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Ursolic acid analogues: non-phenolic functional food components in Jamaican raspberry fruits

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ABSTRACT

The *Rubus* genus produces numerous species that are known for their medicinal properties. *Rubus rosifolius*, called the red raspberry, grows wild in elevated regions in Jamaica. Phytochemical examination of the ethyl acetate extract of the fruit yielded eight compounds of the 19-α-hydroxyursane type: euscaphic acid (**1**), 1-β-hydroxyeuscaphic acid (**2**), hyptatic acid B (**3**), 19α-hydroxyasiatic acid (**4**), trachelosperogenin (**5**), 4-epi-nigaichigoside F1 (**6**), nigaichigoside F1 (**7**), and trachelosperoside B-1 (**8**), as confirmed by NMR spectroscopy. Inhibition of cell proliferation by these compounds were determined by using MCF-7 (breast), SF-268 (CNS), NCI H460 (lung), HCT-116 (colon) and AGS (gastric) human tumour cells. Among the human tumour cell lines assayed, only compounds **3** and **6** displayed significant growth inhibition and was specific to colon tumour cells by 56% and 40%, respectively. These ursolic acid analogues were also tested for anti-inflammatory activity using in vitro cycloxegenase-1 (COX-1) and cycloxegenase-2 (COX-2) enzyme inhibitory assays. Compounds **1**, **2** and **3** showed selective COX-1 enzyme inhibitory activity (13%, 25% and 35%) at 25 μg/ml. In the lipid peroxidation (LPO) inhibitory assays, compounds **2**, **4**, **7** and **6** inhibited LPO by 62%, 60%, 53% and 68%, respectively, at 25 μg/ml.

1. Introduction

Fruits and vegetables are an excellent source of diverse non-nutrient phytochemicals, many of which possess favourable qualities such as the ability to act as cellular antioxidants or as anti-inflammatory agents (Deighton, Brennan, Finn, & Davies, 2000; Seeram, Momin, Nair & Bourquin, 2001). *Rubus* is a diverse plant genus comprised of 250 species. The plants have been traditionally employed in the treatment of several diseases, particularly diabetes (Kanegusuku et al., 2007). The berries are rich in vitamins A, B and C, while the leaves and roots have been used in various medicinal applications (Byamukama, Kiremire, Andersen, & Steigen, 2005; Deighton et al., 2000). *Rubus rosifolius* (Rosaceae) is a red raspberry that is native to Eastern China and is distributed in the Caribbean, Hawaii, Australia and Asia (Adams, 1972). Also called the West Indian raspberry, it is one of the many fruit-bearing plant species in Jamaica that is under utilized, being known and eaten by only a minority of the populace. Much of the work done on the *Rubus* genus to date has been focused on either the anthocyanin content of the fruits or the phytochemistry of the aerial parts (Byamukama et al., 2005; Deighton et al., 2000; Ohtani et al., 1990; Zhou, 1992). This is in contrast to the little research that has been undertaken on the phytochemistry of the fruits. Of the compounds isolated from this genus, triterpenoids of the ursane and oleanane types are among those reported (Ohtani et al., 1990; Zhou, 1992). The methanolic extract of the aerial parts of *R. rosifolius* was shown to possess strong analgesic properties, the active principle being identified as 28-methoxytormentic acid (Kanegusuku et al., 2007). Several sesquiterpenoids including β-caryophyllene, humulene, bicyclygermacrene, and rosifoliol, the latter of which was first isolated from *R. rosifolius*, were isolated from the essential oil Southwell, 1978; Southwell & Tucker, 1996). In the present study, we conducted phytochemical research of the fruits of the plant. Herein we report the isolation of eight 19α-hydroxyursolic acid analogues and their COX-1 and COX-2 enzyme, lipid peroxidation and tumour cell proliferation inhibitory activities. This is the first report on the terpenoidal constituents in *R. rosifolius* fruits, and of their bioactivity.

2. Materials and methods

2.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on an INOVA Varian VRX 500 MHz instrument using CD_3OD. Chemical shifts are in δ (parts per million) relative to δ 3.30 and 49.0 for ^{1}H and ^{13}C NMR, respectively. Merck Silica gel 60 (35–70 μm particle size) was used for medium pressure liquid chromatography (MPLC). The Preparative

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Thin Layer Chromatography (PTLC) plates (20 × 20, 500 μm and 250 μm) were purchased from Analtech, Inc. (Newark, DE). ACS grade solvents were used for isolation and purification of compounds. The COX-1 enzyme was prepared from ram seminal vesicles purchased from Oxford Biomedical Research, Inc. (Oxford, MI). COX-2 enzyme was prepared from insect cells cloned with human PGHS-2 (prostaglandin endoperoxide H synthase-2) enzyme. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), Aspirin, and 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma–Aldrich Chemical Company Co. (St. Louis, MO). Vioxx®, Celebrex™ were provided by Dr. S. Gupta, Sparrow Hospital, MI. 1-Stearoyl 2-linoleoyl *sn*-glycerol 3-phosphocholine (SLPC) was purchased from Avanti Polar Lipids (Alabaster, AL). The fluorescent probe, 3-[*p*-(6-phenyl)-1,3,5-hexatrienyl]-phenylpropionic acid was purchased from Molecular Probes (Eugene, OR).

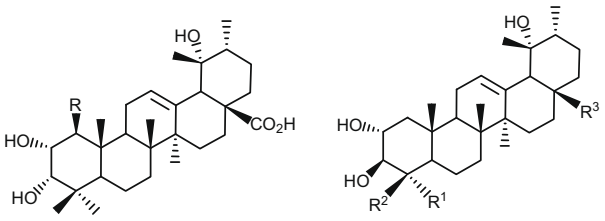
Foetal bovine serum (FBS) and Roswell Park Memorial Institute 1640 (RPMI-1640) medium were purchased from Gibco BRL (Grand Island, NY). Human tumour cell lines SF-268 (Central Nervous System, CNS), NCI H460 (lung), and MCF-7 (breast) were purchased from the National Cancer Institute (NCI, Bethesda, MD). AGS (gastric) and HCT-116 (colon) were purchased from American Type Culture Collection (ATCC, Rockville, MD) and archived in the Bioactive and Natural Products Laboratory at Michigan State University. All cell lines were maintained in a humidified chamber at 37 °C with 5% CO_2 in RPMI-1640 medium containing penicillin–streptomycin (10 units/ml and 10 μg/ml, respectively) and 10% foetal bovine serum (FBS).

2.2. Extraction and isolation

The ripe berries (1.368 kg) were lyophilised, powdered and (171 g) and extracted successively with n-hexane (750 + 550 + 550 ml), EtOAc (550 × 3 ml) and MeOH (750 × 3 + 250 ml). Like fractions were filtered, pooled, and concentrated en vacuo to give 1.6, 4.5 and 71.8 g of extracts, respectively. A portion of the EtOAc extract (1.82 g) was subjected to silica MPLC using $CHCl_3$:MeOH mixtures of increasing polarity (20:1 $CHCl_3$:MeOH to 100% MeOH) as the mobile phase. A total of fourteen fractions were thus obtained. Fraction III (80 mg) was purified by silica PTLC (500 μm) using EtOAc:n-hexane (6.5:3.5) as the mobile phase to yield compound **1** (17.9 mg). The same solvent system was used in the isolation of compound **2** (10.8 mg) from fraction IV (70 mg) by PTLC. Precipitation of fraction VII with $CHCl_3$:n-hexane (7:3) gave compound **3** (25.5 mg). Successive trituration of fraction IX with MeOH gave the MeOH-soluble compound, **4** (29.7 mg). Fraction XII (310 mg) was dissolved in MeOH and centrifuged. The dried supernatant (170 mg) was subjected to PTLC using 4:1 $CHCl_3$:MeOH as the mobile phase. The second band obtained yielded 37.2 mg after elution with MeOH. Subsequent purification by PTLC (250 μm, 4:1 $CHCl_3$:MeOH) afforded compound **5** (3 mg). The eluent of the third band (8.1 mg) was similarly treated and thus gave compound **6** (18.9 mg). Compound **7** (9.7 mg) was obtained after PTLC (250 μm; $CHCl_3$:MeOH (5:1 then 4:1) of fraction XIII. MPLC ($CHCl_3$:MeOH, 8:1–0:1) of fraction XIV (150.8 g) and successive PTLC with 3:1 $CHCl_3$:MeOH as the mobile phase led to the isolation of compound **8** (2.6 mg).

2.3. Compounds *1–8*

Compounds **1**–**8** (Fig. 1) were identified as the known triterpenoids euscafic acid (**1**, 0.032%), 1β-hydroxyeuscaphic acid (**2**, 0.019%), hyptatic acid B (**3**, 0.046%), 19α-hydroxyasiatic acid (**4**, 0.053%), trachelosperogenin (**5**, 0.054%), 4-epi-nigaichigoside F1 (**6**, 0.034%), nigaichigoside F1 (**7**, 0.017%), and trachelosperoside B-1 (**8**, 0.005%). The structures of all the compounds were confirmed further by comparison of spectral data (Tables 1 and 2) with those of reported in literature.



1 R = H

2 R = OH

	R^1	R^2	R^3
3	H	CH_2OH	H
4	CH_2OH	H	H
5	CH_2OH	CH_2OH	H
6	H	CH_2OH	Glc
7	CH_2OH	H	Glc
8	CH_2OH	CH_2OH	Glc

Fig. 1. Structures of compounds **1**–**8**.

2.4. Lipid peroxidation inhibitory assay

The lipid peroxidation inhibitory activity of the samples was determined by using a previously reported method (Jayaprakasam, Zhang, & Nair, 2004). The assay was conducted in a buffer consisting of HEPES (100 μl), NaCl (200 μl), N_2-sparged water (1.64 ml), test sample in DMSO (20 μl) and large unilamellar vesicle (LUV; 20 μl) suspension. The peroxidation was initiated by the addition of $FeCl_2$ (20 μl, 0.5 mM) solution and was monitored by observing the fluorescence at 3 min intervals from 0 to 21 min using a Turner model 450 digital fluorometer (Barnestead Thermolyne, Dubuque, IA) at 384 nm. The decrease of relative fluorescence intensity with time indicated the rate of lipid peroxidation. The compounds isolated were tested at 25 μg/ml. The antioxidant standards BHA, BHT and TBHQ were tested at 1 μg/ml, respectively.

2.5. COX enzyme inhibitory assay

COX-1 and -2 enzyme inhibitory activities were assessed by monitoring the initial rate of O_2 uptake by using an oxygen electrode (Instech Laboratories, Plymouth Meetings, PA) attached to a YSI model 5300 biological oxygen monitor (Yellow springs Instrument, Inc., Yellow Springs, OH) at 37 °C. The assay was conducted in accordance to the previously published procedure (Wang et al., 2000). The test samples and controls were dissolved in DMSO. Each assay mixture contained Tris buffer (0.6 ml, 0.1 M, pH 7), phenol (1 mM), haemoglobin (85 μg), and DMSO or test sample (10 μl). COX-1 or COX-2 enzyme (20–25 μl) was added to the chamber and incubated for 2 min. The reaction was initiated by the addition of arachidonic acid (10 μl of a 1 mg/ml solution). The analysis was done in duplicate for each sample. The data were recorded using QuickLog for windows data acquisition and control software (Strawberry Tree, Inc., Sunnyvale, CA). Compounds **1**–**8** were tested at 25 μg/ml and the positive controls, Aspirin, Celebrex™ and Vioxx® were respectively tested at 60 μM, 26 and 32 nM. The varying concentrations of positive controls used were to yield a 50–100% COX enzyme inhibitory activity by the non-steroidal anti-inflammatory agents (NSAIDs).

Table 1
^{1}H NMR spectral data for compounds **1-8** (CD_3OD, 500 MHz).[a]

H	1	2	3	4	5	6	7	8
H-1		3.45 (d, 10)						
H-2	3.92 (td, 3.5, 11.0)	3.63 (dd, 3.0, 3.95)	3.77 (ddd, 4.0, 9.5, 11.5)	3.68 (ddd, 4.5, 10.0, 12.0)	3.84 (ddd, 4.5, 9.5, 11.5)	3.77 (ddd, 4.0, 9.5, 11.5)	3.68 (ddd, 4.5, 10.0, 12.0)	3.84 (ddd, 4.0, 10.0, 11.0)
H-3	3.32 (d, 2.0)	3.39 (d, 3.0)	3.05 (d, 9.5)	3.35 (d, 10.0)	3.46 (d, 9.5)	3.05 (d, 9.5)	3.35 (d, 10.0)	3.45 (d, 10.0)
H-12	5.28 (bs)	5.34 (t, 3.0)	5.28 (t, 3.5)	5.29 (t, 3.5)	5.28 (t, 3.0)	5.29 (t, 3.5)	5.30 (t, 3.5)	5.28 (t, 3.0)
H-18	2.53 (s)	2.54 (s)	2.50 (s)	2.49 (s)	5.52 (s)	2.51 (s)	2.51 (s)	2.51 (s)
H-23α				3.26 (d, 11.0)	4.05 (d, 11.5)		3.26 (d, 11.0)	4.04 (d, 11.5)
H-23β				3.50 (d, 11.0)	3.50 (d, 12.0)		3.49 (d, 11.0)	3.49 (d, 12.0)
23-Me	0.99 (s)	0.96 (s)	1.23 (s)			1.22 (s)		
H-24α			4.02 (d, 11.5)		3.62 (d, 11.5)	4.03 (d, 10.0)		3.61 (d, 11.5)
H-24β			3.38 (d, 11.0)		3.62 (d, 11.5)	3.37 (d, 9.5)		3.61 (d, 11.5)
24-Me	0.86 (s)	0.87 (s)		0.69 (s)			0.69 (s)	
Me-25	0.99 (s)	1.0 (s)	0.98 (s)	1.02 (s)	1.01 (s)	0.98 (s)	1.03 (s)	1.02 (s)
Me-26	0.80 (s)	0.82 (s)	0.77 (s)	0.79 (s)	0.79 (s)	0.75 (s)	0.77 (s)	0.75 (s)
Me-27	1.33 (s)	1.32 (s)	1.33 (s)	1.34 (s)	1.33 (s)	1.32 (s)	1.33 (s)	1.33 (s)
Me-29	1.18 (s)	1.18 (s)	1.18 (s)	1.19 (s)	1.19 (s)	1.19 (s)	1.20 (s)	1.19 (s)
Me-30	0.92 (d, 7.0)	0.92 (d, 6.5)	0.92 (d, 7.0)	0.92 (d, 6.5)	0.92 (d, 7.0)	0.92 (d, 7.0)	0.92 (d, 7.0)	0.92 (d, 7.0)
H-1′						5.30 (d, 8.5)	5.31 (d, 8.0)	5.31 (d, 8.0)

[a] Values are in ppm (δ). Coupling constants (J, Hz) are in parentheses.

Table 2
^{13}C NMR spectral data for compounds **1–8** in (CD_3OD, 125 MHz).[a]

C	1	2	3	4	5	6	7	8
1	42.5	81.3	47.9	47.9	47.7	47.8	48	47.8
2	67.2	71.8	69.6	69.7	69.9	69.6	69.7	69.9
3	80.1	80.7	86.0	78.3	79.5	86.0	78.3	79.5
4	39.5	38.9	44.4	44.1	48.2	44.4	44.1	48.2
5	49.3	49.0	57.2	48.5	48.8	57.2	48.5	48.7
6	19.3	19.5	20.0	19.2	19.7	19.9	19.2	19.7
7	34.1	34.3	34.4	33.5	33.9	34.4	33.5	33.9
8	41.3	41.8	41.3	41.1	41.1	41.1	41.2	41.2
9	48.3	49.3	48.7	48.2	48.3	48.7	48.2	48.3
10	39.4	44.4	39.0	39.0	39.2	39.0	39.0	38.8
11	24.7	28.3	25.0	24.7	25.0	25.0	24.8	25.0
12	129.1	130.3	129.1	129.2	128.9	129.1	129.5	129.4
13	140.4	139.3	139.7	140.1	140.4	140.1	139.8	139.7
14	42.7	42.6	42.7	42.7	42.8	42.6	42.8	42.7
15	29.7	29.9	29.6	29.6	29.7	29.6	29.6	29.6
16	27.4	26.9	27.2	27.3	27.4	27.3	27.2	27.2
17	49.2	49.3	48.7	49.5	49.5	49.3	49.5	49.5
18	55.3	55.3	54.9	55.1	55.2	55.1	55.0	55.0
19	73.8	73.9	73.6	73.6	73.6	73.6	73.6	73.6
20	43.1	39.3	42.9	43.1	43.1	43.1	42.9	42.9
21	26.8	27.5	26.5	26.6	26.7	26.6	26.5	26.5
22	39.2	39.3	38.2	39.0	38.8	39.0	38.3	38.3
23	29.2	29.1	23.8	66.4	64.5	23.8	66.3	64.5
24	16.9	22.4	66.2	13.9	62.7	66.2	13.9	62.7
25	16.9	12.9	17.6	17.53	17.4	17.5	17.7	17.5
26	17.7	18.0	17.4	17.51	17.4	17.5	17.6	17.5
27	24.9	25.0	24.6	24.9	24.8	24.8	24.7	24.7
28	184.2	184.2	182.3	182.3	182.4	178.5	178.5	178.6
29	27.2	27.2	27.1	27.1	27.1	27.1	27.1	27.1
30	16.7	16.7	16.8	16.6	16.6	16.6	16.6	16.6
1′						95.8	95.8	95.8
2′						73.9	73.8	73.9
3′						78.3	78.3	78.3
4′						71.1	71.1	71.1
5′						78.6	78.6	78.6
6′						62.4	62.4	62.4

[a] Values are in ppm (δ).

2.6. Tumour cell inhibition assay

The assay was performed according to a previously published method (Vareed, Reddy, Schutzki, & Nair, 2006). MCF-7 (breast), SF-268 (CNS), NCI H460 (lung), HCT-116 (colon) and AGS (gastric) human tumour cells were cultured in RPMI-1640 medium containing penicillin–streptomycin (10 units/ml for penicillin and 10 μg/ml for streptomycin) and 10% foetal bovine serum (FBS). The cells were transferred to 96-well microlitre plates and incubated for 24 h, after which time 100 μL of the assay samples of known concentration were added to each well containing the appropriate tumour cells and further incubated for 48 h. Subsequently, an aliquot of MTT solution ((25 μl, 5 mg/ml phosphate-buffered saline solution) was added and the plates were wrapped with aluminium foil and incubated for 3 h at 37 °C. The medium was then removed from each well and DMSO (200 μl) was added. The plates were then shaken and optical density was measured at 570 nm using a microplate reader. Compounds **1–8** were assayed at 100 μg/ml. Adriamycin was used as positive control in this study. Triplicate analyses were performed for each sample.

3. Results and discussion

3.1. Characterization of compounds *1–8*

The structures of compounds **1–8** were elucidated by NMR spectral data (Tables 1 and 2) and identified as euscaphic acid (**1**) (Guang-Yi, Gray, & Waterman, 1989; Mimaki et al., 2001), 1β-hydroxyeuscaphic acid (**2**), (Costa & Carvalho, 2002), hyptatic acid B (**3**), (Yamagishi et al., 1988; Zhou et al., 1992), 19α-hydroxyasiatic acid (**4**), (Houghton & Lian, 1986), trachelosperogenin (**5**), (Abe & Yamauchi, 1987a, 1987b), 4-epi-nigaichigoside F1 (**6**), (Zhou et al., 1992), nigaichigoside F1 (**7**), (Shigenaga, Kouno, & Kawano, 1985) and trachelosperoside B-1 (**8**), (Abe & Yamauchi, 1987a, 1987b). This represents the first report of the isolation of compounds **1–8**

from *R. rosifolius*, and of compound **2** from the *Rubus* genus. The compounds isolated were all of the 19-α-hydroxyursane type. Thin layer chromatographic comparison of the EtOAc extract of *R. rosifolius* with those of other *Rubus* species found in Jamaica (*R. jamaicensis* and *R. race*mosus) resulted in similar profiles for the fruits, indicating that some of these compounds are also present in varying degrees in the other two species investigated. This type of triterpenoids is apparently characteristic of the *Rubus* genus (Ohtani et al., 1990; Zhou et al., 1992). Among the distinctive features of the ^{1}H NMR spectra of these compounds are a doublet at δ 0.9 for the methyl group at position 30, a broad singlet at δ 2.5 (H-18), a *ddd* signal resonating at approximately δ 3.8 for H-2 of compounds **3**–**8**, and an olefinic triplet (H-12) at δ 5.3 (Table 1). Similarly, the characteristic features of the ^{13}C NMR spectra are the methane signal at δ 55 (C-18), the olefinic methylene and quaternary carbon atoms resonating at δ 129 and 140 and the carboxyl carbon at δ 182 in the aglycones, which is shifted upfield to $\sim\delta$ 179 in the glycosides (Table 2). The structures were confirmed further using gradient heteronuclear muiltiple bond coherence (gHMBC) and gradient heteronuclear multiple quantum coherence (gHMQC) spectral data. Both ^{1}H- and ^{13}C-NMR spectra of these compounds are scattered in the literature and hence Tables 1 and 2 provide a side-by-side comparison of the chemical shifts of the isolated ursolic acid analogues from *R. rosifolius* fruits.

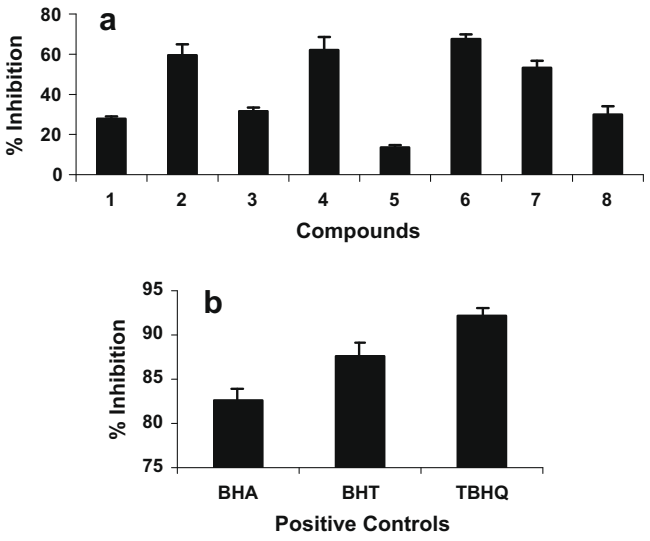


Fig. 2. Cyclooxygenase enzyme inhibitory activities of triterpenoids **1-8** at 25 μg/ml (a) and positive controls Aspirin, Celebrex™ and Vioxx® at 60 μM, 26 and 32 nM respectively (b). Vertical bars represent ±SD for each data point (n = 2). Varying concentrations of positive controls were used in order to obtain inhibition values between 50% and 100%.

3.2. LPO inhibitory assay

The reaction of free radicals with lipids, proteins, and nucleic acids and other cellular molecules result in oxidative damage which is implicated in the aetiology of a number of diseases including cancer and cardiovascular disease and arthritis (Deighton et al., 2000. The LPO assay is a good model for cellular oxidation. Phosphatidylcholines are a major component of biological membranes. The lipid used in our experiments was a phosphatidylcholine containing oleyl and stearoyl side chains. It was used to prepare large unilamellar vesicles (liposomes) that were tagged with DPPH-PA, which served as a fluorescent probe. Peroxidation of the lipid was initiated by the addition of ferrous ions that resulted in the formation of oxygen radicals, which in turn caused peroxidation of the lipid as well as the probe. The latter resulted in loss of fluorescence, which is directly related to the oxidative destruction of the lipid. If the test compound can be oxidised in place of the lipid, it will slow down or prevent lipid peroxidation, which would demonstrate its antioxidant activity. The potential of the eight compounds to inhibit lipid peroxidation was tested at a concentration of 25 μg/ml (Fig. 2). Except for compound **5** which showed negligible activity, all of the compounds exhibited LPO inhibitory activity. Compound **6**, which inhibited LPO by 68%, had the highest ability to inhibit lipid peroxidation. Compounds **4** and **2** followed, showing 60% and 62% inhibitory activity, respectively. An intermediate level of activity (53%) was shown by compound **7**. These compounds show comparative LPO inhibitory activity to β-carotene (73% at 100 μg/ml) and kaempferol-3-*O*-glucuside (61% at 23 μg/ml), which are antioxidants naturally found in food (Reddy, Alexander-Lindo, & Nair, 2005, and Vareed, Reddy, Schutzki, & Nair, 2007). Compounds **1**, **3** and **8** showed similar activity, ranging from 28% (compound **1**) to 32% (compound **3**). Compound **5** did not protect the liposome from peroxidatory degradation, exhibiting similar activity (14%) to that of the reagent control. Compounds **6** and **8** were twice as potent as the corresponding aglycones **3** and **5**. This may be due in part to increased solubility of the glycosides in the assay buffer compared to their counterparts. The same was however not true for compound **7**, which showed slightly lower activity than its aglycone, compound **4**.

3.3. COX-1 and -2 enzyme inhibitory assay

The cyclooxygenase enzyme catalyses the conversion of arachidonic acid to prostanoids including prostaglandins. Inhibition of both COX-1 and COX-2 isozymes can result in alleviation of symptoms of inflammation and pain. The COX-2 isozyme is over expressed in several pathological conditions such as diabetes, Alzheimer's disease and cancer. Studies have shown that its selective inhibition is correlated with delayed onset or reduced progression of disease (Jayaprakasam et al., 2004). Our tests for anti-inflammatory activity were done using COX-1 and COX-2 isozymes. Compounds **1**–**8** were assayed at 25 μg/ml. Varying concentrations of positive controls were used in this assay (as well as in the LPO inhibitory assay) in order to obtain inhibition values between 50% and 100% for each standard. Low cyclooxygenase inhibitory activity was demonstrated by compounds **1**–**8** at 25 μg/ml (Fig. 3). Compound **3,** which showed the highest COX-1 inhibitory activity (35%) did so in a selective manner at the test concentration. Compounds **1** and **2** were also COX-1 enzyme selective (13% and 25%). These two compounds were previously reported to possess marked anti-inflammatory activity (Banno et al., 2005). Very similar COX inhibitory activity was demonstrated by compounds **6** and **8**, which showed moderate selectivity against the COX-1 enzyme. Compound **7**, which like most of the other compounds tested showed less than 15% COX enzyme inhibition at 25 μg/ml, selectively inhibited the COX-2 enzyme. Compound **5** was slightly more effective at inhibiting the COX-2 enzyme than it was for its counterpart.

3.4. Tumour cell proliferation inhibition assay

The ursolic acid analogues isolated were studied at 100 μg/ml for their ability to inhibit the proliferation of five types of human carcinoma cells: colon (HCT-116), breast (MCF-7), lung (NCI-H460), and central nervous system (SF-268). Considering that the compounds did not demonstrate high COX-2 inhibitory activity, it was not expected that they would possess high potential for tumour cell growth inhibition. Compounds **3** and **5** inhibited colon tumour cells by 56% and 40%, respectively. Gastric tumour cell

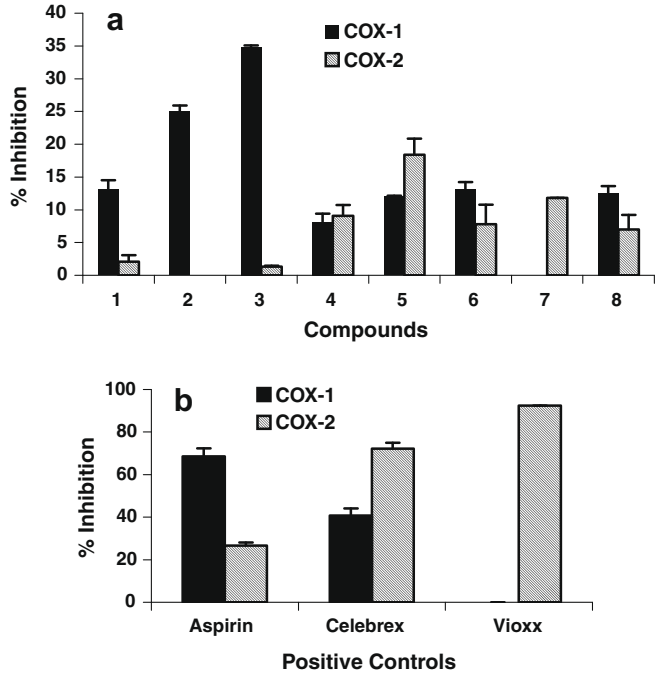


Fig. 3. Lipid peroxidation inhibitory activities of triterpenoids **1-8** at 25 μg/ml (a). The positive controls BHA, BHT and TBHQ were tested at 1 μg/ml (b). The percentage inhibition was the fluorescence intensity at 21 min relative to the DMSO control. Vertical bars represent ±SD for each data point ($n = 2$). A lower concentration of positive controls was used in order to obtain inhibition values between 50% and 100%.

growth was both arrested by 14% by compounds **4** and **6.** Central nervous system and lung tumour cells were respectively inhibited by 13% and 14% by compounds **5** and **6**. It was shown in previous studies that compound **1** is a good inhibitor of tumour growth in mice, while compound **2** demonstrated inhibitory activity towards human oral squamous cell carcinoma (HSC-2) cells (Banno et al., 2005; Mimaki et al., 2001).

4. Conclusion

Terpenoids are ubiquitous plant constituents that are generally considered non-toxic. There are several reports that demonstrate the favourable biological properties of members of this class of compounds (Banno et al., 2005; Kashiwada et al., 1998; Kanegusuku et al., 2007; Jayaprakasam et al., 2004). Of the eight triterpenoids isolated from *R. rosifolius* fruits, four showed over 50% LPO inhibitory activity at 25 μg/ml, indicated their good antioxidant potential. In vitro COX enzyme inhibitory assay results revealed that they also possess anti-inflammatory properties, albeit low. Two of the compounds were effective at arresting the growth of colon tumour cells. These results demonstrate that the triterpenoids in raspberries is another group of phytochemicals, in addition to phenolics compounds, that have the potential to improve health.

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