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Lipoperoxidation and Cyclooxygenases 1 and 2 Inhibitory Compounds from *Iryanthera juruensis*

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Plants from *Iryanthera* genus have been traditionally used as food supplements by South American Indians. The MeOH extract of leaves of *Iryanthera juruensis*, one of the plants endemic to the Amazon region and consumed in Brazil, and the hexane extract from its seeds inhibited lipid peroxidation (LPO) and cyclooxygenase (COX-1 and -2)) enzymes in in vitro assays. Further analyses of these extracts yielded 5-deoxyflavones (**1**−**5**) from the leaf extract and sargachromenol (**6**), sargaquinoic acid (**7**), a novel juruenolic acid (**8**), ω-arylalkanoic acids (**9a**−**c**), and the lignan guaiacin (**10**) from the seed extract. Compounds **3**−**5** inhibited LPO by 86%, 77%, and 88% at 10 ppm, respectively, and compounds **6** and **9a**−**c** showed inhibition at 76% and 78% at 100 ppm, respectively. However, compounds **7** and **8** were inactive and lignan **10** exhibited LPO inhibitory activity by 99% at 100 ppm compared to commercial antioxidants butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and vitamin E. The flavones **1**−**5** also inhibited COX-1 and -2 enzymes by 50−65% at 100 ppm. Compound **6** showed high but nonselective inhibition of COX-1 and COX-2 enzymes, when compared to aspirin and Celebrex, a nonsteroidal anti-inflammatory drug (NSAID). Compounds **7** and **10** inhibited COX-1 by 60% and 65% and COX-2 by 37% and 18%, respectively, whereas compounds **8** and **9a**−**c** showed little or no activity against these enzymes.

KEYWORDS: ***Iryanthera juruensis*; Myristicaceae; lipid peroxidation; cyclooxygenase; flavone; tocotrienol; ω-arylalkanoic acids; lignan**

INTRODUCTION

Plants from Myristicaceae family have been traditionally used as food supplements by South American Indians. The anecdotal health benefits of these plants are implicated in healing stomach infections and wounds and for inducing central nervous system (CNS) alterations when used during religious ceremonies. The *Iryanthera* species are endemic to the Amazon region and their crushed leaves were reported to heal infected wounds and cuts. Also, the latex from the barks, mixed with warm water, was used for treating infections (*1*). The seeds of *Iryanthera* species are valued in Brazil for their high yield in fatty acids, which also explains their general designation as "ucu-uba", or fat-producing tree. The protein-rich aril and roasted seeds as well as beverages prepared from the crushed leaves of *Iryanthera* are consumed by native people in the Amazon region. The major constituent in the seeds of *Iryanthera* species is myristic acid, a C14 fatty acid. It usually co-occurs with antioxidant tocotrienols and is extensively used in the pharmaceutical and cosmetic industries (*2*). In the course of our systematic studies on *Iryanthera* species, we have demonstrated the presence of antioxidant tocotrienols, flavonoids, and flavonolignans in fruits and leaves of *Iryanthera grandis*, *Iryanthera lancifolia*, *Iryanthera sagotiana*, and *Iryanthera juruensis* (*3*−*7*).

Oxidative damage to the cell membrane and its components is the initial event in several human disease processes, including cancer and atherosclerosis. *Iryanthera* spp. are known for their health benefits and hence we have continued the search for natural antioxidants in this plant species. It is a popular notion that regular consumption of antioxidant compounds imparts beneficial effects to biological systems and that such compounds act as chemopreventive agents. It has been established that compounds with radical scavenging activity are beneficial to health since they induce detoxifying enzymes (*8*, *9*).

As part of ongoing projects aimed at the discovery of bioactive compounds from Brazilian flora [Biota-FAPESP (www.biotasp.org.br) and BIOprospecTA (www.bioprospecta.org.br)], the antioxidant and anti-inflammatory activities of 56 plant extracts have been examined by means of in vitro assays measuring the inhibition of lipid peroxidation (LPO) and

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cyclooxygenase (COX-1 and COX-2) enzymes, respectively. The methanol and hexane extracts of *I. juruensis* leaves and seeds, respectively, were subjected to bioassay-directed chromatographic purification. This paper presents the comparative LPO and COX enzyme inhibitory activities of the pure isolates and of crude extracts along with commercial antioxidants butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and vitamin E, as well as clinically used antiinflammatory agents aspirin and Celebrex. Also, this is the first report of flavones **1**−**5** from *I. juruensis* and of the novel juruenolic acid (**8**).

MATERIALS AND METHODS

General Experimental Procedures. UV and IR spectra were recorded on a Perkin-Elmer Lambda 3B and a Perkin-Elmer FT-IR 1750 spectrophotometer, respectively. NMR spectra [^{1}H, ^{13}C, correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond correlation (HMBC) experiments] were measured on Varian INOVA (300 MHz) and VRX (500 MHz) instruments. ^{1}H NMR chemical shifts are reported in parts per million (ppm) relative to residual proton signals of $CDCl_3$ at 7.24 and DMSO-d_6 at 2.50. Coupling constants, *J*, are in Hertz. ^{13}C NMR chemical shifts are reported in parts per million relative to residual ^{13}C signals of $CDCl_3$ at 77.0 and DMSO-d_6 at 39.5. Standard pulse sequences were employed for all 1D [^{1}H, ^{13}C, and distortionless enhancement by polarization transfer (DEPT)] and 2D (COSY, HMBC, and HMQC) NMR experiments. Mass spectra were recorded at the Michigan State University mass spectrometry facility on a JEOL HX-110 double-focusing mass spectrometer (Peabody, MA) operating in the positive ion mode for fast atom bombardment mass spectrometry (FABMS) and high-resolution (HR) FABMS experiments.

Merck silica gel (60 mesh size, 35−70 μm) with particle size of 60 μm was used for preparative medium-pressure liquid chromatography (MPLC). For preparative thin-layer chromatography (TLC) separation, 250, 500, and 1000 μm silica gel plates (Analtech, Inc., Newark, DE) were used. TLC plates were viewed under UV light at 254 and 366 nm or sprayed with 10% sulfuric acid solution.

Solvents were of ACS reagent grade and were purchased from Spectrum Chemical Co. (Gardena, CA). Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), α-tocopherol, dimethyl sulfoxide (DMSO), and acetic acid were purchased from Sigma−Aldrich Chemical Co. (St. Louis, MO). Celebrex was a physician professional sample. 1-Stearoyl-2-linoleoyl-*sn*-glycerol-3-phosphocholine was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL), and 3-[*p*-(6-phenyl)-1,3,5-hexatrienyl]phenylpropionic acid was from Molecular Probes (Eugene, OR). COX-1 and COX-2 enzymes were prepared in the Bioactive Natural Products and Phytoceuticals Laboratory (BNPP) from ram seminal vesicles and prostaglandin endoperoxide H synthase-2 (PGHS-2), from cloned insect cell lysate, respectively (*12*).

Plant Material and Extract Preparation. Leaves and fruits of *I. juruensis* were collected in Reserva do Mocambo, in the Amazon forest in January during the rainy season, and identified as *Iryanthera juruensis* Warburg by botanist Nelson Rosa. A voucher specimen, ID 141860 MG, was deposited at the Herbarium João Murça Pires at Museu Paraense Emílio Goeldi (Belém, PA, Brazil). The dried and ground leaves (111 g) and seeds (53 g) were extracted separately with hexane, ethyl acetate, and methanol at room temperature, using 3 × 300 mL of each solvent for extraction of leaves and 3 × 130 mL of each solvent for extraction of seeds. Solvents were removed under vacuum by use of a rotary evaporator and afforded the crude extracts hexane (1.4 g), EtOAc (1.8 g), and MeOH (6.4 g) from leaves and hexane (13.5 g), EtOAc (14.1 g), and MeOH (3.3 g) from seeds.

Isolation of Constituents. The MeOH extract from the leaves of *I. juruensis* (3.2 g) was stirred with $CHCl_3$ and filtered to remove the precipitate (1.5 g). The $CHCl_3$-soluble part of this extract was concentrated (1.7 g) and subjected to MPLC on silica gel. Elution with hexane/acetone (H/A) solvent system under gradient conditions (H/A 9:1, 200 mL; H/A 4:1, 300 mL; H/A 3:1, 300 mL; H/A 2:1, 300 mL; H/A 1:1, 200 mL; H/A 1:2, 400 mL; acetone 100%, 200 mL; MeOH 100%, 200 mL) afforded 11 fractions (A1−A3, 100 mL each; A4−A6, 200 mL each; A7, 300 mL; A8 and A9, 250 mL each; and A10 and A11, 200 mL each). Fraction A7 was precipitated with acetone and gave pure compound **3** (17 mg) as a pale yellow precipitate. The acetone-soluble portion was concentrated (240 mg) and was further chromatographed on a silica gel MPLC column with $CHCl_3$/MeOH (C/M) under gradient conditions ($CHCl_3$ 100%, 100 mL; C/M 99:1, 100 mL; C/M 49:1, 200 mL; C/M 19:1, 300 mL; C/M 9:1, 200 mL; C/M 4:1, 200 mL; C/M 3:1, 100 mL; C/M 1:1, 100 mL; MeOH 100%, 100 mL) to give 12 fractions (B1−B3, 100 mL each; B4−B5, 150 mL each; B6−B11, 100 mL each; B12, 200 mL). Fractions B2−B4 were combined on the basis of their comparative TLC profiles and submitted to preparative TLC ($CHCl_3$/MeOH 19:1 v/v) to give compounds **1** (2.6 mg, R_f 0.5) and **2** (2.2 mg, R_f 0.4). Similarly, silica gel MPLC of fractions A8−A10 (340 mg) eluted with $CHCl_3$/MeOH solvent systems ($CHCl_3$ 100%, 100 mL; C/M 99:1, 200 mL; C/M 49:1, 300 mL; C/M 19:1, 400 mL; C/M 9:1, 300 mL; C/M 4:1, 200 mL; C/M 3:1, 100 mL; C/M 1:1, 100 mL; MeOH 100%, 100 mL) afforded 13 fractions (C1 and C2, 200 mL each; C3 and C4, 100 mL each; C5−C10, 150 mL each; C11−C13, 100 mL each). Preparative TLC ($CHCl_3$/MeOH 9:1 v/v) of fraction C6 yielded compounds **4** (6 mg, R_f 0.4) and **5** (4 mg, R_f 0.3).

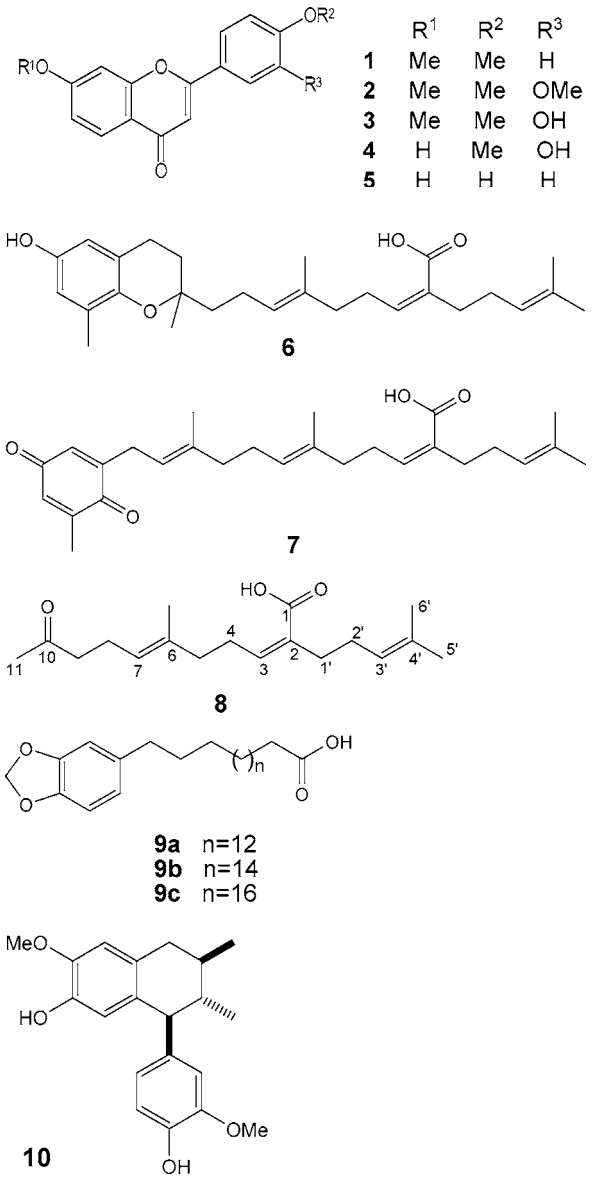
The hexane extract of seeds (5.1 g) was stirred with hot MeOH and was filtered. The MeOH-soluble fraction was concentrated (2.7 g) and subjected to MPLC purification on silica gel with hexane/acetone (H/A) as the mobile phase under gradient conditions (H/A 49:1, 200 mL; H/A 19:1, 200 mL; H/A 9:1, 400 mL; H/A 4:1, 400 mL; H/A 7:3, 400 mL; H/A 3:1, 300 mL; H/A 2:1, 300 mL; H/A 1:1, 200 mL; acetone 100%, 200 mL; MeOH 100%, 100 mL) to afford 28 fractions (D1 and D2, 200 mL each; D3−D6, 100 mL each; D7−D14, 50 mL each; D15−D21, 100 mL each; D22−D25, 50 mL each; D26−D28, 200 mL each). Fraction D12 was precipitated with hexane to give compounds **9a**−**c** (56 mg) as a white precipitate. Fractions D16−D18 (149 mg) were combined and further submitted to MPLC (hexane/acetone gradient: H/A 9:1, 200 mL; H/A 4:1, 300 mL; H/A 3:1, 300 mL; H/A 2:1, 300 mL; H/A 1:1, 200 mL; acetone 100%, 100 mL) to afford five fractions (E1 and E2, 250 mL each; E3−E5, 300 mL each); preparative TLC ($CHCl_3$/MeOH, 49:1, v/v) of fraction E4 afforded compound **8** (10 mg, R_f 0.3). Fractions D22−D24 (1.26 g) were pooled and also submitted to MPLC (hexane/acetone gradient: H/A 9:1, 200 mL; H/A 4:1, 300 mL; H/A 7:3, 400 mL; H/A 3:1, 400 mL; H/A 2:1, 300 mL; H/A 1:1, 300 mL; acetone 100%, 200 mL) to afford nine fractions (F1−F3, 300 mL each; F4−F9, 200 mL each), which gave compounds **6** (44 mg) and **7** (18 mg) as brown oils from fractions F8 and F3, respectively. Preparative TLC (hexane/acetone 3:1 v/v) of fraction F6 gave compound **10** (26 mg, R_f 0.4).

2-(4′-Methylpentenyl-3)-10-oxoundecan-2,6-dienoic acid (juruenolic acid, **8**): Pale yellow oil; UV λ_{max}^{MeOH} nm (ϵ): 246 (3800). IR ν_{max}^{film} cm^{-1}: 3411, 2918, 1728, 1680. ^{1}H and ^{13}C NMR (see **Table 1**). HRFABMS $C_{18}H_{28}O_3$, *m/z* 293.2116 $[M + H]^+$ (calcd 293.4264). FABMS *m/z* 585 $[2M + H]^+$, 293 $[M + H]^+$, 165 (8), 69 (40).

Antioxidant Assays: *β-Carotene Assay.* TLC plates were developed in appropriate solvent systems. After developing and drying, TLC plates were sprayed with a 0.02% solution of β-carotene (Aldrich) in MeOH. Plates were placed under natural light until discoloration of background (*10*). Each plant extract was tested in two elution systems ($CHCl_3$/MeOH, 9:1 v/v, and $CHCl_3$/MeOH/H_2O, 13:6:1 v/v/v) for observation of low-, medium-, and high-polarity compounds. The yellow spots retained on the TLC plates indicated the presence of antioxidant compounds in the extracts tested.

Lipoperoxidation Inhibition Assay. These antioxidant bioassays were conducted by analysis of model liposome oxidation by use of fluorescence spectroscopy (*11*). The lipid, 1-stearoyl-2-linoleoyl-*sn*-glycerol-3-phosphocholine (5 μmol), and fluorescent probe, 3-[*p*-(6-phenyl)-1,3,5-hexatrienyl]-phenylpropionic acid (5 μmol), were combined in *N*,*N*-dimethylformamide (DMF) and dried under vacuum at room temperature. Large unilamellar vesicles (LUVs) were produced by resuspension of the lipid−probe mixture [0.15 M NaCl, 0.1 mM ethylenediaminetetraacetic acid (EDTA) and 0.01 M 3-(*N*-morpholino)-propanesulfonic acid (MOPS) maintained over Chelex 100 resin] followed by 10 freeze−thaw cycles in a dry ice−EtOH bath and

extrusion (29 times) through a 100 nm pore size membrane. The final assay volume was 2 mL, consisting of 100 μL of *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer [50 mM HEPES and 50 mM tris(hydroxymethyl)aminomethane (Tris)], 200 μL of 1 M NaCl, 1.64 mL of N_2-sparged Millipore purified water, 20 μL of test sample or DMSO (solvent control), and a 20-μL aliquot of liposome suspension. Peroxidation was initiated by addition of 20 μL of $FeCl_2 \cdot 4H_2O$ (0.5 mM) to achieve a final concentration of 50 μM Fe^{2+}. The positive controls BHA, BHT, and α-tocopherol/vitamin E were tested at a final concentration of 10 μM. Fluorescence was measured at 384 nm and monitored at 0, 1, and 3 min and every 3 min thereafter up to 21 min on a Turner model 450 digital fluorometer (Barnstead Thermolyne, Dubuque, IA). The decrease of relative fluorescence intensity with time indicated the rate of peroxidation. Relative fluorescence (F_t/F_0) was calculated by dividing the fluorescence value at a given time t (F_t) by that at $t = 0$ min (F_0).



Cyclooxygenase Enzymes Inhibitory Assay. COX-1 enzyme inhibitory assay was conducted with an enzyme preparation from ram seminal vesicles (ca. 0.46 mg of protein/mL in 30 mM Tris buffer, pH 7). COX-2 activity was determined by using a preparation from insect cell lysate and diluting with Tris buffer (pH 7) to give an approximate final concentration of 1.5 mg protein/mL (*12*, *13*). COX assays were measured at 37 °C by observing the initial rate of O_2 uptake with a micro oxygen chamber and electrode (Instech Laboratories, Plymouth Meeting, PA) attached to a YSI model 5300 biological oxygen monitor (Yellow Springs Instrument, Inc., Yellow Springs, OH). Each assay mix contained 0.6 mL of 0.1 M Tris buffer (pH 7), 1 mmol of phenol, and 17 μg of hemoglobin. For each test compound (100 ppm) or standards in DMSO (10 μL), activities were determined following a 2−10 min preincubation of samples with enzyme COX-1 or COX-2 (10−20 μL) in the assay chamber. Celebrex (1.7 ppm) and aspirin (108 ppm) were used as positive controls in this assay. Different concentrations of standards used were due to their difference in inhibitory activities and also to achieve a uniform enzyme inhibition at 50%. Reaction was initiated by adding arachidonic acid (10 μL of a 1.64 μM solution). Data were recorded with Quicklog for Windows data acquisition software (Strawberry Tree Inc., Sunnyvale, CA).

RESULTS AND DISCUSSION

We have evaluated 158 Brazilian plant extracts by TLC for the presence of antioxidant compounds (unpublished data) by spraying the developed TLC plates with β-carotene solution followed by exposure to natural light (*10*). The results indicated the presence of antioxidants in 56 extracts, based on the observation of persistent yellow spots after exposure of the plates to natural light (*10*). These 56 plant extracts were then evaluated for their LPO and COX enzyme inhibitory activities. On the basis of the bioassay results, the methanol and hexane extracts of *I. juruensis* leaves and seeds, respectively, were selected for the isolation of bioactive compounds. In summary, the chromatographic purification of the leaf-extract afforded 5-deoxyflavones (**1**−**5**) and the seeds extract gave sargachromenol (**6**), sargaquinoic acid (**7**), the novel juruenolic acid (**8**), ω-arylalkanoic acids (**9a**−**c**), and guaiacin (**10**).

The identification of isolates was based mostly on NMR, UV, and MS spectral analysis and comparison with literature data. Compounds **1**−**5** belonged to the class of flavones lacking a 5-oxy substituent, which was confirmed by the signal at ca. δ 7.90 (d, $J \sim 8.0$ Hz), assigned to H-5, in their ^{1}H NMR spectra. The position of -OCH_3 groups in flavones **3** and **4** was established by UV spectral analysis with NaOAc/MeOH and NaOMe/MeOH as shift reagents for determination of the substitution patterns in rings A (band I) and B (band II), respectively (*14*, *15*). Also, the NMR data of these compounds were identical to the reported chemical shift values and further confirmed their identities. Therefore, compound **1** was identified as 7,4′-dimethoxyflavone and was formerly isolated from *Virola*, a widely occurring neotropical Myristicaceae genus (*16*), whereas compound **3** was identified as tithonine, previously isolated from *Tithonia* (Asteraceae) (*17*). Compounds **2** and **4**, identified as fasciculiferin and farnisine, respectively, were reported from *Acacia fasciculifera* and *Acacia farnesiana* (Fabaceae) (*18*, *19*). Similarly, compound **5** was identified as 7,4′-dihydroxyflavone, reported from *Pterocarpus marsupium* (*20*).

The structures of sargachromenol (**6**) and sargaquinoic acid (**7**) were also confirmed by NMR and MS spectral analyses. These compounds have been isolated earlier from brown seaweeds from the genera *Sargassum* and *Stipopodium* (*21*, *22*) and as repeated isolates from *I. juruensis* (*7*).

The ^{1}H NMR spectrum of compound **8** showed signals for four methyl groups on sp^2 carbons at δ 1.59, 1.62, 1.68, and 2.13, which showed correlations to signals at δ 17.7, 15.8, 25.6, and 29.9, respectively, in the HMQC spectrum. A triplet at δ 5.98, integrated for one proton, and a multiplet at δ 5.09 for two protons were observed in its ^{1}H NMR spectrum. The ^{13}C NMR signal at δ 172.0, assigned to an α,β-unsaturated carboxyl, and at δ 123.0, 130.0, 132.0, 135.0, and 145.6, assigned to olefinic carbons of an isoprene chain, suggested a partial structure similar to the side chain in compounds **6** and **7**. An

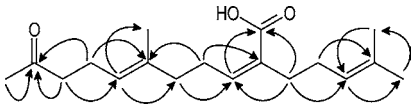


Figure 1. Selected HMBC correlations for compound **8**.

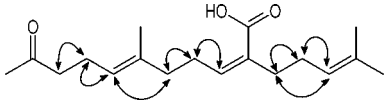


Figure 2. HOMOCOSY correlations for compound **8**.

Table 1. NMR Data for Compound **8**[a]

position	δ_H[b]	δ_C[c]	HMBC	HOMOCOSY
1		172.6 s		
2		135.5 s		
3	5.98 t (7.5)	145.3 d	C-5, C-2′, C-1	H-8
4	2.60 q (7.5)	28.1 t	C-6, C-5, C-3, C-2	H-3, H-3
5	2.08 t (7.5)	39.0 t	C-7, C-4, C-3, C-2, C-12	H-7, H-4
6		130.6 s		
7	5.09 m	123.4 d	C-12, C-5	H-8, H-5
8	2.27 t (7.5)	22.4 t	C-10, C-9, C-7, C-6	H-9, H-7
9	2.45 t (7.5)	43.7 t	C-10, C-8, C-7	H-8
10		208.8 s		
11	2.13 s	29.9 q	C-10	
12	1.62 s	15.8 q	C-5	
1′	2.26 t (6.5)	27.8 t	C-1′, C-3′, C-4′	H-2′, H-3′
2′	2.11 m	34.5 t	C-3, C-2, C-2′, C-3′, C-1	H-1′, H-3′
3′	5.09 m	123.4 d	C-6′, C-5′	H-1′, H-2′
4′		132.3 s		
5′	1.68 s	25.6 q	C-3′, C-4′, C-6′	
6′	1.59 s	17.7 q	C-3′, C-4′, C-5′	

[a] 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR; $CDCl_3$. [b] δ (ppm), multiplicities, *J* (Hz). [c] Assignments were based on HMQC experiments, and multiplicities were mostly taken from a DEPT 135° experiment.

additional signal at δ 208.0, which showed HMBC correlation with the singlet at δ 2.13 and the triplet at δ 2.45, indicated a methyl-carbonyl-methylene moiety in the structure of **8** (**Figure 1**). Also, one quartet at δ 2.60, two triplets at δ 2.08 and 2.11, integrated for two protons each, and one multiplet at δ 2.26, integrated for four protons, were assigned to the allyl-methylene protons. The HMBC correlation between H1′ (δ 2.26) and C1 (COOH, δ 172.6), in addition to correlations of H8 (δ 2.27) to C6 (δ 130.6) and to C10 (C═O, δ 208.8) indicated the position of -COOH moiety at C2. Additional COSY (**Figure 2**) and HMBC correlations led to the complete assignment of all ^{1}H and ^{13}C NMR signals (**Table 1**). HRFABMS and FABMS analysis showed peaks at 293.2116 [M + H$^+$] and 585 [2M + H$^+$], respectively, which confirmed the molecular formula as $C_{18}H_{28}O_3$, whereas peaks at *m*/*z* 69 and 165 in the FAB mass spectrum (positive ion mode) were assigned to the isopentenyl (C_5H_9) moiety and to the ion resulting from the loss of isopentenyl and carboxyl moieties [M − C_5H_9 − COOH]$^+$, respectively. Therefore, the spectral data gave conclusive support to the establishment of the structure of the new compound **8** as 2-(4′-methylpentenyl-3)-10-oxoundecan-2,6-dienoic acid.

ω-Arylalkanoic acids **9a**−**c** were identified on the basis of their UV, ^{1}H and ^{13}C NMR, and FABMS spectra and are probably biosynthetic precursors of γ-lactones known as jurunolides, which occur widely in *Iryanthera* species (*7*, *23*). Compound **10** was identified from its spectroscopic data and by comparison with literature values as guaiacin, an aryltetralin lignan, previously isolated from *Guaiacum officinale* (Zygophyllaceae) (*24*, *25*).

Flavonoids are by far the most investigated class of natural products regarding antioxidant properties. The aromatic -OH

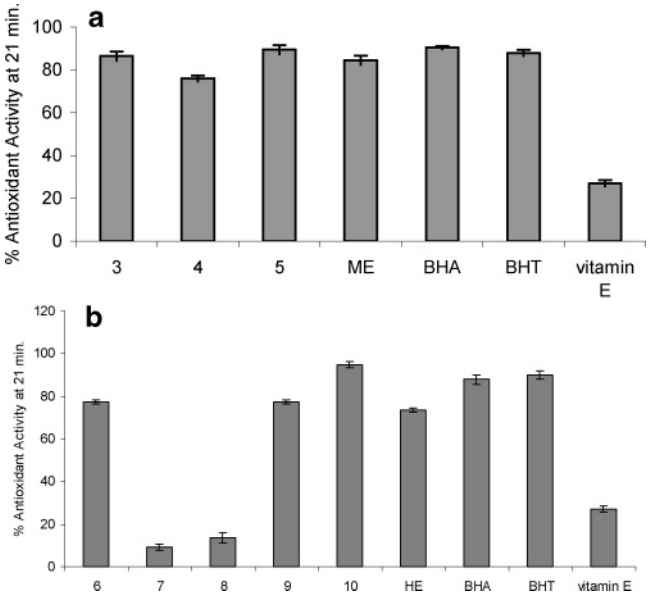


Figure 3. (**a**, top panel) Antioxidant activities of flavones **3**–**5** at 10 ppm and MeOH extract of leaves (ME) at 50 ppm. (**b**, bottom panel) Compounds **6**–**10** at 100 ppm and hexane extract of seeds (HE) at 250 ppm. Positive controls BHA, BHT, and α-tocopherol (vitamin E) were tested at final concentrations of 1.9, 2.2, and 4.3 ppm, respectively. Vertical bars represent the standard deviation of duplicate experiments.

groups and the C2−C3 double bond are some of the features of flavonoids important for their antioxidant activity (*26*). Flavones **3**−**5** showed lipoperoxidation inhibition by 95% when tested at 100 ppm. At lower concentration (10 ppm), they were still highly active (**Figure 3**) and inhibited LPO by 86%, 77%, and 88%, respectively. This suggested that flavones **3**−**5** offer better protection of lipid membranes from oxidation than sargachromenol (**6**) or sargaquinoic acid (**7**).

Flavones **1**−**5** also showed moderate inhibition of COX enzymes. However, the COX-1 enzyme was inhibited the most, by 40−65%, compared to COX-2 enzyme (**Figure 4**). At 100 ppm, flavones **1** and **2** inhibited COX-2 enzyme by 31% and 51%, respectively, whereas flavones **3**−**5** showed <20% inhibition. This activity profile might be dependent on their polarity as well as the substitution pattern in ring B. For example, flavones **1** and **2** are fully methoxylated compounds, whereas compounds **3**−**5** contained free -OH groups in their structures. Moreover, the substitution pattern in ring B seemed to play a major role in determining the COX-2 enzyme activity, as the presence of a 3′,4′-dimethoxy ring B rendered the highest enzyme inhibitory activity among flavones **1**−**5** tested. Also, lack of a methoxy group in ring B led to a reduction in the activity as shown by flavone **5**. The presence of either a 7-OMe or 7-OH group in ring A seemed to be of lesser impact on COX-2 inhibition as shown by compounds **3** and **4**, which showed similar activities.

In previous studies, sargachromenol (**6**) was shown to possess antioxidant and antiinflammatory properties that were assessed by electrochemical studies, by reaction with the stable free radical DPPH, and by inhibition of TPA-induced mouse ear edema (*7*, *27*). In this paper, we present additional support of the biological activity of *I. juruensis* by demonstrating the ability of its isolates to inhibit LPO (76% at 100 ppm) (**Figure 3**) and COX-1 (98%) and COX-2 (84%) enzymes (**Figure 4**). In contrast, plastoquinone **7** exhibited poor inhibitory activities of LPO and COX-1 and -2 enzymes. This was probably due to

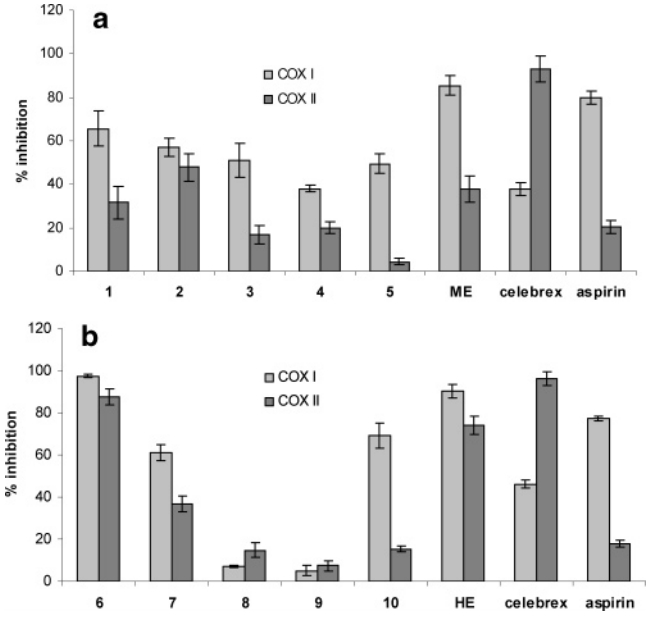


Figure 4. (**a**, top panel) In vitro COX-1 and COX-2 inhibitory activities of flavones **1**–**5** (100 ppm) and MeOH extract of leaves (ME, 250 ppm). (**b**, bottom panel) Compounds **6**–**10** (100 ppm) and hexane extract of seeds (HE, 250 ppm). Positive controls Celebrex and aspirin were tested at 7 and 180 ppm, respectively. Varying concentrations of the standards were used in order to obtain inhibition at around 50%. Vertical bars represent the standard deviation of two independent experiments.

the lack of aromatic hydroxyls in its molecule and/or its oxidizing features associated with the quinone moiety. Poor inhibitory activities toward COX-1 and -2 enzymes were also observed for compounds **8** and **9**. In contrast, arylalkanoic acid **9** inhibited LPO by 79%, whereas compound **8** showed much lower ability to protect liposomes from oxidation (**Figure 3b**). Guaiacin (**10**) presented the highest LPO inhibitory activity (>95% at 100 ppm) among the tested compounds from *I. juruensis* fruits. This was probably due to the presence of two veratryl moieties in its structure, which may increase the hydrogen radical donating ability of the aromatic hydroxyl groups. It also strongly inhibited COX-1 (75% at 100 ppm) enzyme but showed weak activity toward COX-2 (<20% at 100 ppm).

The findings of our research on *I. juruensis* seeds and leaves have clearly shown that the plant parts studied possessed LPO inhibitory activity and were in agreement with the anecdotal reports. In addition, the moderate inhibition of COX-2 enzyme by extracts from seeds and leaves and the isolates from them further suggested the potential health beneficial effects of this plant and validated the ethno uses of *I. juruensis* decoction by Amazon Indians.

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