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Comparison of Anti-oxidative Activity in a Single Serving Size of the Commercial Coffees and Teas

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ABSTRACT - The aim of this work was to study the comparison of anti-oxidative activity in a single serving size of commercial coffees and teas. Commercial regular coffees and teas, including, brand regular coffees (BC_A, BC_B, BC_C, BC_D, and BC_E), green tea (GT_A, GT_B, GT_C, and GT_D), black tea (BT_A, BT_B, and BT_C), pu-erh tea (PT_A, PT_B, and PT_C), chamomile tea (CT_A, CT_B, and CT_C), peppermint tea (P_A, P_B, and P_C), polygonatum odoratum tea (POT_A, POT_B, and POT_C), and jujube tea (JT_A, JT_B, and JT_C) were assayed for the levels of ascorbic acid, caffeine, total content of polyphenols and flavonoids, and ability to scavenge free radicals, using two *in vitro* antioxidant assays. The scavenging abilities of BC_A and BC_C were 664.91 ± 48.87 mg ascorbic acid equivalent/serving size and 624.36 ± 16.18 mg ascorbic acid equivalent/serving size, respectively. The four beverage samples (BC_A, BC_C, GT_D, and BT_A) significantly reduced the production of reactive oxygen species (ROS) and intracellular oxidative stress induced by H_2O_2. These results suggest that the beverages possess significant radical scavenging ability, which may be due to the presence of antioxidants. Furthermore, the significant reducing level of ROS evidences the potential antioxidant effects of these beverages in human cells.

Key words : Antioxidants, Coffee, Polyphenols, Reactive oxygen species, Tea

Free radicals are atoms, molecules, or ions with unpaired electrons. These unpaired electrons are highly reactive towards other substances. Thus, free radicals are highly reactive, and lead to uncontrolled reactions that damage macromolecules, such as proteins, lipids, and DNA. There are several different types of free radicals, derived from oxygen and nitrogen, formed in human body. The oxygen-derived free radicals (referred to as reactive oxygen species, ROS) include the hydroxyl radical ($OH^{\bullet}$), peroxyl radical ($ROO^{\bullet}$), alkoxyl radical ($RO^{\bullet}$), and the superoxide anion ($O_2^{\bullet-}$). The reactive oxygen species (ROS) are continuously generated in human body. For example, ROS are produced as by-products of mitochondrial respiration and processes mediated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidase, and uncoupled NO synthases[1]. The term "antioxidant" refers to any molecule capable of stabilizing or deactivating free radicals before they attack cells[2]. Normally, humans have an antioxidant system, consisting of enzymatic and nonenzymatic components, to protect the cells and organs of the body against the damage caused by free radicals. The enzymatic antioxidants include superoxide dismutase, catalase, and glutathione peroxidase; the nonenzymatic antioxidants involve vitamin C, vitamin E, carotenoids, natural flavonoids, and other compounds[3]. However, an over-production of ROS radicals leads to an imbalance between the generation and elimination of ROS radicals in the body, leading to oxidative stress[4]. Oxidative stress is a causative factor in various diseases, such as age-related degenerative conditions, cancers, cardiovascular diseases, asthma, decline of the immune system, brain dysfunction, liver injury, type 2 diabetes, and the aging process[5].

For those reasons, it is important to find agents that can reduce oxidative stress by directly scavenging free radicals. Several studies report that active dietary ingredients, such as phytochemicals, protect our cells from damage caused by free radicals. These phytochemicals include vitamins C, D,

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and E, alkaloids (caffeine and theobromine), and polyphenols[6]. Vitamin C (ascorbic acid) is a water-soluble free radical scavenger that reduces the levels of ROS radicals and acts as primary defense against aqueous radicals in the blood[7]. Caffeine, and its metabolites in humans, may be highly effective against lipid peroxidation induced by reactive oxygen species[8]. Polyphenols and phenolic compounds are ubiquitous in the plant kingdom. More than 8,000 phenolic compounds have been isolated in a wide variety of forms, all possessing one common structural feature: a phenol, which is an aromatic ring bearing at least one hydroxyl substituent[9]. Because of their antioxidant activity, polyphenols are useful in the prevention of, and symptomatic relief in, such disorders as neurodegenerative and cardiovascular diseases, cancer, and stroke[10]. Flavonoids are a family of polyphenols with strong antioxidant activity in humans. Regular intake of flavonoid-rich foods is associated with a delay in the onset of Alzheimer's disease and a reduction in the risk of developing Parkinson's disease[11].

The consumption of coffee and tea significantly exceeds that of beer, wine, and soft drinks worldwide[12]. Aside from water, coffee and tea (green tea, chamomile tea, peppermint tea, polygonatum odoratum tea, pu-erh tea, black tea, and jujube tea) are the most widely consumed beverages in the Asian regions. Most coffee or tea consumers do not drink it for their health[13,14]. However, coffee and green tea contain a multitude of antioxidants, such as vitamins, caffeine, and polyphenols[15]. Hence, we assessed the content of ascorbic acid, caffeine, total polyphenols, and flavonoids in a single serving size of the commercial regular coffees and teas.

Materials and Methods

Chemicals and reagents

Ascorbic acid, gallic acid, quercetin, Folin-Ciocalteu's phenol reagent, aluminum nitrate nonahydrate, potassium persulfate, DPPH (2, 2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt), MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), DMSO (anhydrous dimethyl sulfoxide), and Hydrogen peroxide (H_2O_2) were all obtained from Sigma-Aldrich (Steinheim, Germany). Caffeine, potassium dihyrogen phosphate, trifluoroacetic acid, metaphosphoric acid, sodium carbonate anhydrous, and potassium acetate were purchased from Wako (Osaka, Japan). Acetonitrile, ethanol, and methanol were purchased from Merck (Darmstadt, Germany). DMEM (Dulbecco's modified Eagle's medium) and 100 × antibiotic-antimycotic reagent were purchased from Thermo Fisher Scientific (Waltham, MA, USA). FBS (Fetal bovine serum) and H_2DCF-DA (Dichlorodihydrofluorescein diacetate) were from Invitrogen Molecular Probes (Eugene, OR, USA). HeLa cell lines were obtained from Korean Cell Line Bank.

Samples and preparation

Samples of commercial regular coffee and tea were purchased at nationwide coffee chain stores, and department stores, large discount stores, in Daejeon metropolitan area, Republic of Korea. The commercial brands (A, B, C, D, and E) of regular coffee, used in this study, were purchased in the quantities of three cups per brand (Table 1). The average capacity (in mL) of a cup of each brand of regular coffee (Americano) was A (284 ± 5), B (319 ± 2), C (304 ± 6), D

Table 1. Commercial regular coffee samples

Coffees	Region	Roasting degree	Coffee powder (g)	Espresso			Addition of water (mL)
				Extraction temperature (°C)	Extraction time (s)	Volume (mL)	
Brand Coffee							
BC_A	Arabica 80%, Robusta 20% (India, Brazil, Ethiopia, Colombia, Peru, El Salvador)	Medium	15	94	25	36	248
BC_B	Arabica (Latin America, Asia/Pacific)	Dark	15	93	21	40	279
BC_C	Arabica (Guatemala 50%, Colombia 30%, Brazil 20%)	Medium	15	93	24	34	270
BC_D	Arabica (Brazil, Ethiopia, Colombia, PNG)	Medium	15	94	24	36	265
BC_E	Arabica (Brazil 50%, Colombia 30%, Guatemala 10%, Ethiopia 10%)	Medium	15	94	27	39	286

Table 2. Commercial tea samples

Teas	Country of origin	Amount (g) and number of teabags, package	Obtained from	Making of tea	Amount (g) used for 1 cup of tea	Best before, month/year
Green Tea						
GT_A	Republic of Korea	Powder, 50.0	Large discount store	Not described	1.0	09/2018
GT_B	Republic of Korea	1.2 × 50 teabags	Large discount store	Not described	1.2	09/2018
GT_C	Republic of Korea	2.0 × 20 teabags	Large discount store	Boiling water, 2~3 min	2.0	08/2018
GT_D	Republic of Korea	Powder, 50.0	Large discount store	Not described	1.0	07/2018
Black Tea						
BT_A	India	3.125 × 80 teabags	Large discount store	Boiling water, 2~3 min	3.125	05/2018
BT_B	Kenya	2.0 × 100 teabags	Large discount store	Boiling water, 2~3 min	2.0	08/2018
BT_C	Sri Lanka	2.5 × 50 teabags	Department store	Boiling water, 4~5 min	2.5	06/2018
Pu-erh Tea						
PT_A	China	0.9 × 40 teabags	Department store	Boiling water (100 mL)	0.9	05/2018
PT_B	China	0.6 × 40 teabags	Department store	Boiling water (100 mL)	0.6	03/2018
PT_C	China	0.7 × 40 teabags	Department store	Boiling water (100 mL), 1~2 min	0.7	03/2018
Chamomile Tea						
CT_A	Germany	0.6 × 20 teabags	Large discount store	Boiling water, 2~3 min	0.6	09/2018
CT_B	Croatia	1.2 × 10 teabags	Large discount store	Boiling water (100~150 mL), 1~2 min	1.2	06/2018
CT_C	Croatia	0.6 × 30 teabags	Large discount store	Boiling water (120 mL), 1~2 min	0.6	09/2018
Peppermint Tea						
P_A	Germany	0.6 × 20 teabags	Large discount store	Boiling water, 1~2 min	0.6	09/2018
P_B	Poland	1.2 × 10 teabags	Large discount store	Boiling water (100~150 mL), 1~2 min	1.2	08/2018
P_C	Poland	0.6 × 30 teabags	Large discount store	Boiling water (120 mL), 1~2 min	0.6	09/2018
Polygonatum Odoratum Tea						
POT_A	Republic of Korea	1.0 × 7 teabags	Large discount store	Boiling water (180 mL), 2 min	1.0	07/2018
POT_B	China	1.2 × 50 teabags	Large discount store	Boiling water (100 mL), 3 min	1.2	07/2018
POT_C	Republic of Korea	Granule, 80.0	Department store	2~3 g of POT, Boiling water	1.0	02/2018
Jujube Tea						
JT_A	Republic of Korea	1.5 × 7 teabags	Large discount store	Boiling water (180 mL), 2 min	1.5	09/2018
JT_B	China	15.0 × 15 teabags	Large discount store	Boiling water (80 mL)	15.0	08/2018
JT_C	Republic of Korea	15.0 × 50 teabags	Large discount store	Boiling water (90 mL)	15.0	08/2018

(301 ± 2), and E (325 ± 5). Twenty kinds of leached tea (four types of green tea, three types of black tea, three types of pu-erh tea, three types of chamomile tea, three types of peppermint tea, three types of polygonatum odoratum tea, and one type of jujube tea), and two kinds of solid-extracted tea (two types of jujube tea), were purchased (Table 2). The infusions were prepared by pouring 120 mL of boiled water at 100°C on one tea bag and brewing for 10 min. To analyze cytotoxicity and the levels of reactive oxygen species (ROS), brand regular coffees and aqueous tea extracts were lyophilized using a FreeZone Plus 2.5 freeze dryer (Labconco, MO, USA) and stored at −20°C until analysis.

Determination of ascorbic acid and caffeine contents in commercial regular coffee and tea extracts by high performance liquid chromatography (HPLC)

The ascorbic acid and caffeine were determined using chromatographic system equipped with analytical HPLC unit model Nanospace SI-2 system (Shiseido Fine Chemicals, Tokyo, Japan) consisting in a 3201 pump, 3202 degasser, Accela PDA detector, 3004 column oven and 3023 auto sampler. The separation of the analytes was carried out using reversed-phase Phenomenex Kinetex column packed with C18 (5 μm particle size, 250 × 4.6 mm). Ascorbic acid was separated by isocratic elution with water-trifluoroacetic acid (99:1, v/v) as the mobile phase. Caffeine was separated using an isocratic elution with acetonitrile-potassium dihydrogen phosphate (10:90). Chromatograms were recorded at 254 nm for ascorbic acid and 274 nm for caffeine.

Determination of total polyphenols and total flavonoids

The total polyphenol contents in the coffee or tea extract were determined by using the Folin-Ciocalteu reagent according to the colorimetric method described by Singleton and Rossi[16]. Briefly, the reaction mixture was composed by 25 μL of coffee or tea extract, 1.6 mL of deionized water, 75 μL of Folin-Ciocalteu reagent and 0.3 mL of 2% sodium carbonate, placed in microtubes. The microtubes were agitated, held for 1 hr, and the absorbance was measured at 700 nm with a Cary 300 UV-visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The total polyphenol contents were expressed as mg gallic acid equivalent (GAE) per serving size. In addition, total flavonoids in the coffee or tea extract were estimated using the colorimetric assay previously described by Chang et al. with some modifications[17]. A volume of 50 μL of each the coffee or tea extract was added in a tube and subsequently, a sequential addition of 450 μL ethanol at 80%, 100 μL aluminum nitrate at 10%, 100 μL potassium acetate (1 M), and 4.3 mL ethanol at 80% to each extract sample was performed. Samples were maintained during 40 min in the dark at room temperature. The absorbance of the mixture was then measured at 415 nm against a blank of deionized water. The content of total flavonoids was expressed as mg quercetin equivalent (QE) per serving size.

Antioxidant capacity

The $DPPH^{\bullet+}$ assay for antioxidant activity was conducted by the method of Gyamfi et al. with slight modifications[18]. Briefly, coffee or tea extract (10 μL) were mixed with $DPPH^{\bullet+}$ (2,990 μL; 0.4 mM) followed by incubation in the dark at room temperature for 10 min. Absorbance at 520 nm was measured against deionized water as blank using a Cary 300 UV-visible spectrophotometer. All results were expressed as mg ascorbic acid equivalent (AE) per serving size. The antioxidant activity measured with ABTS was carried out according to the method described by Re et al. with some modifications[19]. $ABTS^{\bullet+}$ was generated by reacting an ABTS aqueous solution (7.4 mM) with $K_2S_2O_8$ (2.6 mM, final concentration) in the dark for 24 hr and adjusting the Abs 734 nm to 3.1 with water. 0.05 mL of coffee or tea extract was added to 9.95 mL $ABTS^{\bullet+}$ solution and the absorbance were measured at 734 nm after 90 min. Results were expressed as mg ascorbic acid equivalent (AE) per serving size.

Cytotoxicity assay

HeLa cells, human cervical cancer cells, was cultured in DMEM with 10% FBS, 1% antibiotic-antimycotic agent. Cell lines were maintained in an incubator (5% CO_2, 95% relative humidity, 37°C). Evaluation of cytotoxicity was performed by the MTT assay in HeLa cells. Cells were seeded at a density of 2×10^4 cells/well in 96-well plate and were incubated for 1 day before adding the coffee or tea extract. Cells were treated with brand coffee (BC_A and BC_C), green tea D, and black tea A at 1/8 to 1 serving size concentrations for 24 hr at 37°C. Then, 26 μL of MTT stock solution (2 mg/mL) was added and incubated for 4 hr at 37°C. MTT-containing medium was removed and 150 μL DMSO was added to each well to dissolve the formazan crystal formed by live cells. The absorbance of each well was read on a microplate reader (VersaMax, Molecular Devices, US) at 570 nm.

Intracellular ROS level

Intracellular ROS level was determined by using fluorescent probe H_2DCF-DA[20]. HeLa cells were seeded in 96-well plates at 1×10^4 cells/well. To evaluate the direct effect, 24 hr after seeding, exposed for 24 hr to brand coffee (BC_A and BC_C), green tea D, and black tea A at 1/8 to 1 serving size concentrations. Afterwards, 100 μL of H_2DCF-DA (100 μM) in serum- and phenol red-free DMEM was added to each well for 30 min at 37°C, and cells were washed once with PBS. To test the protective effect against oxidative stress, cells were pretreated during 24 hr with brand coffee (BC_A and BC_C), green tea D, and black tea A at 1/8 to 1 serving size concentrations, the H_2DCF-DA (100 μM) probe was added to each well for 30 min at 37°C, and the cells were washed once with PBS and fresh phenol red-free DMEM containing 500 μM H_2O_2 was added to all cultures except controls for 10 min at 37°C. In both experiments, intracellular ROS were measured using a HTS Multi Label Reader (Perkin Elmer, Waltham, MA, USA) at excitation and emission wavelengths of 485 and 538 nm, respectively.

Statistical analysis

Each parameter was analyzed in triplicate. Results are shown as means ± standard deviations. The results were statistically analyzed by Analysis of variance (ANOVA), and the unpaired *t*-test. Significance was accepted at $p \leq 0.05$. All statistical analyses were performed using the SPSS v.12.0 software package.

Results and Discussion

Ascorbic acid, caffeine, total polyphenol, and flavonoid contents of commercial regular coffee and tea

The values of the ascorbic acid, caffeine, total polyphenol, and flavonoid contents of the beverages are shown in Table 3. Green tea (GT_A, GT_B, GT_C, GT_D) and black tea (BT_A, BT_B, BT_C) were not significantly different ($p = 0.1000$) in their content of ascorbic acid, while other beverages did not contain ascorbic acid. This may be because the roasting of coffee, fermentation of pu-erh tea, and drying of other teas destroy the high content of ascorbic acid originally present in the green coffee bean and tea leaves[21]. Therefore, we cannot exclude the possibility that the antioxidant capacity of these beverages is produced by caffeine and phenolic compounds, rather than by ascorbic acid, which is easily destroyed by heat, air, and improper storage and processing of foods. Brand coffees and green, black, and pu-erh teas all

Table 3. The contents of ascorbic acid, caffeine, and phenolic (total polyphenols and flavonoids) in commercial regular coffees and teas.

Beverage Samples	Ascorbic acid (mg/serving size)	Caffeine (mg/serving size)	Total polyphenols (mg GAE/serving size)	Total flavonoids (mg QE/serving size)
Brand Coffee				
BC_A	-	202.75 ± 5.88	265.54 ± 15.97	11.49 ± 0.65
BC_B	-	161.95 ± 7.76	189.97 ± 5.54	7.82 ± 0.09
BC_C	-	165.50 ± 7.19	257.62 ± 8.00	12.14 ± 2.34
BC_D	-	150.17 ± 3.05	220.87 ± 9.78	8.96 ± 4.01
BC_E	-	195.08 ± 9.28	231.95 ± 14.39	6.14 ± 3.12
Green Tea				
GT_A	0.18 ± 0.06	20.15 ± 0.78	98.19 ± 8.17	2.99 ± 0.78
GT_B	1.58 ± 0.15	26.23 ± 0.61	87.04 ± 8.68	2.99 ± 0.09
GT_C	0.14 ± 0.10	14.53 ± 5.13	33.14 ± 10.64	1.34 ± 0.56
GT_D	1.00 ± 0.19	24.27 ± 0.41	107.00 ± 1.02	3.23 ± 0.75
Black Tea				
BT_A	0.07 ± 0.01	63.65 ± 4.27	117.62 ± 10.08	4.46 ± 0.11
BT_B	0.04 ± 0.00	51.44 ± 3.09	90.37 ± 7.43	3.92 ± 0.45
BT_C	0.04 ± 0.00	33.73 ± 4.44	78.24 ± 8.02	3.25 ± 0.89
Pu-erh Tea				
PT_A	-	29.66 ± 1.33	31.93 ± 2.51	0.71 ± 0.14
PT_B	-	21.34 ± 0.38	22.41 ± 0.32	0.70 ± 0.07
PT_C	-	24.28 ± 2.96	20.03 ± 1.88	0.74 ± 0.24
Chamomile Tea				
CT_A	-	-	5.51 ± 0.97	0.52 ± 0.11
CT_B	-	-	11.01 ± 1.15	1.91 ± 0.21
CT_C	-	-	6.98 ± 1.32	0.79 ± 0.06
Peppermint Tea				
P_A	-	-	14.47 ± 1.31	2.05 ± 0.16
P_B	-	-	34.72 ± 3.72	6.10 ± 0.26
P_C	-	-	12.39 ± 0.06	1.62 ± 0.09
Polygonatum Odoratum Tea				
POT_A	-	-	10.56 ± 1.46	0.12 ± 0.13
POT_B	-	-	4.86 ± 0.11	0.13 ± 0.01
POT_C	-	-	5.82 ± 0.42	0.21 ± 0.09
Jujube Tea				
JT_A	-	-	30.21 ± 0.70	0.37 ± 0.15
JT_B	-	-	5.93 ± 1.35	1.30 ± 1.38
JT_C	-	-	8.28 ± 1.18	0.94 ± 0.15

All values are shown as mean ± standard deviation (n = 3)

contain caffeine. The brand coffee A and E had a higher caffeine content ($p < 0.05$) than other coffees. This result may be related to the different species (BC_A, Robusta 20%) of the coffee beans and long extraction time (BC_E, 29 seconds) of the espresso. Other studies showed that Robusta coffee extracts contain twice as much caffeine as Arabica[22]. Weight for weight, tea leaves contain more caffeine than coffee beans; however, a serving size of tea (green, black, or pu-erh tea) contains only 15 to 64 mg caffeine compared with a serving size of brand coffee, which contains between 150 to 203 mg. This is because caffeine is not effectively extracted by brewing, unless the beverage is "stewed". As shown in Table 2, although the amount of comparison is different, our results showed that the three black teas had a higher caffeine content ($p < 0.0001$) than green and pu-erh teas in a single serving size of the teas. Black tea typically has more caffeine than do green and pu-erh teas, but the content of caffeine can vary depending on the type of tea, grade of comminution of tea leaves, and maturity of the leaves; young tea leaves have a higher concentration of caffeine than do mature leaves[23]. All brand coffee samples presented a rich source of phenolic compounds; the total polyphenol content in brand coffees was significantly ($p < 0.05$) higher than that in the other beverages. Total phenolic content was significantly higher ($p < 0.0001$) in jujube tea A than those in jujube tea B and jujube tea C. Jujube teas B and C are solid-extract teas that are made from the concentrated extract of the jujube fruit, and various nuts, such as pine nuts and almonds; conversely, jujube tea A is a leached tea. The percentages of jujube in jujube tea A, B, and C were 100%, 1.6%, and 2.2%, respectively. Hence, the high level of total polyphenol in jujube tea A may be attributable to the content of jujube. The green (GT_A, GT_B, GT_C, GT_D) and black teas (BT_A, BT_B, BT_C) were not significantly different in their polyphenol and flavonoid contents ($p = 0.5490$ and $p = 0.0571$, respectively).

Antioxidant activity of commercial regular coffee and tea

The evaluation of antioxidant activity of commercial regular coffees and teas was conducted *in vitro* by evaluation of DPPH and ABTS radical scavenging ability; the results are shown in Table 4. These methods are widely used to determine *in vitro* antioxidant activity of foods and beverages[24]. The determination of radical scavenging activity using DPPH showed that the brand coffee BC_C had the highest scavenging ability (664.91 mg ascorbic acid equivalent/serving size), while polygonatum odoratum tea (POT_B) had the lowest (1.40 mg AE/serving size). This result indicates that the brand coffee BC_C and polygonatum odoratum tea (POT_B) possess an antioxidant activity equivalent to 664.91 mg and 1.40 mg of ascorbic acid, respectively. The assessment of quenching activity, using DPPH, indicated that the beverages can be ranked in descending order: $BC_C > BC_A > BC_E > BC_D > BC_B > BT_A > GT_D > GT_A > GT_B > BT_B > BT_C > GT_C > P_B > PT_A > PT_C > PT_B > P_C > P_A > JT_A > CT_B > POT_A > CT_A > CT_C > POT_C > JT_C > JT_B > POT_B$. The antioxidative effectiveness of these beverages is due to the presence of antioxidant compounds, which are mainly polyphenols. As shown in Table 3, the phenolic content was higher in the

Table 4. Antioxidant activity of the regular coffees and teas evaluated by the $ABTS^{\bullet+}$ and $DPPH^{\bullet+}$ assays.

Beverage Samples	DPPH (mg AE/serving size)	ABTS (mg AE/serving size)
Brand Coffee		
BC_A	640.14 ± 20.14	604.09 ± 25.95
BC_B	489.88 ± 25.66	524.66 ± 11.58
BC_C	664.91 ± 48.87	624.36 ± 16.18
BC_D	567.04 ± 16.76	544.43 ± 9.26
BC_E	593.27 ± 28.17	594.98 ± 14.88
Green Tea		
GT_A	364.37 ± 16.97	299.16 ± 10.55
GT_B	350.32 ± 24.03	336.82 ± 12.95
GT_C	147.71 ± 41.22	170.23 ± 82.71
GT_D	374.73 ± 18.54	350.83 ± 3.62
Black Tea		
BT_A	433.46 ± 35.42	359.67 ± 29.86
BT_B	345.58 ± 38.36	327.52 ± 12.62
BT_C	268.15 ± 4.43	254.52 ± 31.22
Pu-erh Tea		
PT_A	84.76 ± 4.67	82.04 ± 23.66
PT_B	62.56 ± 1.98	65.37 ± 3.65
PT_C	63.41 ± 12.26	69.48 ± 10.19
Chamomile Tea		
CT_A	31.72 ± 10.92	37.08 ± 18.25
CT_B	35.23 ± 6.44	43.94 ± 18.74
CT_C	25.31 ± 8.87	47.62 ± 45.66
Peppermint Tea		
P_A	49.75 ± 8.39	44.57 ± 36.17
P_B	96.07 ± 20.44	113.53 ± 12.63
P_C	50.09 ± 15.77	39.62 ± 39.22
Polygonatum Odoratum Tea		
POT_A	32.27 ± 7.16	59.51 ± 20.42
POT_B	1.40 ± 2.43	1.38 ± 2.38
POT_C	8.19 ± 12.45	3.11 ± 1.21
Jujube Tea		
JT_A	41.36 ± 4.05	29.49 ± 37.69
JT_B	1.83 ± 1.59	0.20 ± 0.35
JT_C	3.97 ± 6.87	14.50 ± 17.84

All values are shown as mean ± standard deviation (n = 3)

Table 5. Correlation coefficients of between contents of ascorbic acid, caffeine, and total polyphenols, total flavonoids, and antioxidant activity in a serving size of the commercial regular coffees and teas

	Ascorbic acid	Caffeine	Total polyphenols	Total flavonoids	DPPH	ABTS
Ascorbic acid	$r = 1$					
Caffeine	$r = -0.0898$ $p = 0.6559$	$r = 1$				
Total polyphenols	$r = 0.0712$ $p = 0.7241$	$r = 0.9599$ $p < 0.0001$	$r = 1$			
Total flavonoids	$r = -0.0172$ $p = 0.9320$	$r = 0.8778$ $p < 0.0001$	$r = 0.9256$ $p < 0.0001$	$r = 1$		
DPPH	$r = 0.1982$ $p = 0.3217$	$r = 0.8966$ $p < 0.0001$	$r = 0.9756$ $p < 0.0001$	$r = 0.8856$ $p < 0.0001$	$r = 1$	
ABTS	$r = 0.1893$ $p = 0.3442$	$r = 0.9171$ $p < 0.0001$	$r = 0.9798$ $p < 0.0001$	$r = 0.8924$ $p < 0.0001$	$r = 0.9948$ $p < 0.0001$	$r = 1$

coffee brand C than in polygonatum odoratum tea B. The assessment of scavenging ability using ABTS indicated that coffee brand C had the highest scavenging ability, with 624.36 mg AE/serving size, while jujube tea (JT_B) had the lowest value of 0.20 mg AE/serving size. Jujube tea (JT_B) and polygonatum odoratum tea (POT_B) were not significantly different in their DPPH and ABTS values ($p = 0.8085$ and $p = 0.4460$, respectively). The ABTS antioxidant activity assay showed a similar trend to that observed with the DPPH assay.

Correlation between the antioxidant compounds and antioxidant activity

This study aimed to improve the understanding of how antioxidant compounds affect the antioxidative activity of these beverages. The assessment of DPPH and ABTS free radical scavenging ability indicated significant and strongly positive Pearson's correlation between caffeine and total polyphenol and flavonoid content of these beverages (Table 5). Thus, it can be inferred that caffeine, polyphenols, and flavonoids are important contributors to the antioxidant activity of these beverages. Similarly, the correlation between DPPH and ABTS showed a correlation coefficient that approached 1 ($r = 0.9948$, $p < 0.0001$); this is likely because both methods have a common mechanism for eliminating artificially formed free radicals. Contrarily, no linear correlation was confirmed between the concentration of ascorbic acid and DPPH ($r = 0.1982$, $p = 0.3217$) or ABTS assay ($r = 0.1893$, $p = 0.3442$), possibly because of their low concentration.

Cytotoxicity

We have shown that among brand regular coffees (Americano) and leached teas, brand coffees A and C (BC_A, BC_C), and green tea D and black tea A, were those with the highest antioxidant capacity (Table 4). Therefore, we have

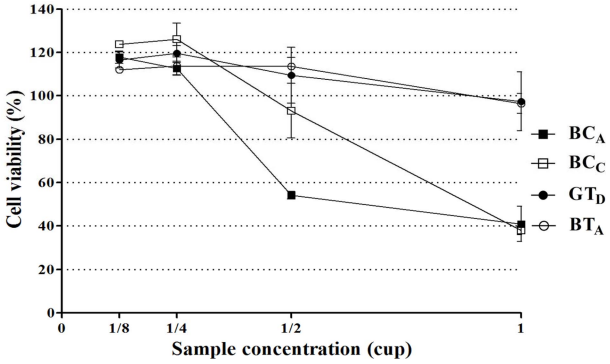

Fig. 1. Viability curves of HeLa cells after 24 hr of incubation with beverage samples. Data were obtained with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Each point indicates the average and standard deviation of three independent experiments.

evaluated the possible antioxidant effect of the four beverages in HeLa cells. First, we examined the cytotoxicity of each beverage in order to choose an adequate concentration of that beverage. Then, we determined the direct effects of each beverage on the level of intracellular ROS using the dichlorofluorescein assay. Finally, we assessed the ability of each beverage to protect against the H_2O_2-induced increase in intracellular ROS. The cytotoxicity of brand coffees BC_A and BC_C, as well as green tea D and black tea A, was measured in HeLa cells, after 24 hr of exposure, using the MTT assay (Fig. 1). The concentrations of each residue, corresponding to 1/8 to 1 cup of each beverage sample, are shown in Table 6. After a 24-hr incubation, the viability of HeLa cells was not affected (> 96%) by green tea D and black tea A at the tested concentrations (~1 cup, Fig. 1). Additionally, brand coffee C did not exert any cytotoxic effects at the concentration of 1/2 cup (cell viability~93.2%), while a clearly toxic effect at the concentration of 1 cup was observed for both brand coffees BC_A

Table 6. Concentrations of total residues (μg mL^{-1} dry weight) of tested beverages.

Samples	Volume (mL)	Residue (mg)	1/8 cup concentration (μg/mL)	1/4 cup concentration (μg/mL)	1/2 cup concentration (μg/mL)	1 cup concentration (μg/mL)
BC_A	284 ± 5	126.33 ± 0.44	55.6 ± 1.2	111.1 ± 2.4	222.2 ± 4.7	444.4 ± 9.4
BC_C	304 ± 6	82.34 ± 2.61	33.9 ± 1.7	67.8 ± 3.3	135.6 ± 6.6	271.3 ± 13.2
GT_D	120 ± 0	7.88 ± 0.53	8.2 ± 0.5	16.4 ± 1.1	32.8 ± 2.2	65.6 ± 4.4
BT_A	120 ± 0	14.16 ± 1.50	14.7 ± 1.6	29.5 ± 3.1	59.0 ± 6.3	118.0 ± 12.5

All values are shown as mean ± standard deviation (n = 3)

and BC_C.

Effect of commercial regular coffee and tea on the intracellular level of ROS

After a 24-hr incubation with the beverage samples, the level of intracellular ROS was evaluated in HeLa cells (Fig. 2(A)). All the tested beverage samples dose-dependently induced a relevant decrease in the levels of ROS at the concentrations of 1/8~1 cup. Additionally, all the tested beverage samples induced a highly significant ($p < 0.001$) decrease in the basal levels of ROS at the concentration of 1/8 to 1 cup (except at 1/8 cup, GT_D). In particular, when HeLa cells were treated with 1/2 cup of BC_C, the level of ROS decreased by approximately 53% compared with that in the control cells. Overall, the inhibition of ROS production by brand coffees BC_A and BC_C was greater than that by the leached teas (at 1/8~1/2 cup, $p < 0.05$). Previous studies found that DPPH and ABTS radical activities of beverages scavenging showed excellent antioxidative effects of beverages due to large amounts of total polyphenols, flavonoids, and caffeine contents. Tables 3 and 4 show that the levels of antioxidant compounds (denoted by the total content of polyphenols, flavonoids, and caffeine) and antioxidative activity of BC_A and BC_C were higher than those of GT_D and BT_A; accordingly, the reduction in the level of ROS, induced by of BC_A and BC_C, was greater than that induced by GT_D and BT_A (Fig. 2(A)). Therefore, we conclude that the high radical scavenging ability of these beverages effectively suppressed basal ROS production in HeLa cells.

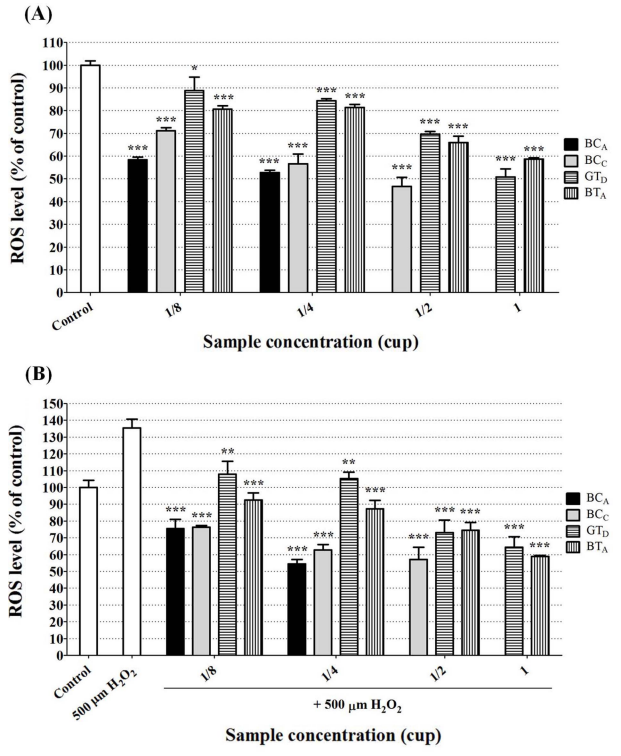

Fig. 2. Intracellular reactive oxygen species (ROS) level of HeLa cells. (A) Direct effect on intracellular ROS generation treated for 24 hr with different concentrations of beverages. (B) Protective effect of the beverages against hydrogen peroxide (H_2O_2)-induced production of ROS, evaluated by the dichlorofluorescein assay. Data are expressed as mean ± standard deviation of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

Protection of commercial regular coffee and tea against oxidative stress by intracellular ROS

We evaluated whether these beverage samples exerted protective activity against an increase in the level of ROS induced by exposure to 500 μM H_2O_2 (10 min, 37°C); the results are shown in Fig. 2(B). In the group treated with H_2O_2, the fluorescence intensity of DCF increased to 135%, compared with 100% observed in the control group. All the tested samples dose-dependently (1/8~1 cup) reduced the intracellular oxidative stress that was induced by H_2O_2, which generates hydroxyl radicals ($p < 0.05$). In particular, at the concentration of 1/4 cup, BC_A showed 54.53%, which is approximately a 60% reduction in the levels of ROS compared to H_2O_2. This protective effect paralleled the ability of the beverage samples to reduce the level of ROS. The ability of the beverages to reduce the levels of ROS was ranked as follows: brand coffees > black tea > green tea; the protective effect exhibited the same trend. These results are likely due to the presence of antioxidant compounds, such as ascorbic acid, caffeine, and total polyphenols and flavonoids, in these beverages. This suggests that these beverage samples can alter the oxidative environment of the cells.

In conclusion, this study revealed that brand regular coffees had the highest antioxidant activity, while green and black tea had adequate antioxidant activity. Among the brand coffees and leached teas, brand coffees BC_A and BC_C, green tea D, and black tea A were found to have higher antioxidant activity. The substances that contribute to the antioxidant capacity of these beverages are polyphenols, flavonoids, and caffeine, but not ascorbic acid. Additionally, brand coffees BC_A and BC_C, green tea D, and black tea A showed high free radical scavenging ability in HeLa cells that had been stimulated by oxidative stress. Further experimental and clinical studies on the relevant antioxidant compounds are needed to expand the significance of these results.

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국문요약

커피와 다류에 대한 소비는 전 세계적으로 해마다 증가하는 추세이며, 한국을 포함하여 아시아권에도 물 다음으로 가장 많은 소비가 이루어지는 음료는 커피와 차(녹차, 케모마일차, 페퍼민트차, 둥글레차, 보이차, 홍차, 대추차)이다. 본 연구는 국내에서 시판되는 커피전문점의 브랜드 커피 5종과 침출차 20종(녹차 4종, 홍차 3종, 보이차 3종, 케모마일차 3종, 페퍼민트차 3종, 둥글레차 3종, 대추차 1종), 고형차 2종(대추차 2종)에 대하여 각 음료 1잔에 함유되어 있는 비타민C, 카페인, 총폴리페놀, 플라보노이드를 분석하였고, 이들의 항산화활성을 측정하여 서로의 상관관계를 살펴보았다. 추가적으로 항산화활성이 높은 커피와 다류 총 4종을 대상으로 과산화수소(H_2O_2)로 유도된 산화적 스트레스로부터 세포 보호효과를 평가하여 시판 커피와 다류의 항산화 연구를 위한 기초자료를 확보하고자 하였다. 녹차와 홍차 각각 1잔당 비타민 C 함량은 0.04~1.58 mg 이었고 커피와 나머지 차는 비타민 C가 검출되지 않았다. 카페인 함량은 브랜드 커피가 1잔당 150.17~202.75 mg으로 다른 종류의 음료보다 높았다. 음료에 함유된 총 폴리페놀 함량은 gallic acid의 등량값(GAE)으로 표시할 때 브랜드 커피 A(BC_A)가 265.54 mg / serving size으로 가장 높았고, 플라보노이드 함량은 quercetin 등량값(QE)으로 브랜드 커피 B(BC_B)가 12.14 mg / serving size으로 가장 높았다. 음료 4종(브랜드 커피 A, C 그리고 녹차 D, 홍차 A) 시료의 농도가 높아질수록 HeLa 세포 내 활성산소종(reactive oxygen species, ROS) 생성 억제효과 및 H_2O_2의 소거활성이 증가하였다. 본 연구결과 브랜드 커피 및 홍차, 녹차는 각각 1잔당 비타민 C의 등량값으로 590, 330, 300 mg의 항산화능을 가지며, HeLa 세포 내에서도 활성산소 감소효과가 확인되는 우수한 항산화 음료로 평가되었다.

References

1. Zorov, D.B., Juhaszova, M., and Sollott, S.J.: Mitochondrial ROS-induced ROS release: an update and review. *Biochim. Biophys. Acta*., **1757**, 509-517 (2006).
2. Rahman, K.: Studies on free radicals, antioxidants, and co-factors. *Clin. Interv. Aging*., **2**, 219-236 (2007).
3. Mates, J.M., Perez-Gomez, C., and Nunez de Castro, I.: Antioxidant enzymes and human diseases. *Clin. Biochem*., **32**, 595-603 (1999).
4. Kovacic, P., and Jacintho, J.D.: Mechanisms of carcinogenesis: focus on oxidative stress and electron transfer. *Curr. Med. Chem*., **8**, 773-796 (2001).
5. Lee, J., Koo, N., and Min, D.: Reactive oxygen species, aging, and antioxidative nutraceuticals. *Compr. Rev. Food. Sci. Food. Saf.*, **3**, 21-33 (2004).
6. Vinod, B.S., Maliekal, T.T., and Anto, R.J.: Phytochemicals as chemosensitizers: from molecular mechanism to clinical significance. *Antioxid. Redox. Signal*., **18**, 1307-1348 (2013).
7. Niki, E.: Action of ascorbic acid as a scavenger of active and stable oxygen radicals. *Am. J. Clin. Nutr.*, **54**, 1119S-1124S (1991).
8. Devasagayam, T., Kamat, J., Mohan, H., and Kesavan, P.: Caffeine as an antioxidant: inhibition of lipid peroxidation induced by reactive oxygen species. *Biochim. Biophys. Acta.*, **1282**, 63-70 (1996).
9. Bravo, L.: Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev.*, **56**, 317-333 (1998).
10. Scalbert, A., Johnson, I.T., and Saltmarsh, M.: Polyphenols: antioxidants and beyond. *Am. J. Clin. Nutr.*, **81**, 215S-217S (2005).
11. Dai, Q., Borenstein, A.R., Wu, Y., Jackson, J.C., and Larson, E.B.: Fruit and vegetable juices and Alzheimer's disease: the Kame Project. *Am. J. Med.*, **119**, 751-759 (2006).
12. Rietveld, A., and Wiseman, S.: Antioxidant effects of tea: evidence from human clinical trials. *J. Nutr.*, **133**, 3285S-3292S (2003).
13. Kim, T.H, Chae, S.J., Kim, C.W.: A study on the coffee consumption behavior by lifestyle. *KJHT*., **22**, 93-112 (2013).
14. Kim, Y.A, Ko, J.Y.: Comparison analysis consensus map of coffee and tea customers using ZMET. *KJHT*., **24**, 99-114 (2015).
15. Ludwig, I.A., Sanchez, L., Caemmerer, B., Kroh, L.W., De Peña, M.P., and Cid, C.: Extraction of coffee antioxidants: impact of brewing time and method. *Food Res. Int.*, **48**, 57-64 (2012).
16. Singleton, V.L., Rossi, J.A.: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.*, **16**, 144-158 (1965).
17. Chang, C.C., Yang, M.H., Wen, H.M., and Chern, J.C.: Esti-

mation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.*, **10**, 178-182 (2002).
18. Gyamfi, M.A., Yonamine, M., and Aniya, Y.: Free-radical scavenging action of medicinal herbs from Ghana: Thonningia sanguinea on experimentally-induced liver injuries. *Gen. Pharmacol.*, **32**, 661-667 (1999).
19. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C.: Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.*, **26**, 1231-1237 (1999).
20. Wang, H., and Joseph, J. A.: Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free. Radic. Biol. Med.*, **27**, 612-616 (1999).
21. Debry, G.: *Coffee and health*. John Libbey, Paris, France, pp. 157-249 (1994).
22. Jeszka-Skowron, M., Sentkowska, A., Pyrzyńska, K., and De Peña, M.P.: Chlorogenic acids, caffeine content and antioxidant properties of green coffee extracts: influence of green coffee bean preparation. *Eur. Food Res. Technol.*, **242**, 1403-1409 (2016).
23. Boros, K., Jedlinszki, N., and Csupor, D.: Theanine and caffeine content of infusions prepared from commercial tea samples. *Pharmacogn. Mag.*, **12**, 75-79 (2016).
24. Pellegrini, N., Serafini, M., Salvatore, S., Del Rio, D., Bianchi, M., and Brighenti, F.: Total antioxidant capacity of spices, dried fruits, nuts, pulses, cereals and sweets consumed in Italy assessed by three different *in vitro* assays. *Mol. Nutr. Food Res.*, **50**, 1030-1038 (2006).