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Antioxidant and Anti-inflammatory Activities of Bean (*Phaseolus vulgaris* L.) Hulls

B. Dave Oomah,*,† Amélie Corbé,‡ and Parthiba Balasubramanian§

†National Bioproducts and Bioprocesses Program, Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada V0H 1Z0, and §Crop Genetic Enhancement Program, Lethbridge Research Centre, Agriculture and Agri-Food Canada, 5403-1 Avenue South, P.O. Box 3000, Lethbridge, Alberta, Canada T1J 4B1. ‡ Present address: Institut supérieur des sciences agronomiques, horticoles et du paysage (INSFA), Agrocampus Ouest, 65 rue de Saint Brieuc, CS 84215, 35042 Rennes Cedex, France

Hulls obtained by mechanical abrasive dehulling from four bean cultivars were extracted with two solvents, aqueous (70%) acetone and water, and the extracts evaluated for antioxidant and anti-inflammatory activities in relation to their phenolic contents. Total phenolic content and antioxidant activity of bean hulls, measured using oxygen radical absorbance capacity (ORAC) values, were 6−8-fold those of corresponding whole beans. Aqueous acetone (70%) extracted over twice the amount of total phenolics from hulls that exhibited significantly higher antioxidant and stronger inhibitory effect on both cyclooxygenases, COX-1 and COX-2, than water. Acetone extract of black bean hull exhibited strong COX-1 (IC_{50} = 1.2 μg/mL) and COX-2 (IC_{50} = 38 μg/mL) inhibitory effects, even outperforming aspirin. Bean hull water extracts were stronger inhibitors of lipoxygenase, 15-LOX, than corresponding acetone extracts. Anti-inflammatory activity of bean hulls was dependent on their phenolic content and antioxidant activity that were significantly affected by cultivar and extracting solvent.

KEYWORDS: Anti-inflammatory activity; hulls; antioxidant activity; phenolics; beans; cultivar; market class; (*Phaseolus vulgaris* L); COX; LOX

INTRODUCTION

The seed coat or hull of dry beans has been the focus of current research due to its various health-promoting advantages (benefits) because of high fiber, phytochemical, and low caloric contents. Although the hull is a relatively small portion of the seed (7−13 g/100 g) on a weight basis (*1*−*4*), it is rich in dietary fiber (*5*,*6*), minerals, particularly calcium (*7*), and phenolic compounds exhibiting strong antioxidant activity (*3*, *8*) essential for the development of novel food products.

Recently, studies identified the polyphenolic compounds of black Jamapa bean seed coat consisting of flavonoids with known biological and physiological activities (*9*). The primary phenolic compounds in beans and their hulls are flavonoids, mainly as caffeic, *p*-coumaric, ferulic, and sinapic esters (*10*−*13*). Recently, bean market classes were differentiated by the presence of three anthocyanins (delphinidin, petunidin, and malvidin) in black beans, kaempferol in pintos (including Othello), and quercetin and kaempferol in pink bean, whereas flavonoids were undetected in navy bean (*14*).

The flavonoids in a methanolic extract of black bean seed coat are presumed to reduce liver injury in animal models by modulating type I and IV collagen gene expression (*15*). Earlier studies showed that these hepatoprotective effects were probably due to the antioxidative properties exerted by the water extract from adzuki bean hulls (*16*). Supplementation of hot water extract from pinto bean hull improved bone metabolism in mice, and this favorable effect was also ascribed to the antioxidant activity of polyphenols (*17*). Similar water-soluble black bean tannins (extracted with 70% acetone consisting mostly of catechin or epigallocatechin) extract inhibited cancer cell proliferation by suppressing angiogenic factors (*18*). Methanolic (80%) extracts of black bean hulls rich in antioxidants were generally more effective than whole-seed extracts against colon, breast, and prostate cancer cell proliferation (*4*). These and several other studies suggest that bean hull antioxidants may be responsible for their physiological effects.

Previously we demonstrated that phenolics are concentrated in bean hulls and exert antioxidant properties by scavenging free radicals or suppressing lipid peroxidation (*8*). The yield of hull was also found to be totally dependent on the choice of bean cultivar. This investigation extends our recent study on mechanical dehulling of Canadian dry bean cultivars using the tangential abrasive dehulling device (TADD) (*19*) and demonstrates the anti-inflammatory activity of bean hulls. The bean cultivars for this study were selected on the basis of their dehulling characteristics and represent different market classes. Emphasis on regulation of inflammation in addition to antioxidant activity provides further efficacy for application of bean hull as an important functional food and nutraceutical ingredient.

*Corresponding author [fax (250) 494-0755; phone (250) 494-6399; e-mail oomahd@agr.gc.ca].

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MATERIALS AND METHODS

Seeds of dry beans (*Phaseolus vulgaris* L.) used in this study were grown and harvested at Lethbridge, a semiarid location in southern Alberta, Canada, in 2007 and were kindly provided by Agriculture and Agri-Food Canada (Lethbridge, AB). The cultivars used in this study included four bean market classes, black (AC Black Violet), great northern (AC Polaris), pink (Viva), and pinto (Othello). The names of the cultivars are used hereafter without the prefix. Hulls were obtained by abrasive dehulling carried out on a model 4E-230 TADD (Venables Machine Works Ltd., Saskatoon, SK, Canada) with an eight-cup cover plate as described previously (*19*). Beans (25 g) were placed in two of the cups and dehulled for a given time interval, seeds were then removed from the sample cups using the vacuum aspirating device, and the dehulled seeds and hulls were separated by air aspiration. Samples were cryomilled in a 6800 SPEX freezer/mill (SPEX, Metuchen, NJ) at 1 min of grinding time, two cycles, and impactor speed of 10 s^{-1} (stainless steel diameter ≅ 19 mm, length ≅ 70 mm).

Extraction and Analysis of Phenolics. Ground samples (200 mg) were extracted with aqueous acetone 70% (v/v) (8 mL), using a Reacti-Therm heating and stirring module (N 18970, Pierce, Rockford, IL) for 2 h and filtered (Acrodisc 0.45 μm, VWR International, Mississauga, ON, Canada). Similar extractions were carried out with distilled water. The water extracts were centrifuged (Sorvall model RC 5B plus; DuPont Co. Wilmington, DE) at 11000*g* for 30 min and filtered through glass wool. The recovered supernatant was stored at −20 °C in the dark until analysis. All extractions were carried out in triplicates.

Phenolic content of extracts was determined according to a procedure described previously (*8*). Briefly, the method consisted of adding 100 μL of sample with 150 μL of a solution of 2% HCl in 80% ethanol in a 96-well ultraviolet flat-bottom plate (Greiner Bio-One Inc., Longwood, FL). The absorbance of the solution was monitored at 280, 320, 360, and 520 nm after mixing for 2 min with a spectrophotometer (Spectramax Plus 384, Molecular Devices Corp., Sunnyvale, CA) using (+)-catechin (0−100 μg/mL), caffeic acid (0−20 μg/mL), quercetin (0−30 μg/mL), and cyanidin-3-glucoside (0−8 μg/mL) as standards for total phenolics, tartaric esters, flavonols, and anthocyanins, respectively. Standards were prepared in aqueous ethanol 80% (v/v). The absorbance was also read at 710 nm for turbidity correction, and the results were expressed in milligrams of (+)-catechin, caffeic acid, quercetin, or cyanidin-3-glucoside equivalents per gram of sample. The acetone extract was evaporated in the Reacti-Therm module under nitrogen and dissolved in aqueous ethanol 80% (v/v) prior to phenolic assay.

Antioxidant Assay. Antioxidant activity was measured using the oxygen radical absorbance capacity ($ORAC_{FL}$) described previously (*20*), according to established procedure (*21*). A SpectraMax GeminiEM microplate fluorescence reader (Molecular Devices Co.) was used with excitation and emission wavelength at 485 and 530 nm, respectively. Sample extracts and Trolox standards were diluted with 75 mM phosphate buffer (pH 7.4) prior to transfer to a 96-well microplate (Fluotrac 200, Greiner Bio-One Inc.). A peroxyl radical was generated by 2,2′-azobis(2-methylpropionamide) dichloride (AAPH) (Sigma Aldrich, USA) during measurement, and fluoresceine was used as the substrate. Measurements were taken every 2 min for 120 min upon addition of AAPH. Final ORAC values were calculated by using a regression equation between the Trolox concentration (0−4 μg/mL) and the net area under the curve (AUC in μM Trolox equivalents (TE)/g sample) and converted to milligrams per gram of sample.

Anti-inflammatory Activity. Anti-inflammatory activity was determined by measuring the inhibition of COX enzymes using a colorimetric COX (ovine) inhibitor screening kit (Cayman Chemical Co., Ann Arbor, MI) according to the manufacturer's instructions. The assay measures the peroxidase activity of COX by monitoring the appearance of oxidized *N*,*N*,*N*,*N*-tetramethyl-*p*-phenylenediamine (TMPD) (Sigma, St. Louis, MO). Briefly, 10 μL of extract was incubated with 160 μL of 0.1 M Tris-HCl buffer (pH 8), 10 μL of heme, and 10 μL of ovine COX-1 or COX-2, for 5 min at 25 °C. After preincubation, 20 μL of TMPD was added to each well of the 96-well plate, and the reactions were started with 20 μL of arachidonic acid (1.1 mM). Absorbance of the cleaved TMPD substrate was monitored at 590 nm after 5 min of incubation with a spectrophotometer (Spectramax Plus 384, Molecular Devices Corp.). Each extract and appropriate positive controls were tested for COX-1 and COX-2 inhibition in triplicate, at room temperature at different dilutions (0−600 μg/mL), in 95% ethanol. The inhibitor concentration versus percentage inhibition was plotted, and the 50% inhibitory concentration (IC_{50}) was determined by taking the half-maximal point along the isotherm and intersecting the concentration on the *x*-axis. Percent inhibition was calculated by comparison of compound treated to control incubations. Aspirin (Cayman Chemical Co.) and celecoxib (Pfizer Canada, Kirkland, PQ, Canada) were used as reference standards.

The anti-inflammatory activity of bean extracts was also measured using a lipoxygenase (15-LOX) inhibitor screening assay kit (Cayman Chemical Co.) according to the manufacturer's instructions in a 96-well plate. The assay measures hydroperoxides produced from the incubation of purified 15-LOX with arachidonic acid. Briefly, 10 μL of extract was incubated with 90 μL of 15-LOX, 10 μL of 0.1 M Tris-HCl buffer (pH 7.4), and 10 μL of 1 mM arachidonic acid for 5 min. Chromogen (100 μL) was added to stop the reaction. The plate was placed on a shaker for 5 min, and the absorbance was monitored at 500 nm (Spectramax Plus 384, Molecular Devices Corp.,) using nordihydroguaiaretic acid (NDGA; 0.45−9.52 μg/mL). The hull extracts were assayed at different dilutions in 95% ethanol.

Statistical Analysis. At least three determinations were made for all assays except for antioxidant activity, which was determined in duplicate. Analysis of variance by the general linear models (GLM) procedure, means comparison by Duncan's test, and Pearson correlation were performed according to Statistical Analysis System (*22*).

Table 1. Phenolic Contents and Antioxidant Activities of Bean Extracts

	concentration[a,b]				ORAC (Trolox)[a]	
extract	total phenolics	tartaric esters	flavonols	anthocyanins	mg/g	μmol/g
acetone						
Black Violet	10.77c	0.96a	0.56a	0.709a	34.20a	136.63a
Othello	13.26a	0.83b	0.43b	0.029b	36.61a	146.25a
Polaris	4.15d	0.59c	0.20d	0.012c	16.69b	66.69b
Viva	11.89b	0.96a	0.36c	0.033b	37.84a	151.18a
mean	10.02Q	0.83Q	0.39Q	0.196P	31.56P	126.08P
water						
Black Violet	13.33y	1.15x	0.66x	0.156w	18.14x	72.50x
Othello	10.04z	0.79z	0.43z	0.042z	13.66yz	54.59yz
Polaris	13.83x	0.80z	0.44z	0.073x	10.49z	41.90z
Viva	13.09y	1.00y	0.50y	0.057y	14.53y	58.08y
mean	12.56P	0.93P	0.51P	0.083Q	14.34Q	57.31Q
overall mean	11.27	0.88	0.45	0.14	22.95	91.70

[a] Means in a column with different letters are significantly different ($p < 0.05$). [b] Concentrations of phenolic compounds are expressed as milligram equivalents of (+)-catechin, caffeic acid, quercetin, or cyanidin-3-glucoside per gram of sample for total phenolics, tartaric esters, flavonols, and anthocyanins, respectively.

RESULTS

The bean cultivars for this study were selected on the basis of their dehulling parameters that segregated the cultivars into three major abrasive hardness index (AHI) groups: AC Black Violet (AHI, 12.7−14.9 s; yield, 96.9 g/kg); Othello and AC Polaris (AHI, 11.7−12.5 s; yield, 98.7 and 103.8 g/kg, respectively); and Viva (AHI, < 11 s; yield, 112.1 g/kg). The phenolic contents of beans differed significantly among cultivars independent of extracting solvent (**Table 1**), with Black Violet and Polaris expressing the highest and lowest total phenolics, tartaric esters, and flavonols, respectively. Water generally extracted significantly higher amounts of phenolic compounds from bean cultivars than aqueous 70% acetone. This was clearly demonstrated in the extreme with cultivar Polaris, where water extracted phenolics were significantly higher (> 3× total phenolics, > 2× flavonols, and > 6× anthocyanins) than those from acetone. Othello was the only cultivar that had significantly higher phenolic components in

Table 2. Phenolic Content and Antioxidant Activity of Bean Hull Extracts

	concentration[a,b]				ORAC (Trolox)[a]	
extract	total phenolics	tartaric esters	flavonols	anthocyanins	mg/g	μmol/g
acetone						
Black Violet	108.68c	3.32a	3.48a	7.55a	282.25b	1127.69b
Othello	158.20a	3.23b	3.04b	0.18c	340.51a	1360.46a
Polaris	2.07d	0.30d	0.07d	0.01d	4.92c	19.64c
Viva	125.17b	2.26c	1.22c	0.30b	342.57a	1368.71a
mean	98.53P	2.28P	1.95P	2.01P	230.50P	920.94P
water						
Black Violet	53.11x	2.76x	2.38w	3.47x	140.86y	562.78y
Othello	66.02w	1.66y	1.18x	0.08yz	200.16w	799.70w
Polaris	4.26z	0.31z	0.11z	0.02z	10.28z	41.08z
Viva	50.27y	1.65y	0.83y	0.14y	179.89x	718.72x
mean	43.41Q	1.60Q	1.13Q	0.93Q	137.98Q	551.27Q
overall mean	70.97	1.94	1.54	1.47	185.95	742.95

[a] Means in a column with different letters are significantly different ($p < 0.05$). [b] Concentrations of phenolic compounds are expressed as milligram equivalents of (+)-catechin, caffeic acid, quercetin, or cyanidin-3-glucoside per gram of sample for total phenolics, tartaric esters, flavonols, and anthocyanins, respectively.

its acetone than in its water extract. This is similar to the total phenolic contents of all bean (cotyledon) types obtained by water extraction reported recently (*23*). Acetone was highly selective in extracting phenolic compounds from whole beans indicated by the significantly different amounts of phenolics obtained from each bean cultivar. The acetone extracts of Black Violet and Polaris contained the most and least anthocyanin contents, respectively. The white great northern pinto bean (Polaris), considered as a market class, contained significantly less phenolics than colored beans in accordance with previous studies (*23*−*25*).

Total phenolic acids and ORAC values of acetone extract from black bean were >2-fold higher than those reported (*26*) using various acetone concentrations. The antioxidant activity of water extract from Polaris was within the range (39−67 μmol of Trolox/g) reported for water-soluble protein extract of white bean (*27*), suggesting the participation of protein in eliciting antioxidant activity. ORAC values of acetone extracts from Black Violet and Othello were similar to those of beans from the same market class reported previously (*20*). However, the acetone-extracted phenolics exhibited significantly higher antioxidant activity than the corresponding water extract. Thus, the potency of the phenolic antioxidant (PAOXI), calculated as the ratio of total phenolics to antioxidant activity, for the acetone extract was >2-fold (2.75×) that of the water extract.

Overall total phenolic content and antioxidant activity (ORAC) values of bean hulls were 6−8-fold those of corresponding whole beans. For black beans, the total phenolic and flavonol contents of bean hulls were 10 and 6 times, respectively, those of whole beans, similar to those reported earlier (*28*). Total phenolic content of hulls differed significantly among cultivars, independent of extracting solvent, decreasing in the following order: Othello > Viva > Black Violet > Polaris (**Table 2**). However, Black Violet and Polaris hulls had the highest and lowest contents of tartaric esters, flavonols, and anthocyanins, respectively, similar to the trend found in whole beans, irrespective of the extracting solvent. Aqueous acetone generally extracted over twice the amount of total phenolics from hulls as water, a contrast to that observed with whole beans. This was evidently consistent for hulls from all cultivars except Polaris and thereby reflected in their superior ORAC values. Total phenolic content of water-extracted hulls decreased in the following order: Othello > Black Violet > Viva > Polaris, the reverse order of that observed with whole beans. The flavonol content of bean and hull extracts, a major contributor to seed coat color (*29*), decreased in the following order: Black Violet > Othello > Viva > Polaris (**Tables 1** and **2**).

Total phenolic contents of hulls were within the range of those reported previously for manually separated hulls extracted with 70% acetone from 12 black bean cultivars (*4*). However, black bean hulls, according to Gutierrez-Uribe (*4*), contained up to 20 times more phenolic compounds (84.9−277 mg/g catechin equiv) than their respective whole grains compared to the 10 times more phenolics observed in this study. Total phenolic contents of acetone extracted whole beans and hulls were similar to those of Flor de Mayo extracted with methanol (*30*). Anthocyanin values (except for Black Violet) were within the 0.13−2.1 mg/g range reported for manually peeled (removed) hulls extracted with acidified methanol (70% MeOH with 0.5% HCl) (*31*). Total phenolic contents of our acetone extracts from hulls and whole beans, except Polaris, were two-fifths to two-thirds the values for hulls and one-eighth to one-fourth for whole beans reported previously (*32*). The higher values probably result from the overestimation of phenolic content by Folin−Ciocalteu used in that study (*33*). In our study, total phenolic contents of acetone hull extracts were 10−11 times those of whole beans, except Polaris, whereas that ratio was only 2−6 for unspecified varieties of colored beans extracted with 80% acetone under reflux (*32*).

The antioxidant activity of dry bean hulls, measured by the ORAC procedure, showed significant variations in scavenging activity of peroxyl radical among cultivars (**Table 2**). Cultivar, extracting solvent, and their interaction significantly ($P < 0.0001$) affected the antioxidant activity of bean hulls. Acetone extracted 160% more antioxidant than water from bean hulls. Hulls from all cultivars, except Polaris, exhibited high [>550 μM Trolox equiv (TE)/g of dry matter] antioxidant capacity irrespective of extracting solvent. These values are considerably higher than those reported previously for bean hulls (*4*). ORAC values for water extracts of bean hulls were higher than those reported for most natural products and herbs (*34*).

Hull extracts were selected for anti-inflammatory activity on the basis of their relatively high antioxidant activity. Acetone extract of black bean hull exhibited strong COX-1 (IC_{50} = 1.2 μg/mL) and COX-2 (IC_{50} = 38 μg/mL) inhibitory effects, even outperforming aspirin, followed by Othello and Viva (**Table 3**). This anti-inflammatory trend of the acetone extracts follows the same descending order of their abrasive hardness index. Inhibition results for acetone extract of Othello hulls showed approximately 2-fold more COX-1 versus COX-2 inhibition when IC_{50} values were compared, resulting in a COX-1/COX-2 ratio of 0.6. The potency of acetone hull extracts of Othello and Viva compared to Celebrex, a known COX-2 inhibitor, was similar to those

Table 3. Selective Inhibition of COX Enzymes by Bean Hull Extracts

	COX-1		COX-2		specific activity[a]
extract	IC_{50} (μg/mL)	potency (%)	IC_{50} (μg/mL)	potency (%)	COX-1/COX-2
acetone					
Black Violet	1.2 (97.0)[b]	3333.3	38.1 (96.8)	147.0	0.03
Othello	58.2 (85.2)	69.0	100.6 (73.7)	55.7	0.58
Viva	69.5 (80.2)	57.6	96.8 (67.6)	57.9	0.72
water					
Black Violet	52.7 (78.2)	75.9	188.5 (54.8)	29.7	0.28
Othello	186.0 (48.0)	21.5	315.7 (48.4)	17.7	0.59
Viva	379.6 (33.7)	10.5	245.8 (46.9)	22.8	1.54
aspirin	40 (92.8)		273 (33.0)		0.15
Celebrex			56 (64.6)		

[a] COX-1-selective inhibitor will have a ratio of <1, whereas a COX-2-selective inhibitor will have a ratio of >1. The higher this number the more selective the extract is for COX-2 as opposed to COX-1. COX-2 potency was calculated relative to IC_{50} of Celebrex. [b] Values in parentheses represent (%) inhibition at 227 μg/mL concentration of extract or standard.

Table 4. LOX Inhibition by Hull Extracts

	concentration	LOX inhibition[a] (%)		IC_{50}	potency
sample	(μg/mL)	acetone	water	(μg/mL)	(%)
Black Violet	238	20.6B ± 1.5	54.5B ± 2.8	186.9	2.07
	119	28.3c ± 0.7	41.8b ± 5.3		
	59	35.5y ± 2.4	21.8z ± 2.8		
Othello	238	39.8A ± 1.6	71.5A ± 1.1	15.6	24.83
	119	43.0a ± 0.7	64.4a ± 1.4		
	59	44.2x ± 0.7	60.9x ± 1.4		
Viva	238	39.1A ± 1.2	67.3AB ± 8.4	116.1	3.33
	119	40.7b ± 0.8	50.0b ± 2.8		
	59	43.2x ± 1.2	34.4y ± 1.8		
NDGA[b]	60.48	43.8 ± 1.2		3.87	

[a] Means with standard deviations in a column at the same concentrations with different letters are significantly different ($P < 0.05$). [b] NDGA, nordihydroguaiaretic acid.

of fruit extracts, particularly chokeberry, reported previously (*35*). Overall, the water extracts exhibited weak inhibitory effect on both COX-1 and COX-2. However, the water extract of Viva, despite its high IC_{50} values, revealed the best COX-2 selectivity index (COX-1/COX-2 ratio) of 1.54, whereas Othello extracts showed an almost similar index of 0.58, independent of extracting solvent. The specific activity (0.28) of water extract from Black Violet hulls was similar to that reported for pure catechin (*36*). IC_{50} values for COX inhibitions of bean extracts were similar to those of anthocyanin-rich fruits and natural products (*35*), except for the water extracts of Othello and Viva hulls. The acetone extract of the great northern bean (Polaris) hulls exhibited extremely low COX inhibition (3−5% COX-1 and 5−14% COX-2), whereas the water extract had no inhibition. Thus, the results of our study indicate that black bean hull extracts act as nonselective inhibitors of the COX enzyme and therefore possess strong anti-inflammatory activity. The acetone extract of Black Violet hulls could potentially play a significant role in the prevention of atherosclerosis akin to aspirin by protecting vascular damage caused by oxygen radicals (*37*) because it exhibited strong antioxidant activity and COX inhibitory activity superior to aspirin.

Generally, water extracts from bean hulls demonstrated stronger inhibition toward 15-LOX compared to the acetone extract (**Table 4**). Black Violet hull extracts inhibited 15-LOX dose dependently, with inhibition increasing and decreasing significantly ($P < 0.001$) with concentration for the water and acetone extracts, respectively. The inhibitory effect of the water extracts at the lowest concentration (59 μg/mL) was significantly different ($P < 0.001$) among cultivars. However, this variability was reduced at higher concentrations, with Othello extract displaying the strongest effect, followed by those of Viva and Black Violet. This order of LOX inhibition paralleled their antioxidant activity and was reflected in their respective IC_{50} values and potency. The water extract of Othello hulls had an IC_{50} value similar to those of catechin and epicatechin reported elsewhere (*38*). LOX inhibition by acetone extracts of hulls differed significantly ($P < 0.0001$) among cultivars at the highest concentration, with Black Violet extract exhibiting the weakest inhibitory effect at all concentrations. However, IC_{50} values of the acetone extracts could not be determined due to their weak inhibitory effect and negligible dose response. Under this condition, NDGA, a nonspecific LOX inhibitor, had an IC_{50} value of 3.9 μg/mL, 4 times stronger than the water extract from Othello. The results strongly suggest that the water extracts, particularly from Othello, exert anti-inflammatory activity, at least in part, via 15-LOX inhibition. Because the water extract of hulls from Othello had no meaningful effect on COX-2, an important pro-inflammatory enzyme, it may not be effective on inflammatory conditions in which prostanoids play an important role. It can instead act as a 15-LOX inhibitor, modulating the lipoxygenase pathway. This inhibition may influence the inflammation processes because 15-LOX participates in oxidative modifications of low-density lipoproteins (LDL) and in the development of atherosclerotic lesions (*38*).

Comparison of the phenolic content of bean hull extracts with antioxidant and anti-inflammatory activities revealed strong correlation. Anthocyanin content of hull extracts was positively correlated ($r = 0.998$, $P \leq 0.04$) with antioxidant activity of water extracts and anti-inflammatory activity of acetone extracts ($r = 0.999$, $P \leq 0.02$) evaluated as COX-1 and COX-2 potencies. Lipoxygenase (LOX) activity was also negatively correlated with anthocyanin content ($r \geq -0.993$, $P \leq 0.08$), flavonol content ($r \geq -0.998$, $P \leq 0.04$), and antioxidant activity ($r = -0.998$, $P \leq 0.04$) of water extracts. Flavonol content of the acetone extracts was inversely related to their antioxidant activity ($r = -0.999$, $P \leq 0.01$) and COX-1 inhibition ($r = -0.998$, $P \leq 0.04$). COX-2 potency was related to COX-1 potency ($r = 0.999$, $P \leq 0.02$) but negatively correlated with LOX inhibition ($r = -0.999$, $P \leq 0.007$) of the acetone extracts. Tartaric esters were inversely related ($r = -0.999$, $P \leq 0.03$) to IC_{50} of COX-2 of the water extracts. COX-1 inhibition correlated positively ($r = 0.852$, $P \leq 0.03$) with COX-2 inhibition, but inversely ($r = -0.908$,

$P \leq 0.02$) with LOX, influenced mostly by the acetone extracts. Thus, phenolic compounds of colored bean hulls function mostly as free radical scavengers rather than inhibitors of lipoxygenase generating free radicals from fatty acid oxidation.

DISCUSSION

This investigation in addition to our earlier (*3*, *8*) and numerous other studies confirm that most of the bioactive phenolics and antioxidants in common beans are concentrated in the hulls. The hulls of almost all previous studies were primarily obtained manually or by inefficient and ineffective dehulling systems in contrast to the tangential abrasive dehulling device used in this report. The TADD is already used in screening dehulling characteristics of other crops including grain legumes (*2*, *4*), and this platform is analogous to potential industrial bean hull production for the functional foods and nutraceutical markets.

Overall, both acetone and water extracts exhibited anti-inflammatory activity; the extent of this protective effect can be ascribed to their phenolic constituents and antioxidant activities. This is evident from the weaker anti-inflammatory potency of the water extract associated with its lower antioxidant activity and phenolic content compared to the potent acetone extracts (end point IC_{50} values $< 100\,\mu g$/mL). As inflammatory reactions often include the formation of tissue-damaging oxidation products, compounds with high antioxidant activity may inhibit inflammation. Our results with bean hulls support previous studies in which antioxidant and anti-inflammatory activities of extracts are associated with polyphenols capable of inhibiting COX and LOX (*39*−*41*), the two major metabolic routes controlling eicosanoid biosynthesis. Cyclooxygenases COX-1 and COX-2 differentially regulate cardiovascular and renal function, with COX-2 inhibitors increasing cardiovascular risk as opposed to the reduced cardiovascular risk associated with COX-1 inhibitors (*42*). Hence, the prominent COX-1 inhibition by Black Violet hull extract surpassing low-dose aspirin can aid in the prevention of cardiovascular events without affecting renal function. The COX inhibitory activities, particularly of the Black Violet acetone extract, provide the biochemical basis for the beneficial physiological and chemopreventive effects observed with black bean hulls in cellular and animal models (*4*).

Both 70% acetone and water were excellent solvents for extracting phenolic compounds that elicited antioxidant and anti-inflammatory activities in colored bean hulls. However, water might be a preferred option for pharmaceutical and food grade commercial processes and regulatory agencies. Black Violet representing the black bean market class has a high abrasive index (difficult to mechanically dehull relative to Viva), although its extracts elicited the most potent antioxidant and anti-inflammatory response. The activity of the acetone extract of Black Violet hull is due to the presence of bound anthocyanins and condensed tannins localized in the seed coat (*43*).

Extracts of bean hulls, particularly enriched in phenolics able to modulate inflammation, can be an important dietary factor in developing new therapeutic products associated with reduced inflammation and overall risk reduction of CVD. Our findings, one of the few reports on COX and LOX inhibitory activities, raise the possibility that the use of bean hulls in foods may help protect against some diseases associated with chronic inflammation by virtue of its aspirin-like COX-inhibiting activity.

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