the same structure type but different stereo configurations. The most remarkable difference was the anomeric proton of glucopyranosyl residue (**1**: δ_H = 4.60, **2**: δ_H = 3.97). The CD curve of the two compounds showed Cotton effects at 324 and 290 nm (Table 2). The absolute configurations were elucidated to be (2S, 3S) for **1** and (2R, 3R) for **2** according to the literatures (Sakushima, Ohno, Coskun, Seki, & Ohkura, 2002; Yu et al., 1992).

To our knowledge, (2S,3S)-dihyrokaempferol 3-*O*-β-D-glucoside (**1**) and its isomer (2R,3R)-dihydrokaempferol 3-*O*-β-D-glucoside (**2**) , velutin (**4**) and 5,4′-dihydroxy-7,3′,5′-trimethoxyflavone (**5**) were identified from acai pulp for the first time.

3.2. Antioxidant capacities of the flavonoids

ORAC is a chemical antioxidant assay that is based on the inhibition of the peroxyl-radical induced oxidation initiated by thermal decomposition of AAPH. It is one of the most widely used *in vitro* antioxidant capacity assays (Prior, Wu, & Schaich, 2005). Similar to what we observed from the previous study (Kang et al., 2010), the ORAC values of these five compounds varied distinctly, from 4458.0 ± 408.8 μmol TE/g for 5,4′-dihydroxy-7,3′,5′-trimethoxyflavone (**5**) to 22404.5 ± 1322.1 μmol TE/g for isovitexin (**3**) (Table 3). The numbers and positions of the hydroxyl groups and/or other substitutes, such as through glycosylation have been shown to affect the antioxidant capacity of flavonoids (Cao, Sofic, & Prior, 1997; Kang et al., 2010; Prasad, Divakar, Shivamurthy, & Aradhya, 2005). Methylation of hydroxyl groups on benzene rings dramatically decreased the antioxidant capacities of flavonoids, which was clearly demonstrated through 5,4′-dihydroxy-7,3′,5′-trimethoxyflavone (**5**). In addition, our data suggested for the first time that stereo configuration could also dramatically affect the antioxidant capacity of flavonoids. The ORAC value of (2S,3S)-dihydrokaempferol 3-*O*-β-D-glucoside (**1**) is about 35% higher than its isomer (2R,3R)-dihydrokaempferol 3-*O*-β-D-glucoside (**2**). Nevertheless, how stereo configuration affects the antioxidant capacity of flavonoids requires further investigation.

3.3. Anti-inflammatory activities of flavonoids

NF-κB is one of the principal inducible transcription factors in mammals and has been shown to play a pivotal role in the mammalian innate immune response and chronic inflammatory conditions (Bremner & Heinrich, 2002; Li & Stark, 2002). Active NF-κB participates in the control of transcription of over 150 target genes, including the expression of various inflammatory cytokines, chemokines, immunoreceptors, and cell adhesion molecules (Pahl, 1999). NF-κB has thus often been termed a "central mediator of the human immune response" (Pahl, 1999). A number of plant-derived substances including certain flavonoids, such as quercertin (Nair et al., 2006) and luteolin (Kim & Jobin, 2005) are currently known as NF-κB modulators. In this study, the activity and potency of five flavonoids as NF-κB inhibitors were evaluated by the SEAP reporter assay in RAW-Blue™ cells. RAW-Blue™ cells are derived from RAW 264.7 macrophages. They stably express a SEAP gene inducible by NF-κB. However, basal expression of the SEAP gene is too low, so it was usually induced by stimuli, such as lipopolysaccharides (LPS). The SEAP protein secreted to the culture media was measured by a SEAP assay kit as a read-out. Among all five compounds being screened, velutin (**4**) was found to strongly inhibit SEAP secretion in RAW-Blue™ cells induced by LPS (Fig. 2). Velutin (**4**) is a flavone that is structurally similar to luteolin, which was also isolated in acai pulp by our lab (Kang et al., 2010). Luteolin is one of the most common flavones has its good anti-inflammatory activities, including inhibition of NF-κB activation (Lopez-Lazaro, 2009). In this study, luteolin was adopted as a positive control. Dose response studies of both velutin (**4**) and luteolin in inhibiting SEAP secretion were further conducted to compare the effectiveness of these two compounds (Fig. 3). The IC_{50} were calculated as 2.0 μM for velutin (**4**) and 12.4 μM for luteolin, respectively. Velutin (**4**) exhibited much greater activity in inhibiting NF-κB activation than luteolin did. The chemical structures of velutin (**4**) and luteolin are very similar with the only difference on two hydroxyl/methoxy groups. Velutin (**4**) bears two methoxyl groups at 7- and 3'-positions, whereas luteolin has two hydroxyl groups at 7- and 3'-positions. Substitution of methoxyl groups appears to be a significant factor that determines inhibition effects. However, exactly how and to what extent the methoxyl groups modulate the inhibition effects of flavones compounds are yet to be determined.

Acai juice has showed effects in the prevention of atherosclerosis in an *in vivo* animal study (Xie et al., in press). NF-κB activation is thought to be involved in the initiation and progression of atherosclerosis through its role as a direct regulator of pro-inflammatory and anti-inflammatory genes and as a regulator of cell survival

Table 3
ORAC values of compounds **1**–**5**.

Compounds	ORAC (μmol TE/g)[a]
(2S,3S)-dihyrokaempferol 3-*O*-β-D-glucoside (**1**)	15198.9 ± 502.6
(2R,3R)-dihydrokaempferol 3-*O*-β-D-glucoside (**2**)	11218.9 ± 432.8
Isovitexin (**3**)	22404.5 ± 1322.1
Velutin (**4**)	13643.3 ± 1119.5
5,4′-dihydroxy-7,3′,5′-trimethoxyflavone (**5**)	4458.0 ± 408.8

[a] Data was expressed as mean ± SD, n = 3.

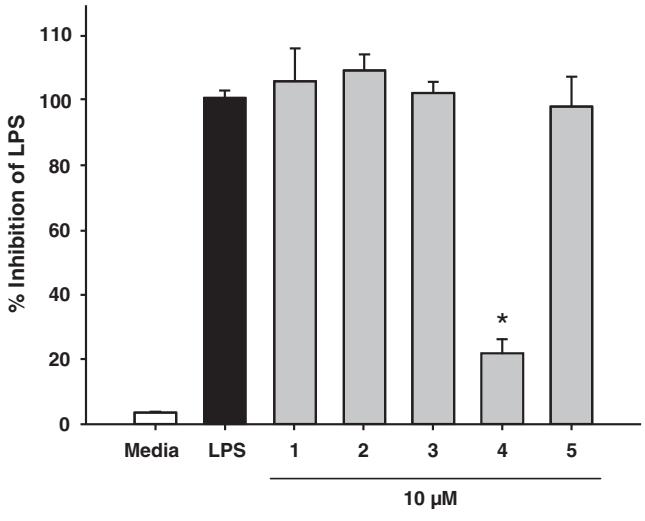

Fig. 2. Results of the SEAP reporter assay induced by LPS on compounds **1**–**5** isolated from acai pulp (initial screening). The bars represent the mean ± SD (n = 3) from three independent experiments, $^*P < 0.05$.

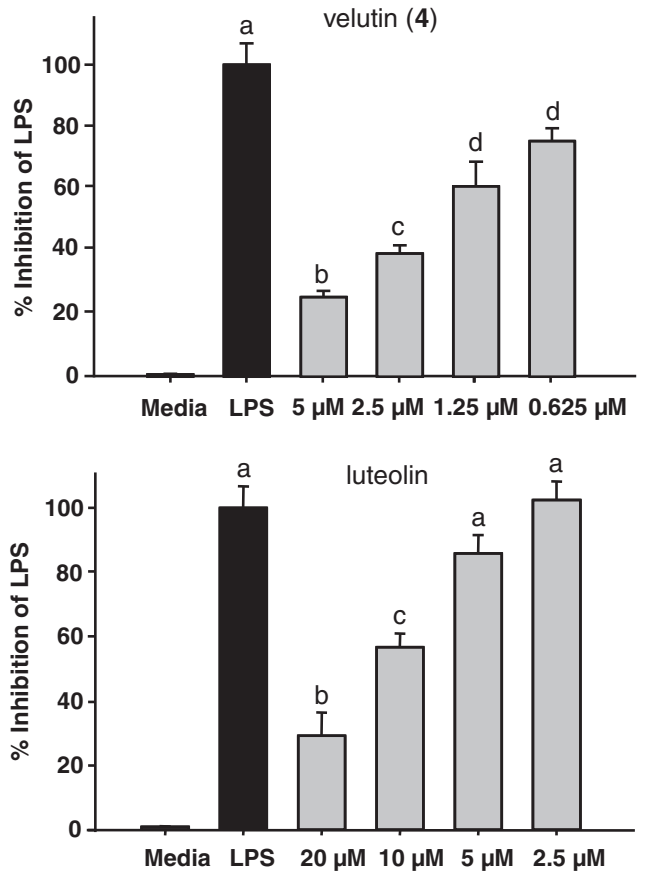

Fig. 3. Results of dose–response experiments of velutin (**4**) and luteolin from SEAP reporter assay induced by LPS. The bars represent the mean ± SD ($n = 3$) from three independent experiments. Means with different letters are different ($P < 0.05$).

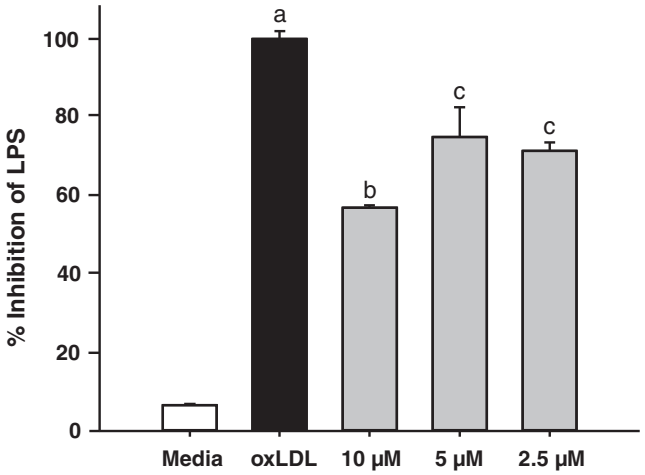

Fig. 4. Results of the SEAP reporter assay induced by oxLDL on velutin (**4**). The bars represent the mean ± SD ($n = 3$) from three independent experiments. Means with different letters are different ($P < 0.05$).

and proliferation (de Winther, Kanters, Kraal, & Hofker, 2005; Xanthoulea, Curfs, Hofker, & de Winther, 2005). It is well recognised that oxLDL plays a crucial role in the initiation and progression of atherosclerosis (Kita et al., 2001). Therefore, the inhibitory effect of velutin (**4**) in oxLDL induced NF-κB activation was also examined. Velutin (**4**) was found to inhibit NF-κB activation induced by oxLDL (Fig. 4) at concentration as low as 2.5 μM. Since the oxidation of LDL is a key step in the early stage of atherosclerosis development, this suggests possible athero-protective effects of this compound. However, clinical validation of these findings is needed. This result may also contribute to our understanding of why adding acai to high fat diet could increase the lifespan of *Drosophila melanogaster* challenged by oxidative stress (Sun et al., 2010).

4. Conclusions

In this study, five flavonoids were isolated and structurally identified from freeze-dried acai pulp. Four of them, (2S,3S)-dihyrokaempferol 3-*O*-β-D-glucoside (**1**) and its isomer (2R,3R)-dihydrokaempferol 3-*O*-β-D-glucoside (**2**) , velutin (**4**) and 5,4′-dihydroxy-7,3′,5′-trimethoxyflavone (**5**), have never been reported from this plant before. The antioxidant capacities of these compounds were evaluated by ORAC assay. The ORAC values of the five compounds varied significantly based on their chemical structures. The methoxyl groups and stereo configuration appeared to be important structural factors that determined their antioxidant capacities.

The potential effects of these five flavonoids in inhibiting NF-κB activation were screened by SEAP reporter assay. Velutin (**4**), an uncommon flavone, was found to strongly inhibit SEAP secretion in RAW-blue cells induced by LPS or oxLDL at low micromole levels, implicating potential anti-inflammatory effects. Further studies are warranted to reveal the mechanisms underlying its inhibitory effects.

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