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Original Article

Anthocyanin content, antioxidant, anti-inflammatory and anticancer properties of blackberry and raspberry fruits

Camille S. Bowen-Forbes [a,b], Yanjun Zhang [c], Muraleedharan G. Nair [b,*]

[a] Department of Chemistry, The University of the West Indies, Mona, Kingston, Jamaica
[b] Bioactive Natural Products and Phytoceuticals, Department of Horticulture and National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI, USA
[c] UCLA Center for Human Nutrition, David Geffen School of Medicine, Los Angeles, CA, USA

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ABSTRACT

Fresh or processed berries are considered to be beneficial for health by many consumers. Fruits of closely related species of plants sometimes possess strikingly different phytochemistry and biological activities. Therefore, even though similar research has been conducted on a number of *Rubus* berries, there is much relevance associated with the investigation of species that have not been previously studied. In the current report, the fruits of three wild Jamaica-grown species: *Rubus jamaicensis*, *Rubus rosifolius* and *Rubus racemosus*, and of the Michigan-grown *Rubus acuminatus*, *Rubus idaeus* cv. Heritage and *Rubus idaeus* cv. Golden were analyzed for their anthocyanin contents, and lipid peroxidation, cyclooxygenase enzyme and human tumor cell proliferation inhibitory activities. It was revealed that the fruits contained superior levels of anthocyanins (146–2199 mg/100 g fresh weight) to those previously reported for other raspberry and blackberry species, and their hexane, EtOAc and MeOH extracts showed good antioxidant activity, the majority of the extracts exhibiting over 50% lipid peroxidation inhibitory activity at 50 μg/mL. The hexane extracts of the Jamaican *Rubus* spp. demonstrated moderate COX inhibitory activity (27.5–33.1%) at 100 μg/mL, and exhibited the greatest potential to inhibit cancer cell growth, inhibiting colon, breast, lung, and gastric human tumor cells by 50, 24, 54 and 37%, respectively. The high anthocyanin content and biological activities of these fruits indicate that their consumption would be beneficial to health, and that they may be useful in the production of functional foods containing an efficacious dose of anthocyanins.

1. Introduction

Blackberries, raspberries and other small fruits are an excellent source of natural antioxidants, which is one of the major reasons for their increasing popularity in the human diet (Moyer et al., 2002; Mylnikov et al., 2005; Pantelidis et al., 2007; Seeram et al., 2006). Most of these fruits belong to the diverse *Rubus* genus, which consists of 250 species. Many *Rubus* fruits are consumed fresh or as processed products such as jams, jellies, syrups and wines. The leaves and roots have been used in various medicinal applications (Byamukama et al., 2005).

The anthocyanins present in blackberries and raspberries are important for the beneficial health effects associated with their antioxidant, anti-inflammatory, and chemopreventative properties; the biological activity of black raspberry against esophageal, colon, and oral cancers has been demonstrated (Tulio et al., 2008). It has long been established that cyanidin-3-glucoside and cyanidin-3-rutinoside are the respective major and minor anthocyanins in blackberries (Fan-Chiang and Wrolstad, 2005). It is also known that cyanidin-3-sambubioside, cyanidin-3-glucoside, cyanidin-3-xylosylrutinoside and cyanidin-3-rutinoside are commonly found in black raspberries.

In addition to anthocyanins, these fruits are also a rich natural source of other chemopreventative phytochemicals such as flavonols, phenolic acids, ellagic acid, vitamins C and E, folic acid and β-sitosterol. It is not uncommon for fruits or any other plant material of related species to demonstrate varying degrees of biological activities ranging from some species being totally inactive to others exhibiting exceptional activity (Boivin et al., 2007).

Keeping these factors in mind and in our continued quest to find new edible plants with functional properties, we undertook chemical and biological research on three species of wild Jamaican *Rubus* berries that have not previously been studied: *R. jamaicensis* (blackberry), *R. rosifolius* (red raspberry), and *R. racemosus* (black raspberry). Their anthocyanin contents and antioxidant, anti-inflammatory and anticancer activities were investigated. We also compared them with three Michigan-grown species, namely *R.*

* Corresponding author. Tel.: +1 517 884 2018; fax: +1 517 432 2310.
E-mail address: nairm@msu.edu (M.G. Nair).

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acuminatus (black raspberry), and the Heritage and Golden cultivars of *R. idaeus* (red and yellow raspberry, respectively). *R. acuminatus* is being investigated for its anthocyanin and biological properties for the first time here.

2. Materials and methods

2.1. General experimental

Fluorescence measurements were performed on a Turner model 450 digital fluorometer (Barnestead Thermolyne, Dubuque, IA) set at 384 nm. COX data were recorded using QuickLog for windows data acquisition and control software (Strawberry Tree, Inc., Sunnyvale, CA). ACS grade solvents were used for isolation and purification of anthocyanins. COX-1 enzyme was prepared from ram seminal vesicles purchased from Oxford Biomedical Research, Inc. (Oxford, MI), and COX-2 enzyme was prepared from insect cells cloned with human 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. Subash, 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). Fetal bovine serum (FBS) and Roswell Park Memorial Institute 1640 (RPMI-1640) medium were purchased from Gibco BRL (Grand Island, NY). Human tumor 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.

2.2. Plant material

Ripe fruits of *Rubus jamaicensis*, *Rubus rosifolius* and *Rubus racemosus* were collected from various locations in Jamaica from April to December 2007. *Rubus jamaicensis* fruits were collected from St. Catherine in May–June, *Rubus rosifolius* from St. Andrew in April–June and December, and *Rubus racemosus* from Mandeville in June–August. Ripe fruits of *R. idaeus* cv. Heritage, *R. idaeus* cv. Golden and *R. acuminatus* were collected from established orchards of the Michigan State University Horticultural Farm during August–September 2008.

2.3. Preparation of fruit extracts

Fresh fruits of *R. jamaicensis* (718 g), *R. rosifolius* (8096 g), *R. racemosus* (1027 g), *R. idaeus* cv. Heritage (132 g), *R. idaeus* cv. Golden (393 g) and *R. acuminatus* (114 g) were lyophilized to yield 158, 1011, 198, 22, 52 and 34 g of dried material, respectively. The lyophilized and powdered fruits of *R. jamaicensis* (149 g), *R. rosifolius* (171 g) and *R. racemosus* (120 g) were extracted successively with hexane (3 × 550 mL), ethyl acetate (3 × 550 mL) and methanol (3 × 750 mL + 250 mL). The lyophilized and powdered fruits of *R. idaeus* cv. Heritage (20 g), *R. idaeus* cv. Golden (20 g) and *R. acuminatus* (19.9 g) were extracted successively with hexane (3 × 80 mL), ethyl acetate (3 × 80 mL) and methanol (3 × 80 mL). Each extraction process lasted for 2 h. Like fractions were filtered, pooled, and concentrated in vacuo to respectively give hexane extracts (0.5, 1.63, 0.31, 0.08, 0.08 and 0.11 g), EtOAc extracts (0.91, 4.51, 0.44, 0.24, 0.30 and 0.15 g) and methanolic extracts (57.3, 71.8, 66.6, 9.0, 10.2 and 5.5 g).

2.4. Extraction of berries for anthocyanin quantification

The lyophilized, powered fruits of *R. jamaicensis* (105.3 mg), *R. rosifolius* (516.8 mg), *R. racemosus* (98.7 mg), *R. acuminatus* (101.4 mg) and *R. idaeus* cv. Heritage (495.2 mg) were each extracted with 5 mL of 0.08% HCl in MeOH for 2 h at room temperature with periodic vortexing. The extracts (200 μL) were passed through C-18 Sep pak cartridges and eluted with 2 mL 0.08% HCl in MeOH. An aliquot of 40 μL was analyzed by HPLC.

2.5. HPLC–MS analyses

HPLC–MS analyses were carried out using a Survey HPLC system equipped with a diode array absorbance detector and a Thermo-Finnigan LCQ Advantage MS system fitted with an Electrospray Interface (ESI). An Agilent Zorbax SB C18 column (2.1 mm × 150 mm) column was used. Solvent A consisted of 1% acetic acid in water, and solvent B was 1% acetic acid in methanol. The flow rate was 190 μL/min, and the linear gradient elution system was 95% A to 95% B over a 50 min period. The positive ion mode (*m*/*z* M+H+) was employed for the detection of anthocyanins. Preliminary analyses were carried out using full scan, data dependent MS/MS scanning from *m*/*z* 250–1000. The capillary temperature was set at 275 °C and the sheath and auxiliary gas at 45 and 0 units/min, respectively. The source voltage was 4 kV. MS/MS and fragmentation carried out with 50% energy.

2.6. Identification of anthocyanins

The anthocyanins were identified based on HPLC–MS spectral analysis of all the anthocyanins, and on comparison of the retention times of anthocyanins **1–3** with authentic samples.

2.7. Isolation of anthocyanins 4 and 5 from R. rosifolius

The methanolic extract of *R. rosifolius* (20 g) was dissolved in water (100 mL) and centrifuged. The water-soluble extract was partitioned with ethyl acetate (3 × 70 mL), and the resultant aqueous portion was partitioned with n-butanol (3 × 70 mL). The n-butanol partition was concentrated in vacuo to give a gummy, red extract (2.98 g). A portion of this extract (2.51 g) was further purified by C-18 medium pressure liquid chromatography (350 mm × 40 mm) using water:methanol solvent systems for elution (70:30 then 80:20). Two successive HPLC (Capcell-Pak C-18 column; 250 mm × 10 mm) analyses of fractions I (49.4 mg) and IV (9 mg) yielded pure anthocyanin **4** (2.1 mg) and 90.6% anthocyanin **5** (2.4 mg, containing anthocyanin **4** as the impurity). HPLC–MS spectral data confirmed their structures, the molecular ions being found as *m*/*z* 433 ($C_{21}H_{21}O_{10}$+: **4**) and *m*/*z* = 579 ($C_{27}H_{31}O_{10}$+: **5**).

2.8. Quantification of anthocyanins

The *Rubus* anthocyanins were quantified in accordance with the protocol published by our laboratory (Mulabagal et al., 2007). The mobile phase consisted of (A) 0.1% TFA:water and (B) 50.4% water/48.5% acetonitrile:1% acetic acid:0.1% TFA. The gradient elution was executed as follows: 20–40% B in 26 min, then 40–20% B in 4 min. The latter condition was then maintained for 10 min. The flow rate and injection volume were 0.8 mL/min and 40 μL. The anthocyanins were detected at 520 nm. Standard solutions of anthocyanins **1–6** in MeOH containing 0.08% HCl were prepared by serial dilutions ranging from 0.0078 to 1.0 mg/mL in concentration. Calibration curves were obtained by plotting the average peak area from three injections of each standard against concentration. Each sample was injected three times, and the average value used for the determination of anthocyanin content.

2.9. Lipid peroxidation inhibitory activity

The lipid peroxidation inhibitory activity of the samples was determined by using a previously reported method (Mulabagal et al., 2007). The decrease of relative fluorescence intensity with time indicated the rate of lipid peroxidation. Anthocyanins **4** and **5** were tested at 10 μM in H_2O, while the *Rubus* extracts were analyzed at 50 μg/mL in DMSO. The antioxidant standards BHA, BHT and TBHQ were tested at 1 μg/mL in DMSO.

2.10. 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 analysis was carried out in duplicate for each sample. All extracts were tested at 100 μg/mL, pelargonidin-3-glucoside and pelargonidin-3-rutinoside at 50 μg/mL, and the positive controls – aspirin, Celebrex™ and Vioxx® – were tested at 60 μM, 26 and 32 nM, respectively. The extracts that exhibited no activity or very low activity relative to the associated error were not included in the data. The varying concentrations of positive controls used were to yield a 50–100% COX enzyme inhibitory activity by the commercial anti-inflammatory agents.

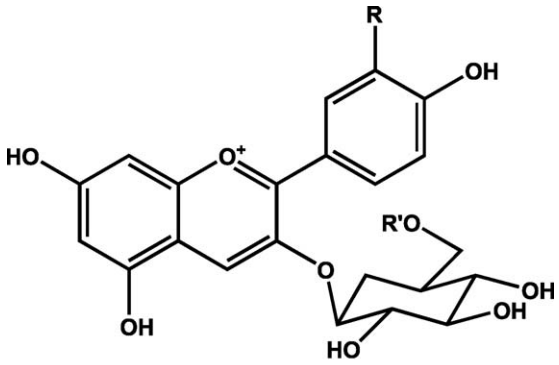
2.11. Tumor cell proliferation inhibitory activity

The MTT assay which was employed was performed according to a previously published method (Vareed et al., 2006). MCF-7 (breast), SF-268 (CNS), NCI-H460 (lung), HCT-116 (colon) and AGS (gastric) human tumor cells 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% fetal bovine serum. The extracts were assayed at 250 μg/mL. Triplicate analyses were performed for each sample. Extracts exhibiting no activity or very low activity relative to the associated error were not included in the data.

3. Results and discussion

3.1. Quantification of anthocyanins

A number of reports have shown that cyanidin and pelargonidin are the only anthocyanin aglycones in *Rubus* berries, the former being much more common. The pelargonidins are usually found in trace amounts alongside the cyanidins, although at times they occur as the major pigments, as is the case for *R. pileatus*. The fruits that possess pelargonidin glycosides as the major anthocyanins usually have an orange-red color, rather than a true red color (Jennings and Carmichael, 1980). It has been found that black raspberries possess higher anthocyanin contents (up to 400 mg/100 g) than blackberries (<150 mg/100 g), followed by the red raspberries (20–60 mg/100 g), orange raspberries (0.3–8.7 mg/100 g) and finally, the yellow raspberries (0–3.4 mg/100 g). The orange and yellow raspberries are usually cultivars of red raspberries (Deighton et al., 2000; Fan-Chiang and Wrolstad, 2005; Jennings and Carmichael, 1980; Pantelidis et al., 2007).



	R	R'
1	OH	Glu-Rha
2	OH	H
3	OH	Rha
4	H	H
5	H	Rha
6	OH	Mal

Fig. 1. Anthocyanins from *Rubus* fruits. (**1**) Cyanidin-3-glucosylrutinoside; (**2**) cyanidin-3-glucoside; (**3**) cyanidin-3-rhutinoside; (**4**) pelargonidin-3-glucoside; (**5**) pelargonidin-3-rutinoside; (**6**) cyanidin-3-glucosylmalonate.

Cyanidin-3-glucosylrutinoside (**1**), cyanidin-3-glucoside (**2**), cyanidin-3-rutinoside (**3**), pelargonidin-3-glucoside (**4**), pelargonidin 3-rutinoside (**5**), and cyanidin-3-glucosylmalonate (**6**) were identified in the fruits (Fig. 1, Table 1). *R. acuminatus* was found to possess the highest amount of anthocyanins, followed by *R. racemosus*, *R. jamaicensis*, *R. idaeus* cv. Heritage, and *R. rosifolius* (2199, 1882, 1672, 314 and 146 mg/100 g fresh weight respectively). The cultivated Michigan species possessed higher

Table 1
The anthocyanin concentration (mg/100 g FW) in *Rubus* fruits.

Anthocyanins	*Rubus* fruits				
	R. jamaicensis	*R. rosifolius*	*R. racemosus*	*R. acuminatus*	*R. idaeus* cv. Heritage
1: Cyanidin-3-glucosylrutinoside	–	–	–	–	163 ± 9
2: Cyanidin-3-glucoside	1501 ± 42	17 ± 0.4	1437 ± 40	1639 ± 38	151 ± 8
3: Cyanidin-3-rhutinoside	–	–	445 ± 7	560 ± 14	–
4: Pelargonidin-3-glucoside	–	81 ± 4	–	–	–
5: Pelargonidin-3-rutinoside	–	48 ± 0.1	–	–	–
6: Cyanidin-3-glucosylmalonate	172 ± 6	–	–	–	–
Total	1673	146	1812	2199	314

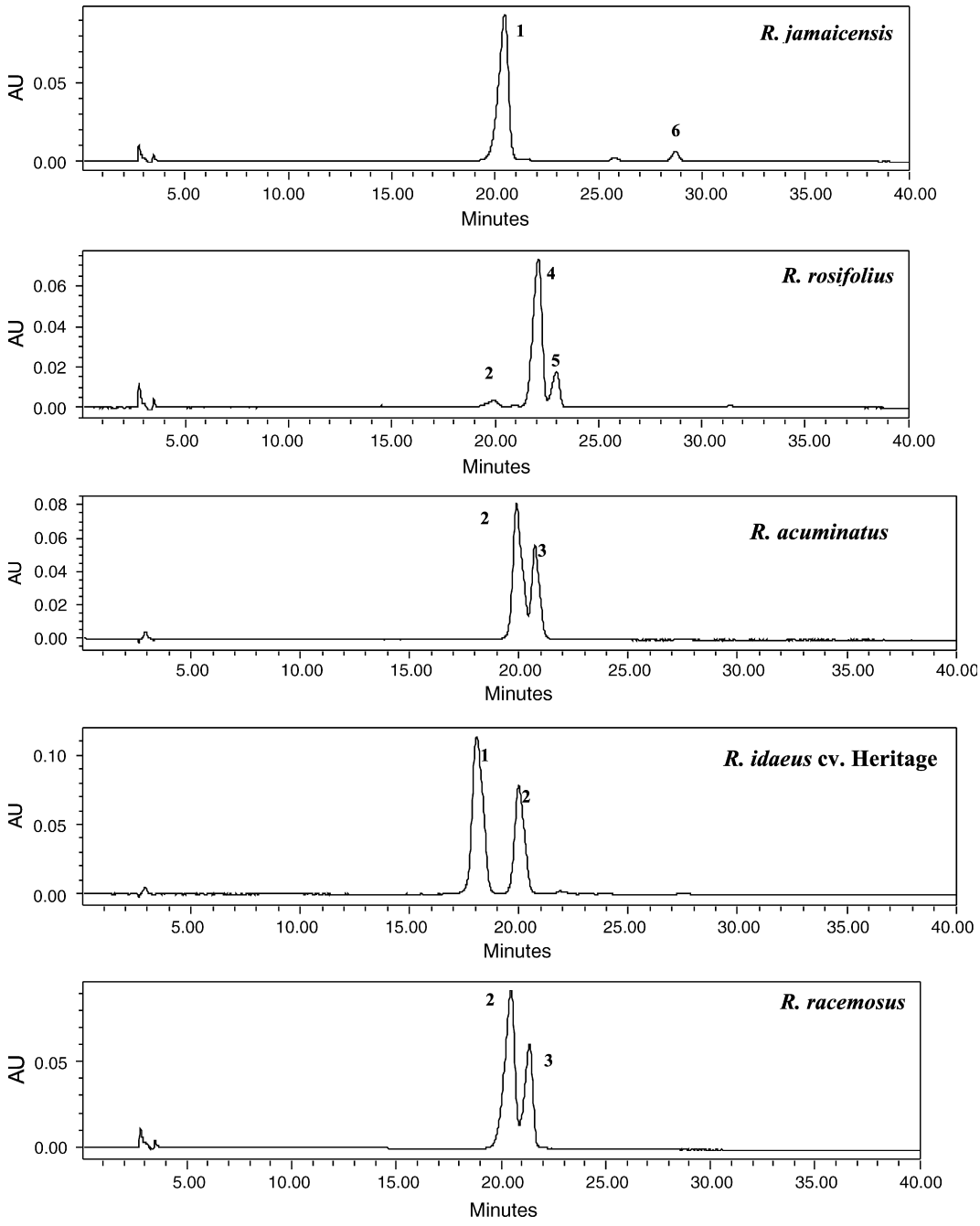


Fig. 2. HPLC profiles of *Rubus* berries.

quantities than their wild Jamaican counterparts. We did not detect any anthocyanins in the yellow raspberry. The HPLC profiles of the berries are shown in Fig. 2. In a previous study, *R. idaeus* cv. Heritage was shown to contain 48 mg/100 g anthocyanins (Pantelidis et al., 2007). This is markedly less than the quantities we now report. In fact, the quantities we now report are more than five times greater than those previously reported for other *Rubus* spp. With the exception of *R. idaeus* cv. Heritage, this is the first report of the anthocyanin content in these fruit species.

3.2. LPO inhibitory activity

With the exception of the hexane extracts of the Heritage and Golden cultivars of *R. idaeus*, all extracts were analyzed at 50 μg/mL (Fig. 3). All three extracts of the six *Rubus* berries analyzed demonstrated the ability to inhibit lipid peroxidation. Of the hexane extracts, those of *R. jamaicensis* and *R. acuminatus* exhibited the highest activity, resulting in 74 and 71% inhibition, respectively. The hexane extracts of *R. rosifolius* and *R. racemosus* showed 47 and 64% inhibition, respectively. The test solutions containing the hexane extracts of *R. idaeus* cv. Heritage and *R. idaeus* cv. Golden were analyzed at lower concentrations due to interference with the analysis due to turbidity. When assayed at 12.5 μg/mL, the extracts of *R. idaeus* cv. Heritage and *R. idaeus* cv. Golden showed 45 and 37% activity, respectively (data not shown in Fig. 3). The EtOAC extracts were generally the most active extracts at LPO inhibition, with activities ranging from 53% (*R. jamaicensis*) to 90% (*R. idaeus* cv. Heritage). Extracts of *R. racemosus*, *R. acuminatus* and *R. idaeus* cv. Golden also showed greater than 80% activity.

The MeOH extract of *R. racemosus* showed the greatest potential for LPO inhibition (84%), followed respectively by those of *R. idaeus*

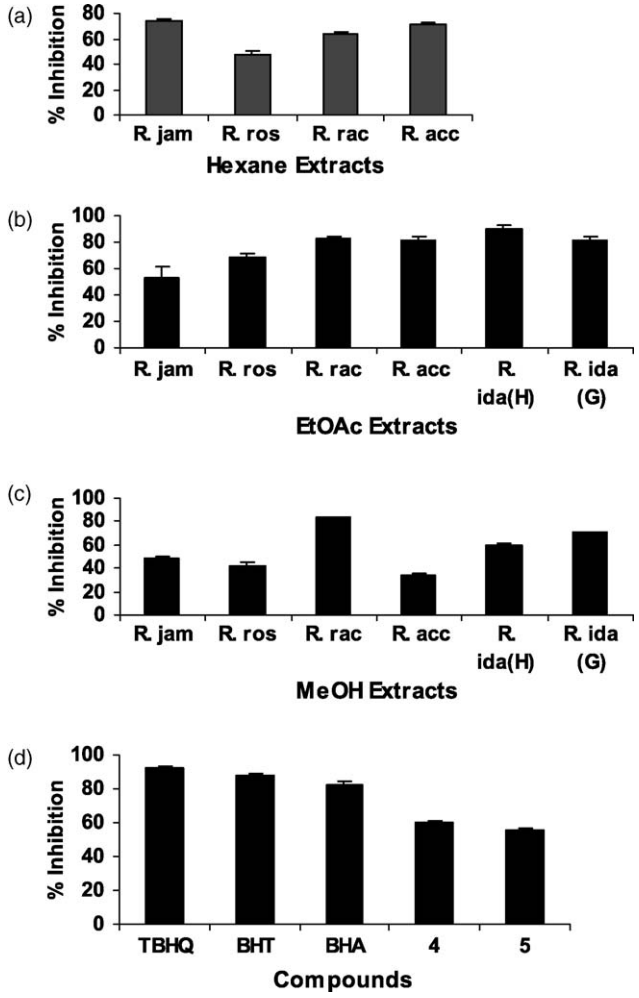


Fig. 3. Antioxidant activities of *Rubus* berry extracts at 50 μg/mL assayed in a liposomal model system. *R. jamaicensis*, *R. rosifolius*, *R. racemosus*, *R. acuminatus*, *R. idaeus* cv. Heritage and *R. idaeus* cv. Golden are represented as *R.* jam, *R.* ros, *R.* rac, *R.* acu, *R.* ida (H) and *R.* ida (G), respectively. (a) Hexane extracts, (b) EtOAc extracts, (c) MeOH extracts, (d) compound **4** (pelargonidin-3-glucoside) and compound **5** (pelargonidin-3-rutinoside) were tested at 10 μM, and the positive controls BHA, BHT and TBHQ were tested at 1 μg/mL (d). The percentage inhibition was the fluorescence intensity at 21 min relative to the DMSO control. Vertical bars represent ±SD for two data points. Varying concentrations of positive controls were used in order to obtain inhibition values between 50 and 100%.

cv. Golden and *R. idaeus* cv. Heritage, which showed 70 and 60% activity. *R. jamaicensis* and *R. rosifolius* inhibited lipid peroxidation by 48 and 42%, respectively. We analyzed anthocyanins **3** and **4** at 10 μM for LPO inhibitory activity. They demonstrated 60 and 56% activity, respectively.

Considering that the MeOH extracts account for over 90% of the total fruit extract, our LPO inhibition results indicate that the black raspberry, *R. racemosus*, has the highest antioxidant potential of the six berries studied, followed by the yellow cultivar of *R. idaeus* raspberries. It is interesting that the black raspberry, *R. acuminatus*, which had the highest anthocyanin content, exhibited the lowest antioxidant activity (34%), whereas the yellow raspberry which had no anthocyanins possessed greater antioxidant activity than all except one of the berries studied.

This finding corroborates the fact that anthocyanins are just one of the many types of compounds within a plant system that contributes to its antioxidant capacity. However, studies suggest that the majority of the antioxidant capacity of small fruits is due to phenolic compounds. Previous reports show that up to 80% of the total phenolic content of yellow cloudberries (*R. chamaemorus*) is

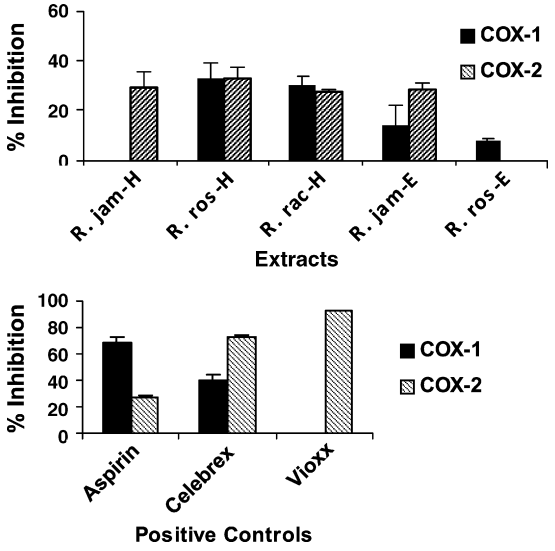


Fig. 4. Cyclooxygenase enzyme inhibitory activities of hexane, MeOH, and EtOAc extracts (indicated by -H, -E and -M) of *R. jamaicensis*, *R. rosifolius* and *R. racemosus* at 100 μg/mL (a), and positive controls Aspirin, Celebrex™ and Vioxx® at 60 μM, 26 and 32 nM respectively (b). Vertical bars represent ±SD for two data points. Varying concentrations of positive controls were used in order to obtain inhibition values between 50 and 100%.

derived from ellagitannins (Mylnikov et al., 2005). A similar condition could possibly exist in the yellow raspberries. There are a number of reports on the antioxidant capacity of the anthocyanins from fruits. Wang et al. (1999) found that anthocyanin **2**, the major anthocyanin in most *Rubus* species, exhibited 75% LPO inhibitory activity at 10 μg/mL.

3.3. Cyclooxygenase enzyme inhibitory activity

The expression of the COX-1 isozyme is common in most tissues. On the other hand, COX-2 is upregulated in inflamed cells, and is mediated by cytokines and human growth factors (Adhikari et al., 2005; Aggarwal et al., 2006). The inhibition of COX-2 is therefore an indicator of possible anticancer properties. All berry extracts were tested at 100 μg/mL. The only Michigan-grown berry extract that demonstrated activity was the hexane extract of *R. acuminatus*, which inhibited COX-2 by 71%. All the hexane extracts of the Jamaican *Rubus* berries were COX-active. The hexane extracts of *R. jamaicensis*, *R. rosifolius* and *R. racemosus*, inhibited COX-2 by 18–33%. The hexane extract of *R. jamaicensis* selectively inhibited COX-2 at the test concentration. The said extracts of *R. rosifolius* and *R. racemosus* inhibited COX-1 by 33 and 30%, respectively. The EtOAc extracts of *R. jamaicensis* and *R. rosifolius* also demonstrated some COX inhibitory activity, as shown in Fig. 4. The Michigan-grown *Rubus* extracts were, in general, not COX-active, while the hexane extracts of the Jamaican spp. were the most active. The MeOH extracts showed no activity at the concentration tested. Considering that the inhibition of COX-2 is an indication of possible anticancer properties, it was expected that the latter extracts would possess the highest anticancer potential, and this is what was observed.

3.4. Tumor cell proliferation inhibitory activity

The extracts were assayed at 250 μg/mL (Fig. 5). The hexane extracts, particularly those of the Jamaican species, demonstrated the greatest potential to inhibit the growth of tumor cells. The hexane extract of *R. jamaicensis* had the greatest overall capacity to inhibit the progression of tumor cell growth, inhibiting colon, breast, lung, and gastric human tumor cells by 50, 24, 54, and 37%,

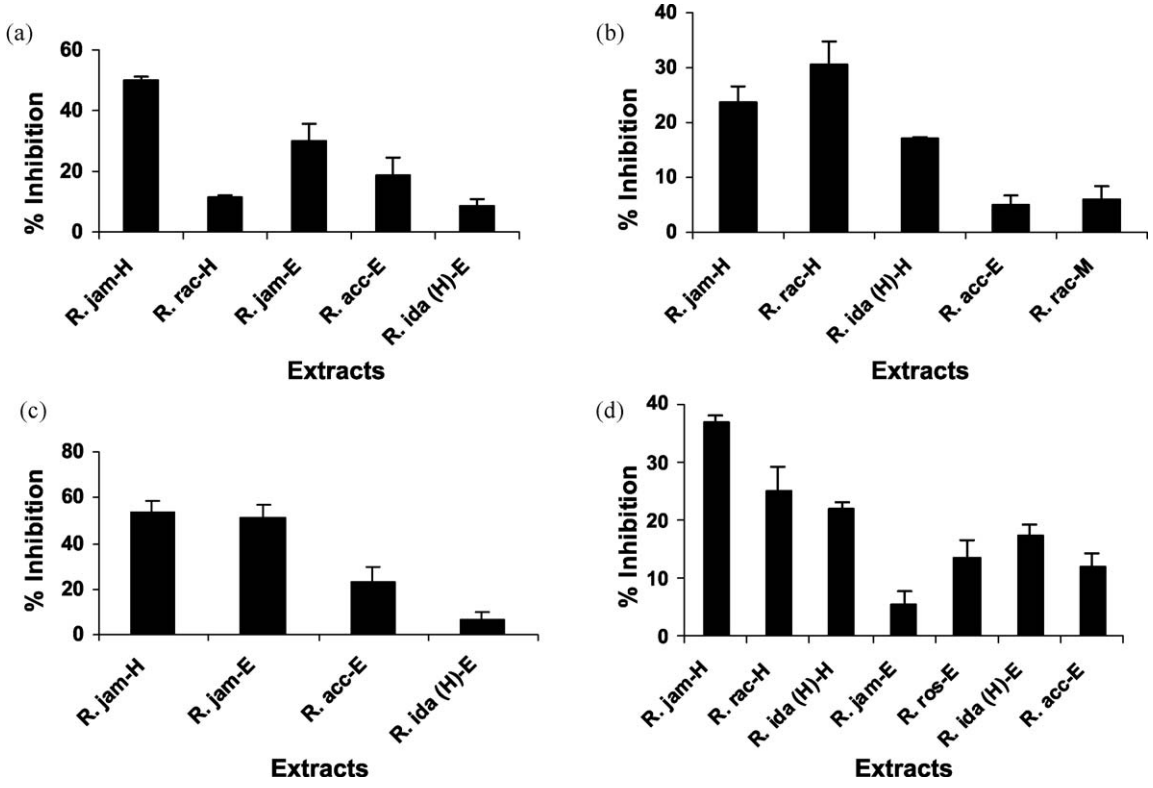


Fig. 5. Tumor cell proliferation activities of *Rubus* berry hexane, MeOH, and EtOAc extracts (indicated by -H, -E and -M) at 250 μg/mL (a) on human colon (HCT-116), (b) breast (MCF-7), (c) lung (NCI-H460), and (d) gastric (AGS) cancer cell lines. Vertical bars represent ±SD for three data points.

respectively. The hexane extracts of *R. racemosus* inhibited colon, breast and gastric cancer cells by 11, 31, and 25%, respectively. The hexane extract of *R. idaeus* cv. Heritage resulted in inhibition of breast and gastric cancer cells by 17 and 22%. The EtOAc extracts showed a lower capacity to inhibit cancer cell growth, while the MeOH extracts were generally not active. The most active EtOAc extracts were those of *R. jamaicensis* and *R. acuminatus*. The former extract respectively inhibited colon, lung and gastric tumor cells by 30, 51, and 6%, while the latter inhibited colon, breast and lung cells by 19, 5 and 24%. The growth of central nervous system cancer cells were inhibited only by the EtOAc extract of *R. idaeus* cv. Golden (14%). The MeOH extracts in general showed no activity at the concentration tested.

Rubus berry seeds account for 9–12% of the fruit on a fresh weight basis, and contain 10–23% oils (dry weight). It has been found that the oils possess good lipid oxidation stability, have superior levels of tocols compared to grape seed and safflower oils, and have excellent anti-inflammatory activity compared to grape seed, wheat germ and avocado oils (Oh et al., 2007). The anticancer properties of the hexane extracts of the berries are likely to be due mainly or in part to those favorable qualities of the seed oils. With the exception of *R. idaeus*, this is the first report of the antiproliferative activity of these *Rubus* spp.

4. Conclusions

Our results demonstrate that *R. acuminatus* possessed the highest amount of anthocyanins, followed by *R. racemosus*, *R. jamaicensis*, *R. idaeus* cv. Heritage and *R. rosifolius*. This finding corroborates previous reports that with respect to anthocyanin content, black raspberries are superior, followed by blackberries, and then red raspberries. The anthocyanin levels reported herein are higher than those previously reported for other *Rubus* fruits. This may have been partly due to increased extraction efficiency. Cyanidin-3-glucoside was found to be the major anthocyanin in all the berries except *R. rosifolius* and *R. idaeus* cv. Heritage, in which pelargonidin-3-glucoside and cyanidin-3-glucosylrutinoside were the major anthocyanins. *R. idaeus* cv. Golden was void of anthocyanins. Based on the high antioxidant potential of the fruits, as indicated by the LPO inhibitory activity of their extracts, their incorporation in the diet is highly recommended. Of all the extracts tested, the hexane extracts, particularly those of the Jamaican berries, exhibited the highest anti-inflammatory and tumor cell proliferation inhibitory activities, an indication that these berries may be good candidates for alternative functional food products that would be beneficial to health.

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