

Comparison of Antioxidant Potency of Commonly Consumed Polyphenol-Rich Beverages in the United States

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A number of different beverage products claim to have antioxidant potency due to their perceived high content of polyphenols. Basic and applied research indicates that pomegranate juice (PJ), produced from the Wonderful variety of *Punica granatum* fruits, has strong antioxidant activity and related health benefits. Although consumers are familiar with the concept of free radicals and antioxidants, they are often misled by claims of superior antioxidant activity of different beverages, which are usually based only on testing of a limited spectrum of antioxidant activities. There is no available direct comparison of PJ's antioxidant activity to those of other widely available polyphenol-rich beverage products using a comprehensive variety of antioxidant tests. The present study applied (1) four tests of antioxidant potency [Trolox equivalent antioxidant capacity (TEAC), total oxygen radical absorbance capacity (ORAC), free radical scavenging capacity by 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP)]; (2) a test of antioxidant functionality, that is, inhibition of low-density lipoprotein (LDL) oxidation by peroxides and malondialdehyde methods; and (3) evaluation of the total polyphenol content [by gallic acid equivalents (GAEs)] of polyphenol-rich beverages in the marketplace. The beverages included several different brands as follows: apple juice (3), açai juice (3), black cherry juice (3), blueberry juice (3), cranberry juice (3), Concord grape juice (3), orange juice (3), red wines (3), iced tea beverages (10) [black tea (3), green tea (4), white tea (3)], and a major PJ available in the U.S. market. An overall antioxidant potency composite index was calculated by assigning each test equal weight. PJ had the greatest antioxidant potency composite index among the beverages tested and was at least 20% greater than any of the other beverages tested. Antioxidant potency, ability to inhibit LDL oxidation, and total polyphenol content were consistent in classifying the antioxidant capacity of the polyphenol-rich beverages in the following order: PJ > red wine > Concord grape juice > blueberry juice > black cherry juice, açai juice, cranberry juice > orange juice, iced tea beverages, apple juice. Although in vitro antioxidant potency does not prove in vivo biological activity, there is also consistent clinical evidence of antioxidant potency for the most potent beverages including both PJ and red wine.

INTRODUCTION

Pomegranate (*Punica granatum* L.) fruits are popularly consumed in beverage forms such as pomegranate juice (PJ). Several studies have been conducted on a well-characterized PJ made from the Wonderful variety of *P. granatum* fruits (1–6). Basic and applied research in animals and humans indicates that this PJ has potent antioxidant activity, which has been linked to a diverse group of polyphenols including ellagitannins,

gallotannins, ellagic acid, and flavonoids, such as anthocyanins (1). Whereas there are numerous phytochemicals consumed in our diet, polyphenols constitute the largest group and have attracted much attention due to their antioxidant properties (7). In fact, the potential health benefits of plant foods are commonly linked to their polyphenol content.

Currently, there are a number of commercial ready-to-drink (RTD) polyphenol-rich beverages, which base their marketing strategies on antioxidant potency. Apart from PJ, other popularly consumed RTD polyphenol-rich beverages that claim high antioxidant potency include red wine, berry fruit juices (e.g., blueberry, black cherry, Concord grape, cranberry, etc.), apple juice, bottled tea beverages, and, recently, the Amazonian palm

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Table 1. Antioxidant Potency of Major Brands of Leading Ready-to-Drink Polyphenol-Rich Beverages Available in the United States^a

beverage	brand	DPPH (% inhibited)	ORAC (μmol of TE/mL)	FRAP (μmol of FE/mL)	TEAC (μmol /mL)
pomegranate juice	A	50.1 \pm 1.9	25.0 \pm 1.0	8.1 \pm 0.3	41.6 \pm 1.8
red wine	A	37.2 \pm 1.8	26.7 \pm 3.5	4.6 \pm 0.1	19.8 \pm 0.4
	B	31.7 \pm 3.3	24.0 \pm 2.0	3.8 \pm 0.5	17.1 \pm 0.9
	C	36.8 \pm 1.4	26.5 \pm 0.7	4.5 \pm 0.2	19.2 \pm 0.7
	av	35.2 \pm 2.2	25.7 \pm 2.1	4.3 \pm 0.3	18.7 \pm 0.7
Concord grape juice	A	27.4 \pm 4.6	26.4 \pm 1.9	5.0 \pm 0.2	14.9 \pm 0.6
	B	32.0 \pm 8.4	30.5 \pm 1.4	5.4 \pm 1.1	21.7 \pm 1.4
	C	25.1 \pm 5.2	20.8 \pm 2.2	4.4 \pm 0.9	14.9 \pm 1.1
	av	28.2 \pm 6.1	25.9 \pm 1.8	4.9 \pm 0.8	17.2 \pm 1.0
blueberry juice	A	31.3 \pm 2.2	23.5 \pm 2.6	4.7 \pm 0.4	17.1 \pm 0.5
	B	17.5 \pm 0.8	23.9 \pm 2.4	4.3 \pm 0.1	14.7 \pm 0.5
	C	13.0 \pm 1.2	14.5 \pm 3.8	3.6 \pm 1.1	13.3 \pm 0.6
	av	20.6 \pm 1.4	20.6 \pm 2.9	4.2 \pm 0.5	15.0 \pm 0.5
black cherry juice	A	10.9 \pm 1.2	22.1 \pm 2.0	3.3 \pm 0.4	11.4 \pm 0.5
	B	8.2 \pm 1.5	22.1 \pm 3.0	3.0 \pm 0.6	11.6 \pm 0.8
	C	14.8 \pm 0.6	31.7 \pm 4.9	3.8 \pm 0.1	17.8 \pm 0.2
	av	11.3 \pm 1.1	25.3 \pm 3.3	3.4 \pm 0.4	13.6 \pm 0.5
acai juice	A	21.3 \pm 1.0	16.6 \pm 0.6	4.3 \pm 0.4	12.8 \pm 0.4
	B	22.2 \pm 1.9	22.9 \pm 2.8	4.1 \pm 0.6	16.2 \pm 0.8
	C	14.8 \pm 0.8	17.1 \pm 1.2	3.5 \pm 2.0	11.4 \pm 0.6
	av	18.3 \pm 1.2	19.5 \pm 1.5	3.8 \pm 1.0	12.8 \pm 0.7
cranberry juice	A	19.2 \pm 0.7	9.1 \pm 1.0	2.2 \pm 0.2	6.7 \pm 0.3
	B	21.4 \pm 0.6	21.5 \pm 3.1	3.8 \pm 0.8	14.8 \pm 0.5
	C	17.1 \pm 0.6	15.9 \pm 2.1	2.2 \pm 0.6	9.6 \pm 0.4
	av	19.2 \pm 0.6	15.4 \pm 2.1	2.7 \pm 0.5	10.4 \pm 0.4
orange juice	A	12.9 \pm 1.0	9.2 \pm 0.3	1.5 \pm 0.1	3.4 \pm 0.4
	B	14.4 \pm 0.6	6.1 \pm 0.7	1.5 \pm 0.5	4.4 \pm 0.2
	C	10.9 \pm 1.3	6.9 \pm 0.4	1.5 \pm 0.1	4.8 \pm 0.3
	av	12.7 \pm 1.0	7.4 \pm 0.5	1.5 \pm 0.2	4.2 \pm 0.3
iced green tea	A	21.0 \pm 0.5	5.8 \pm 0.8	1.7 \pm 0.1	6.8 \pm 0.5
	B	24.3 \pm 6.5	6.1 \pm 3.9	1.9 \pm 0.8	10.5 \pm 0.5
	C	26.6 \pm 2.3	6.0 \pm 0.4	1.9 \pm 0.4	7.5 \pm 0.4
	D	17.5 \pm 0.8	3.2 \pm 2.4	1.5 \pm 0.2	4.8 \pm 0.2
	av	22.3 \pm 2.6	5.3 \pm 1.9	1.7 \pm 0.4	7.4 \pm 0.4
iced black tea	A	19.7 \pm 0.9	5.9 \pm 0.2	1.0 \pm 0.2	5.3 \pm 0.3
	B	11.1 \pm 2.4	1.7 \pm 0.4	0.5 \pm 0.1	4.0 \pm 0.2
	C	8.1 \pm 1.7	1.8 \pm 0.0	0.1 \pm 0.0	1.5 \pm 0.1
	av	13.0 \pm 1.7	3.1 \pm 0.2	0.5 \pm 0.1	3.6 \pm 0.2
iced white tea	A	21.6 \pm 8.7	2.3 \pm 0.3	1.1 \pm 0.1	4.2 \pm 0.4
	B	19.6 \pm 0.9	4.8 \pm 0.5	1.3 \pm 0.2	6.9 \pm 0.9
	C	5.1 \pm 0.3	1.0 \pm 0.1	0.2 \pm 0.0	1.1 \pm 0.5
	av	15.4 \pm 3.3	2.7 \pm 0.3	0.9 \pm 0.1	4.1 \pm 0.6
apple juice	A	15.4 \pm 2.1	2.5 \pm 0.3	1.4 \pm 0.2	4.3 \pm 0.3
	B	9.8 \pm 2.8	5.7 \pm 2.0	1.1 \pm 1.2	3.6 \pm 0.4
	C	10.2 \pm 0.8	6.2 \pm 0.8	1.0 \pm 0.1	2.7 \pm 0.3
	av	11.8 \pm 1.9	4.8 \pm 1.0	1.2 \pm 0.5	3.6 \pm 0.3

^a TEAC, Trolox equivalent antioxidant capacity; ORAC, oxygen radical absorbing capacity; FRAP, ferric reducing antioxidant capacity; DPPH, free radical scavenging properties by diphenyl-1-picrylhydrazyl radical.

berry, *Euterpe oleraceae* Mart. (açaf), juice. However, to the best of our knowledge, data on the direct comparison of PJ's antioxidant activity to those of these widely available leading beverage products are unavailable. It is of great interest to the general public to know the antioxidant capacity of the beverages that they consume. However, it should be cautioned that because of the inherent complexity of food matrices, the use of one antioxidant capacity method to determine antioxidant potency is ineffective. This is because antioxidants respond to different reactive species in different tests, which is partially attributed to multiple reaction mechanisms and reaction phases (8, 9).

The aim of the current study was to compare the antioxidant potency of PJ to other leading brands of RTD polyphenol-rich beverages, available either nationally or regionally. Because no single antioxidant assay can accurately reflect the antioxidant potency of any beverage, we utilized four tests used to measure antioxidant potency [(1) Trolox equivalent antioxidant capacity (TEAC), (2) oxygen radical absorbing capacity (ORAC), (3) ferric reducing antioxidant power (FRAP), and (4) free radical scavenging properties by the diphenyl-1-picrylhydrazyl (DPPH) radical] and one test of antioxidant antioxidant functionality, namely, the inhibition of low-density (LDL) oxidation by

Table 2. Antioxidant Functionality of Major Brands of Leading Ready-to-Drink Polyphenol-Rich Beverages Available in the United States as a Measure of Ability To Inhibit Oxidation of Low-Density Lipoprotein (LDL) by the Peroxides and Malondialdehyde Methods

beverage	brand	inhibition of LDL oxidation (peroxides)	inhibition of LDL oxidation (malondialdehyde)
pomegranate juice	A	97.1 ± 0.0	97.2 ± 0.7
red wine	A	86.5 ± 5.6	69.7 ± 5.3
	B	70.2 ± 12.8	57.6 ± 12.5
	C	73.5 ± 7.3	56.6 ± 6.2
	av	76.7 ± 8.6	61.3 ± 8.0
grape juice	A	35.1 ± 10.5	31.1 ± 18.7
	B	46.5 ± 10.2	51.0 ± 18.4
	C	40.0 ± 6.0	26.7 ± 2.3
	av	40.5 ± 8.9	36.3 ± 13.1
blueberry juice	A	77.1 ± 10.6	59.1 ± 8.1
	B	42.9 ± 12.3	59.4 ± 6.1
	C	35.1 ± 5.9	17.8 ± 6.0
	av	51.7 ± 9.6	45.5 ± 6.8
black cherry juice	A	27.6 ± 3.1	10.0 ± 0.0
	B	25.1 ± 3.0	7.5 ± 1.6
	C	52.2 ± 2.1	82.9 ± 1.2
	av	35.0 ± 2.7	33.4 ± 0.9
acai juice	A	24.3 ± 0.9	14.5 ± 1.4
	B	29.2 ± 15.5	20.4 ± 6.7
	C	20.2 ± 1.2	14.2 ± 0.5
	av	21.7 ± 5.9	13.0 ± 2.8
cranberry juice	A	18.8 ± 2.4	21.2 ± 2.4
	B	58.2 ± 17.8	50.1 ± 11.6
	C	39.4 ± 10.0	45.6 ± 7.0
	av	38.8 ± 10.1	39.0 ± 7.0
orange juice	A	11.4 ± 7.6	9.8 ± 5.4
	B	16.9 ± 3.9	8.0 ± 2.6
	C	10.6 ± 0.6	5.3 ± 0.0
	av	12.9 ± 4.0	7.7 ± 2.7
apple juice	A	0.2 ± 0.3	-0.9 ± 2.7
	B	2.1 ± 3.2	0.6 ± 0.0
	C	2.7 ± 2.1	3.7 ± 2.7
	av	1.7 ± 1.9	1.1 ± 1.8
iced green tea	A	9.4 ± 5.1	11.4 ± 2.8
	B	12.0 ± 4.8	18.7 ± 0.3
	C	10.4 ± 0.7	3.9 ± 2.4
	D	4.9 ± 3.8	6.7 ± 3.1
	av	9.2 ± 3.6	10.2 ± 2.2
iced black tea	A	9.8 ± 1.9	13.7 ± 2.6
	B	6.9 ± 4.1	16.5 ± 3.3
	C	5.7 ± 1.8	17.9 ± 2.9
	av	7.4 ± 2.6	16.1 ± 2.9
iced white tea	A	9.5 ± 2.2	12.9 ± 1.3
	B	5.3 ± 1.7	9.5 ± 5.3
	C	1.5 ± 0.4	11.4 ± 2.0
	av	5.4 ± 1.4	11.3 ± 2.9

peroxides and malondialdehyde determinations. In addition, the beverages were also evaluated for polyphenol content as gallic acid equivalents (GAEs). The leading brands of beverages that PJ was compared to included apple juices (3), açai juices (3), black cherry juices (3), blueberry juices (3), cranberry juices (3), Concord grape juices (3), orange juices (3), red wines (3), and iced tea beverages (10), consisting of black tea (3), green tea (4), and white tea (3).

MATERIALS AND METHODS

Ready-to-Drink Polyphenol-Enriched Beverages. The products used for the study are among the top brands in the selected beverage categories either nationally or regionally as follows: pomegranate juice (1), A, POM Wonderful 100% pomegranate (POM Wonderful LLC, Los Angeles, CA; 15MAY07Y0038, 16MAY07Y1804, 10MAY07Y0137); red wines (3), A, Merlot Beringer (Beringer Vineyards, Napa, CA; lot Founders Estate 2004-L11306B110, lot 3RD Century 2004-1101611, lot Founders Estate 2004-1 1130 6B 117), B, Zinfandel Robert Mondavi (Robert Mondavi, Woodbridge, CA, lot Private Selection 2005-LW34760602, lot Private Selection 2005-L2020106, lot Private Selection 2005-LW3450602), C, Cabernet Sauvignon Turning Leaf (Turning Leaf Vineyards, Modesto, CA, lot 2005-LB300107HE, lot 2005-LB171006DE, lot 2005-LB060206HH); Concord grape juices (3), A, RW Knudsen-Just Concord (Knudsen & Sons Inc., Chico, CA; lot NOV 29 2008 6 333 003, lot DEC 08 2007 5 342 003, lot DEC 09 2007 5 343 003), B, Lakewood-Pure Concord Grape (Lakewood, Miami, FL; lot 146S FEB232009, lot OCT252008, 4/10/2007, lot MAR012008), C, Welch's Grape Juice (Welch's, Concord, MA; lot DEC-11-07 6NL11L1451, lot 7N17A1048 JAN-17-08, lot PL 22 B11 FEB-23-08); blueberry juices (3), A, RW Knudsen-Just Blueberry (Knudsen & Sons Inc.; FEB 14 2009 7 045 003, NOV 16 2008 6 320 003, SEP 20 2008 6 263 003), B, Trader Joe's Just Blueberry (distributed by Trader Joe's, Monrovia, CA; JAN 23 2008 7 023 003, JAN 10 2008 7 010 003, JUL 13 2007 6 194 003), C, Wyman's Wild Blueberry (Jasper Wyman & Son, Milbridge, ME; 8036 CA1 0903 CT 803, C136CA 1 0534 CT 803, 1267CA 0214 1 CT 803); black cherry juices (3), A, RW Knudsen-Just Black Cherry (Knudsen & Sons Inc.; JAN 18 2009 6 199 003, DEC 6 2008, DEC 14 2008 6 165 003), B, Lakewood-Pure Black Cherry (Lakewood; regular, red cap with vitamin C, 3/23/2007, organic, 141 VADA, organic, gold cap), C, Trader Joe's Just Cherry (distributed by Trader Joe's; JAN 23 2008 7 023 003, 3/23/2007, DEC 13 2007 6 347 003, DEC 21 2007 6 355 003); acai juices (3), A, Bolthouse Bom Dia Acai-Mangosteen (Bolthouse Juice Products LLC, Bakersfield, CA; lot 061107, lot 051107, lot 062607), B, Bossa Nova Acai Original (Bossa Nova Beverage Group Inc., Los Angeles, CA; lot 09 16 07, lot 10 10 07, lot 10 09 07), C, Sambazon Mango Uprising (Sambazon, San Clemente, CA; lot ASA07029 APR 2007, lot 0610T HA16PTK13, 4/07/2007, lot ASA07073 12 JUN 2007); cranberry juices (3), A, Northland 100% Juice Cranberry (distributed by Northland Products LLC, Port Washington, NY; lot 02/28/08, lot 02/13/08, lot 03/06/08), B, RW Knudsen-Just Cranberry (Knudsen & Sons Inc.; lot NOV 21 2008 6 325 003, lot JAN 11 2009 7 011 003, lot FEB 07 2009 7 038 003), C, Ocean Spray-Pure Cranberry (Ocean Spray Cranberries Inc., Lakeville-Middleboro, MA; lot MAY 28 07 CT841 CPI); orange juices (3), A, Florida's Natural Orange Juice (Florida's Natural Growers, division of Citrus World Inc., Lake Wales, FL; lot JUN 05 07, lot JUN 08 07, lot MAY 15 07), B, Tropicana Pure Premium Orange Juice (Tropicana Products Inc., Bradenton, FL; lot MAY 15 07 48NK0713, lot JUN 29 07 48NK0647, lot JUN 15 07 46NL1446), C, Minute Maid Premium Orange Juice (Minute Maid, produced for Coca-Cola Co., Atlanta, GA; lot APR 30 07 DN, lot MMOJ 02810 APR 30 07 1030 DN1CR, lot MAY 7 07 DN); iced green tea (4), A, Tazo Iced Green Tea (bottled for Tazo, Portland, OR; lot BB08NOV2007 L08NOV20060315, lot BB08JAN2008 L08JAN20070313, lot BB18OCT2007 L18OCT20060312), B, Honest Just Green Tea (Honest Tea Inc., Bethesda, MD; lot H06314, lot H06122), C, Lipton Original Green Tea-Honey (manufactured for Pepsi-Lipton Tea Partnership, Purchase, NY; lot MAY0707V 2237 YY 10306 3, lot AUG2707 0742 YY 02227 3, lot SEP2407 59745 2336 YY 03247 3), D, Snapple Green Tea-Mango (distributed for Snapple Beverage Corp., Rye Brook, NY; lot FC292 LPG 111506 D, lot 6200 12 1228, lot 6300 12 1230); iced black tea (3), A, Tazo Iced Tea with Lemon (bottled for Tazo; lot BB08MAY2007, L08MAY20060316, lot BB29JUN2007 L29JUN20060319, lot BB13NOV2007 L13NOV20060317), B, Lipton Original Iced Tea (manufactured for Pepsi-Lipton Tea Partnership; lot OCT2907 0926 YY 01257 3, lot NOV2607 0759 YY 02217 3, lot SEP 10 07 YY 120863), C, Nestea Sweetened Lemon Flavored Iced Tea (Nestle USA or Beverage Partners Worldwide, lot OCT1507TRB 08103, lot NOV0507TRC 19133, lot

Table 3. Phenolic Content, as Gallic Acid Equivalents (GAEs), of Commonly Consumed Beverages and Their Primary Antioxidant Phytochemicals As Reported in the Literature

beverage	GAEs (mg/mL)	primary antioxidant phytochemicals
pomegranate juice	3.8 ± 0.2	ellagitannins and anthocyanins (30)
red wine	3.5 ± 0.1	proanthocyanidins, anthocyanins, catechins, and flavonoids (31)
Concord grape juice	2.6 ± 0.4	proanthocyanidins, anthocyanins, catechins, flavonoids, and vitamin C added (31–33)
blueberry juice	2.3 ± 0.4	proanthocyanidins, catechins (34), anthocyanins (35), and other phenolic acids (36)
black cherry juice	2.1 ± 0.1	anthocyanins, flavonoids, flavan-3-ols, and other phenolic compounds (37)
acai juice	2.1 ± 0.1	proanthocyanidins, flavonoids, and anthocyanins (38)
cranberry juice	1.7 ± 0.2	proanthocyanidins, flavonoids, and anthocyanins (39)
orange juice	0.7 ± 0.1	flavonoids, phenolic acids, and vitamin C (40)
apple juice	0.4 ± 0.1	polyphenolic acid, flavonoids, proanthocyanidins (41, 42)
iced green tea	0.8 ± 0.1	catechins and phenolic acids (43)
iced black tea	0.4 ± 0.0	theaflavones, catechins, and phenolic acids (44)
iced white tea	0.9 ± 0.0	catechins and phenolic acids (45)

SEP1707TRC 11033); iced white tea (3), A, Snapple White Tea Nectarine (distributed for Snapple Beverage Corp.; lot LLP11C7 17022, lot FC292 LPG 111706 004421, lot FC292 LPG 121806), B, Honest Mango White Tea (Honest Tea Inc.; lot H06361, lot H06333), C, Inko's White Tea Original (bottled for Inko's White Iced Tea, New York, NY; lot CT90 A03308, lot CT90 A103208); apple juices (3), A, Dole Apple Juice (manufactured for Pepsico, Purchase, NY; lot AUG 06 07 JL 12066 2, lot NOV 05 07 JL 03057 2, lot SEP 10 07 JL 01097), B, Tree Top Apple Juice (Tree Top Inc., Selah, WA; 3067C LN1 03/05/08, 02/04/08 2047X 0503, 10/20/08 A206A), C, Mott's Apple Juice (manufactured for Motts LLP, Rye Brook, NY; lot 020807TA APR 08 08, lot 020707TA APR 07 08, lot 021207TA APR 12 08). All fruit juices, wines, and iced tea beverages were analyzed in late March or early April prior to their expiration dates as stated on their packages. All beverages were kept at storage conditions as specified on their labels prior to analyses. Chain-of-custody forms to verify unopened products within the same dates codes were generated for all test samples and are archived in our laboratory.

Determination of Total Polyphenols. Total polyphenols were determined spectrophotometrically according to the method of Singleton (10), modified for small volumes (11), and are reported as gallic acid equivalents (GAEs). Gallic acid stock solution was prepared in ethanol at a concentration of 1 mM.

Antioxidant Assays. Trolox Equivalent Antioxidative Capacity. The assay was performed as previously reported (12). Briefly, 2',2'-azobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical cations were prepared by adding solid manganese dioxide (80 mg) to a 5 mM aqueous stock solution of ABTS^{•+} (20 mL using a 75 mM Na/K buffer of pH 7). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analogue of vitamin E, was used as an antioxidant standard. A standard calibration curve was constructed for Trolox at 0, 50, 100, 150, 200, 250, 300, and 350 μM concentrations. Samples were diluted appropriately according to antioxidant activity in Na/K buffer pH 7. Diluted samples were mixed with 200 μL of ABTS^{•+} radical cation solution in 96-well plates, and absorbance was read (at 750 nm) after 5 min in a ThermoMax microplate reader (Molecular Devices, Sunnyvale, CA). Samples were assayed in six replicates. TEAC values were calculated from the Trolox standard curve and expressed as Trolox equivalents (in millimolar).

Total Oxygen Radical Absorbance Capacity. The ORAC assays were performed at Covance Analytical Laboratories, Inc. (Madison, WI), and were conducted as previously described (12). Briefly, a mixture of 0.125 mL of fluorescein (0.16 μM) was used as the target of free radical attack, and 0.250 mL of 2,2'-azobis(amidinopropane) dihydrochloride (AAPH) (147 mM) was used as a peroxy radical generator at 37 °C combined with 0.250 mL of each sample extract. Trolox standards ranged from 5 to 40 μM. The decrease in fluorescence of fluorescein was determined by collecting readings at excitation of 535 nm and emission of 595 nm every minute for 45 min in a Molecular Devices SpectraMax M2 plate reader. The ORAC value was evaluated as the area under curve (AUC) and calculated by taking into account the Trolox reading using the following equation: (AUC_{sample} - AUC_{buffer})/

(AUC_{Trolox} - AUC_{buffer}) × dilution factor of sample × initial Trolox concentration (mM). For each sample, four serial dilutions in phosphate buffer (pH 7.4) were measured.

Free Radical Scavenging Capacity. The free radical scavenging capacity was analyzed by the DPPH assay (13, 14). DPPH is a radical-generating substance that is widely used to monitor the free radical scavenging abilities (the ability of a compound to donate an electron) of various antioxidants. The DPPH radical has a deep violet color due to its impaired electron, and radical scavenging can be followed spectrophotometrically by the loss of absorbance at 517 nm, as the pale yellow nonradical form is produced. Aliquots from the analyzed compounds were mixed with 1 mL of 0.1 mM DPPH/L in ethanol and the change in optical density at 517 nm was continuously monitored using a Molecular Devices SpectraMax M2 plate reader.

Ferric Reducing Antioxidant Capacity Assay. The FRAP assays were performed using established standardized methods previously described at Covance Analytical Laboratories, Inc. (15). Reaction mixtures were prepared by combining 10 mM 2,4,6-tri[2-pyridyl-s-triazine] (TPTZ), 20 mM ferric chloride, and 300 mM (pH 3.6) sodium acetate buffer in a 1:1:10 ratio. Ferrous sulfate standards ranged from 100 to 1000 μM. A 0.3 mL portion of reaction solution was heated at 37 °C for 10 min, and then 0.020 mL of aqueous sample extracts was added. Sample absorbance was then read at 593nm in a Molecular Devices SpectraMax M2 plate reader and using linear regression results are in terms of millimolar ferric ions converted to the ferrous form per milliliter.

Inhibition of Low-Density Lipoprotein Oxidation. LDL was isolated from plasma derived from healthy normolipidemic volunteers, by discontinuous density gradient ultracentrifugation (16). The LDL was washed at $d = 1.063$ g/mL and dialyzed against 150 mmol/L NaCl and 1 mmol/L Na₂EDTA (pH 7.4) at 4 °C. The LDL was then sterilized by filtration (0.45 μM), kept under nitrogen in the dark at 4 °C, and used within 2 weeks. LDL (100 μg of protein/mL) was incubated for 10 min at room temperature with the beverages. Then, 5 μmol/L of CuSO₄ was added, and the tubes were incubated for 2 h at 37 °C. Cu²⁺-induced oxidation was terminated by the addition of butylated hydroxytoluene (BHT, 10 μM) and an immediate storage at 4 °C. At the end of the incubation, the extent of LDL oxidation was determined by measuring the generated amount of lipid peroxides and also by the thiobarbituric acid reactive substances (TBARS) assay at 532 nm, using malondialdehyde (MDA) for the standard curve (17, 18).

Statistical Analysis. Antioxidant capacity values were determined in six replicates for each sample tested, and the mean values ± standard deviation (SD) are reported. An overall antioxidant potency composite index was determined by assigning all assays an equal weight, assigning an index value of 100 to the best score for each test, and then calculating an index score for all other samples within the test as follows: antioxidant index score = [(sample score/best score) × 100]; the average of all seven tests for each beverage was then taken for the antioxidant potency composite index. All assays were given equal weight, and an overall mean index value was calculated on a normalized basis for each beverage. A simple rank order was reported, and where the values were close to each other, an equal rank was assigned.

Table 4. Antioxidant Potency Composite Index of Major Brands of Leading Ready-to-Drink Polyphenol-Rich Beverages Available in the United States^a

beverage	brand	DPPH index	ORAC index	FRAP index	TEAC index	antioxidant potency composite index ^b
pomegranate juice	A	100.0	78.9	100.0	100.0	95.8
red wine	A	74.3	84.2	56.8	47.6	72.0
	B	63.3	75.7	46.9	41.1	63.3
	C	73.5	83.6	55.6	46.2	69.6
	av	70.3	81.1	53.1	45.0	68.3
Concord grape juice	A	54.7	83.3	61.7	35.8	57.6
	B	63.9	96.2	66.7	52.2	70.0
	C	50.1	65.6	54.3	35.8	57.5
	av	56.3	81.7	60.5	41.3	61.7
blueberry juice	A	62.5	74.1	58.0	41.1	60.8
	B	34.9	75.4	53.1	35.3	52.4
	C	25.9	45.7	44.4	32.0	40.7
	av	41.1	65.0	51.9	36.1	50.9
black cherry juice	A	21.8	69.7	40.7	27.4	42.4
	B	16.4	69.7	37.0	27.9	40.2
	C	29.5	100.0	46.9	42.8	57.0
	av	22.6	79.8	42.0	32.7	46.5
acai juice	A	42.5	52.4	53.1	30.8	46.8
	B	44.3	72.2	50.6	38.9	54.4
	C	29.5	53.9	43.2	27.4	44.0
	av	36.5	61.5	46.9	30.8	46.2
cranberry juice	A	38.3	28.7	27.2	16.1	27.3
	B	42.7	67.8	46.9	35.6	52.8
	C	34.1	50.2	27.2	23.1	33.7
	av	38.5	48.9	33.3	24.8	38.0
orange juice	A	25.7	29.0	18.5	8.2	20.0
	B	28.7	19.2	18.5	10.6	19.1
	C	21.8	21.8	18.5	11.5	17.9
	av	25.3	23.3	18.5	10.1	19.1
apple juice	A	30.7	7.9	17.5	10.3	15.2
	B	19.5	17.9	14.0	8.7	14.1
	C	20.3	19.5	12.3	6.5	13.8
	av	23.6	15.1	14.8	8.7	14.6
iced green tea	A	41.9	18.3	21.0	16.3	23.2
	B	48.5	19.2	23.5	25.2	27.5
	C	53.1	18.9	23.5	18.0	29.0
	D	34.9	10.1	18.5	11.5	17.1
	av	44.5	16.7	21.0	17.8	24.2
iced black tea	A	39.3	18.6	12.3	12.7	19.8
	B	22.2	5.4	6.2	9.6	10.8
	C	16.2	5.7	1.2	3.6	6.4
	av	25.9	9.8	6.2	8.7	12.2
iced white tea	A	43.1	7.3	13.6	10.1	23.8
	B	39.1	15.1	16.0	16.6	21.1
	C	10.2	3.2	2.5	2.6	4.7
	av	30.7	8.5	11.1	9.9	16.8

^a TEAC, Trolox equivalent antioxidant capacity; ORAC, oxygen radical absorbing capacity; FRAP, ferric reducing antioxidant capacity; DPPH, free radical scavenging properties by diphenyl-1-picrylhydrazyl radical. ^b Antioxidant index score = [(sample score/best score) × 100], averaged for all seven tests for each beverage for the antioxidant potency composite index.

RESULTS

Table 1 shows the antioxidative potency (by TEAC, DPPH, ORAC, and FRAP assays) and **Table 2** shows the antioxidant functionality (by inhibition of LDL oxidation) of the leading types of RTD polyphenol-rich antioxidant beverage categories sold in the United States. **Table 3** shows the phenolic content, as GAEs, of the commonly consumed beverages, and their primary antioxidant phytochemicals as reported in the literature. **Table 4** shows the antioxidant potency composite index

determined for the beverages based on ranking of all four antioxidant assays, TEAC, DPPH, ORAC, and FRAP.

Generally, it is known that total polyphenols are highly correlated with antioxidant activity, and the bioavailability of polyphenols has been reported (19). As shown in **Figure 1**, as a group, the ordering of the average amounts of total phenolics for the beverages was as follows: PJ > red wine > Concord grape juice > blueberry juice > black cherry juice, acai juice, cranberry juice > orange juice, iced tea beverages, apple juice.

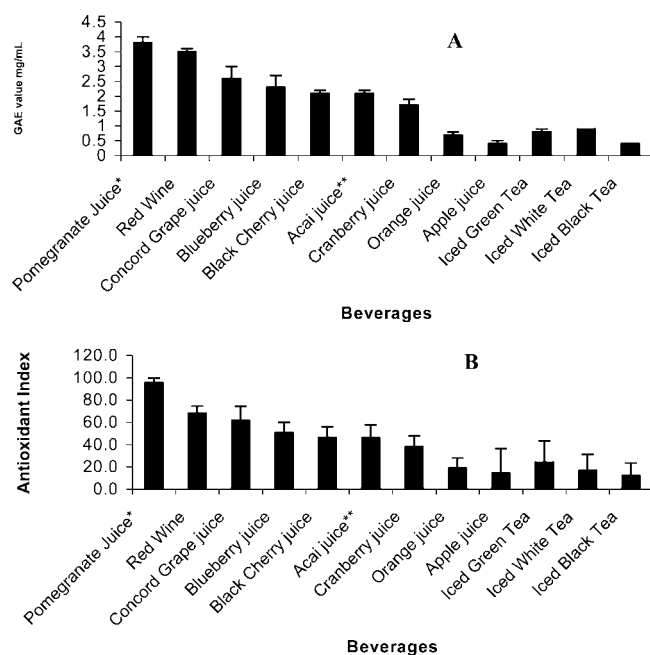


Figure 1. (A) Total phenolics in ready-to-drink polyphenol-rich antioxidant beverages as gallic acid equivalents (GAEs) and (B) antioxidant potency composite index of ready-to-drink polyphenol-rich antioxidant beverages calculated as a percentage of average antioxidant activities compared to the highest one in each assay and sum of the individual index divided by the number tested (four assays in total: DPPH, ORAC, TEAC, and FRAP). Each beverage had three different batches, and each sample was analyzed in triplicate ($n = 3$). *, POM Wonderful 100% pomegranate juice. **, the acai juices in **Figure 1** and **Tables 1** and **2** did not include Mona Vie, the premier acai blend, because it is a blend of acai and 18 other fruit juices. The Mona Vie data show the polyphenol and antioxidant index to be in the same range as for the acai juices reported or in the midrange for all beverages analyzed in this study (unpublished data).

The order of antioxidant potency showed the same trend as the total phenolic content in the beverages as follows: PJ > red wine > Concord grape juice > blueberry juice > black cherry juice, acai juice, cranberry juice > orange juice, iced tea beverages, apple juice (see **Figure 1**). Similarly, as a test of antioxidant functionality, the LDL susceptibility to oxidation values was highly correlated with both total polyphenol content and antioxidant capacity assays as follows: PJ > red wine > blueberry juice > Concord grape juice, cranberry juice, black cherry juice > acai juice, orange juice, iced tea beverages, apple juice. However, the most widely marketed method of determining antioxidant capacity is the ORAC method, and this method gave a rank order different from the above methods as follows: Concord grape juice, red wine, PJ, black cherry juice > blueberry juice, acai juice, cranberry juice > orange juice, iced tea beverages, apple juice. Nevertheless, when all of the methods were combined into a single index of antioxidant activity (**Table 4**), the rank order was as shown in **Figure 1**: PJ > red wine > grape juice > blueberry juice > black cherry juice, acai juice, cranberry juice > iced tea beverages, orange juice, apple juice. PJ's overall antioxidant index was at least 20% higher than any of the other beverages tested, thereby displaying the most complete free radical neutralizing range/bandwidth.

DISCUSSION

Being a polyphenol-rich food with health benefits has become a more common element in food marketing. The public is highly aware of the term "antioxidant", which has been defined by the

Institute of Medicine of the National Academy of Sciences as follows: "a substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiologic function in humans." Therefore, the marketing of many so-called "superfoods" is commonly based on their antioxidant potential. In fact, a number of antioxidant foods claim to have superior antioxidant activity with health benefits based on *in vitro* antioxidant assays, and a limited number also have clinical evidence demonstrating effects on physiological function that can be related to oxidant protection.

In the present study, among the most popular national brands of polyphenol-rich antioxidant beverages including 100% fruit juices, iced tea beverages, and red wine, PJ had the most potent antioxidant capacity followed by red wine and grape juice (see **Figure 1** and **Table 4**). The order of antioxidant capacity was very consistent across the different methods with the exception of the ORAC method. The ORAC method is the most widely recognized assay used by food manufacturers but has significant internal variability. Using the ORAC assay, the antioxidant activity is determined as area under the curve of a 60 min measurement of the protection from oxidation by free radicals (AAPH) generated in a temperature-dependent reaction. On the basis of technical issues related to temperature gradients across the plate in commonly used plate readers, this assay can have significant internal variabilities.

The ORAC method has been applied extensively to evaluate the antioxidant capacity of a large variety of foods (20, 21), and many supplement and functional food companies compare their products, including juices, favorably to fruits and vegetables using the ORAC results from those studies. In fact, Prior et al. also evaluated some of the fruit juices used in our study, and there is a good agreement with the ranking (22). However, our laboratory has demonstrated that temperature variation in the plate readers used in this assay leads to increased variability of this method (unpublished data). Although this technical issue does not pertain to the end point determinations used in the TEAC, FRAP, and DPPH assays or to the assays of LDL oxidation, we chose to include the data from the ORAC assay in our overall determination of an antioxidant index for fruit juice beverages. Therefore, in our view, it is important to run multiple antioxidant methods rather than just the ORAC method to get a better estimate of antioxidant capacity and to substantiate *in vitro* results with clinical studies. Furthermore, because *in vitro* results are not necessarily translated into *in vivo* effects, issues such as the bioavailability and metabolism of phenolic compounds should be taken into account in the overall evaluation of the impact of "phenolic/antioxidant-rich" foods on human health.

Multiple assays with different sensitivities and specificities for antioxidant activity are being used separately to justify health claims. At the 2007 meeting of the Institute of Food Technologists, a number of new polyphenol-rich fruits were being identified as "superfruits" including acai, mangosteen, noni, sea buckthorn, and Chinese wolfberry (goji). Consumers have a difficult time distinguishing among the various antioxidant claims for widely available antioxidant beverages even without considering these newer entries to the marketplace. Therefore, the present study was significant in comparing the most commonly available national brands of RTD beverages for antioxidant activity using the most well-known laboratory methods for determining antioxidant capacity.

PJ had the highest antioxidant capacity and the most complete antioxidant coverage *in vitro*. In addition, there is extensive evidence of physiological activity of this juice in humans with regard to intima media thickness (5), cardiac blood flow (23),

prostate cancer progression (2), erectile dysfunction (24), and type 2 diabetes mellitus (25). All of these can arguably be related to protection from oxygen radicals or closely related anti-inflammatory effects of antioxidant phytochemicals (26). The bioavailability of PJ polyphenols and active metabolites has also been extensively studied to support the translation of this in vitro research into in vivo bioactivity (1, 27).

This study applied in vitro antioxidant capacity testing to reflect the multiple antioxidant capacity tests using different reagents to provide a more complete profile of antioxidant capacity. The present research demonstrates that although a number of popular beverages have evidence of antioxidant activity in vitro, there are clear differences in antioxidant potency. Some beverages with lower potency would need to be consumed in much larger amounts to equal the antioxidant potency of PJ. Whereas antioxidant potency in vitro may not always correlate with antioxidant effects in humans, research with PJ (1–6, 23–25) and red wine (28, 29) suggests that these two potent antioxidant beverages do have effects in humans including anti-inflammatory effects.

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