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Full Length Research Paper

Antibacterial and antioxidant activities of tannins extracted from agricultural by-products

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This study was aimed at evaluating antibacterial and antioxidant activity of tannins extracted by several solvents from agricultural by-products, including green tea waste and acorn, chestnut, and persimmon hulls. Tannin content was the highest in acetone extracts from all types of hulls, but not green tea waste ($p<0.05$). Tannin extracts from green tea waste had higher antibacterial activity than those from acorn, chestnut, and persimmon hulls extracted with distilled water, ethanol, or acetone. With ethanol extraction, 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of green tea tannin extracts was significantly higher than that of tannins extracted from acorn, chestnut, and persimmon hulls ($p<0.05$). In particular, tannins extracted from green tea waste had a greater IC_{50} value for DPPH scavenging activity than did vitamin C. Antioxidant activity was also significantly higher in ethanol extracts from green tea waste than in distilled water and acetone extracts ($p<0.05$). Therefore, tannins extracted from green tea waste could be a natural source for substitutes to present antioxidants as well as antibiotics.

Key words: Acorn, antibacterial activity, antioxidant activity, chestnut, green tea, persimmon.

INTRODUCTION

Animal products contribute significantly to the human protein and energy needs. As people increase their standard of living, the per capita consumption of animal products also increases (Taylor and Field, 2001). Therefore, the vegetarian food consumption pattern of the past has changed to a pattern of higher consumption of animal products in the present, and consumers have become more deeply concerned about the quality, safety, variety, and functionality of food than its quantity (Worsley, 2000). Due to their high interest in food sources, consumers want high quality brand meats raised in an eco-friendly manner. They are also worried about using antibiotics and animal growth promoters (AGPs), which have been used to improve animal productivity in the past.

Use of AGPs tends to be restricted in developed Countries, especially in the EU. Denmark and Sweden in EU first began regulating AGP applications in animal production in early 2000, and Korea is also scheduled to legally restrict use of all antibiotics to produce concentrated feed in 2012. Because problems including reduced daily gain and feed efficiency, increased death rate, and reduced productivity by restriction of antibiotics (Inborr, 2000) are expected, alternative options for diversified eco-friendly and efficient choices are needed.

Nature has been a source of medicinal agents for millennia, and an impressive number of modern drugs have been isolated from natural sources. Plant-based, traditional medicine continues to play an essential role in the health care of human and animals (Doughari et al., 2009).

Plant compounds have potentially significant therapeutic applications against human and animal pathogens, including bacteria, fungi, and viruses (Perez,

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2003), and plant secondary compounds, particularly tannins and other polyphenols, have attracted attention. Tannins are polyphenolic compounds found in most plants and are generally thought to function as chemical defenses against pathogens and herbivory (Gedir et al., 2005). Two major tannin structural classes exist, hydrolysable tannins and condensed tannins, with the latter being more widely distributed in nature. Although attention has been primarily directed at understanding tolerance to tannins, recent work points to a number of beneficial effects of tannins at moderate concentrations (Barry and McNabb, 1999; Hoskin et al., 1999; Kaitho et al., 1998), including being a natural detergent to reduce bloat (Tanner et al., 1995), and a suitable substitute for synthetic anthelmintics (Niezen et al., 1995; Robertson et al., 1995) and having antimicrobial activity (Doughari et al., 2008) and antioxidant activity (Zargham and Zargham, 2008). An enormous amount of agricultural wastes derived from tannin-containing plants is produced every year in Korea, and some of these wastes may have medicinal value.

Therefore, this study was aimed at evaluating antibacterial and antioxidant activities of tannins extracted with several solvents from agricultural by-products, including green tea waste and acorn, chestnut, and persimmon hulls.

MATERIALS AND METHODS

Preparation of agricultural by-products

A sample was collected from wastes produced from green tea processing, and acorn, chestnut, and persimmon hulls were collected from factories processing acorn starch, chestnut, and cured persimmon, respectively. Collected samples were dried for 2 weeks at room temperature, ground with a Cyclotec mill (Foss Tecator, Sweden) and used for extraction of tannin.

Tannin extraction

For ethanol and aqueous extractions, 100 g ground samples of green tea waste or hulls were added to 1 L of ethanol or distilled water and boiled for 1 h in 90°C water bath. Boiled samples were mixed with ethanol or distilled water and filtered through filter paper (No. 1), decompressively concentrated by evaporator at 60–65°C for ethanol or 85–90°C for distilled water, and then freeze-dried. As for the acetone extraction, 100 g ground samples of waste or hulls were added to 1 L of acetone, boiled for 24 h, and centrifuged at 1690 × *g* for 30 min. This process was repeated four times, and supernatants were gathered, evaporated, and freeze-dried (Ahn et al., 2002).

Determination of tannin concentration

The tannin content was determined according to a previous procedure (Lowenthal, 1877) with a slight modification. A potassium permanganate solution was made up as required by dissolving 0.79 g of analytical grade potassium permanganate in 1 L of water to yard a 0.005 M solution. Indigo carmine indicator was prepared as a 0.1% working solution by dissolving 1 g of indigo carmine in 1 L of water to which 50 ml of concentrated sulphuric acid had been added. The samples (2–5 g) were mixed with 400 ml of distilled water and the mixtures were boiled for 30 min. The supernatant was then analyzed by adding 10 µl of the sample and 20 ml of indigo carmine to a 1 L flask and then adding 750 ml water. This was titrated against the permanganate solution until the royal blue faded to a light green.

Further titration was drop wise until the lime green changed to yellow. Record this value as X ml. Additionally 50 ml of a 1% gelatin solution, 100 ml of a 10% saturated salt solution, and 10 g of kaotine were added to 100 ml mixtures of the sample and distilled water. The resulting solutions were boiled for 30 min and then the supernatant from the tannin extraction using 20 ml of indigo carmine alone in 750 ml water was used in a blank titration. The blank value should be 10 µl and should be recorded as Y ml.

Total tannin content (%) = (X – Y)/10 expressed as 'tannic acid' equivalents

Where X (ml) is Volume used, Y (ml) is Blank titration value.

Antimicrobial activity test

Six pathogenic bacteria, including *Listeria monocytogenes (*ATCC 19111), *Bacillus coagulans* (ATCC 51232), *Escherichia coli* (ATCC 10536), *Shigella flexneri* (ATCC 9199), *Methicillin-Resistant Staphylococcus Aureus* (MRSA, ATCC 25923), and *Vancomycin-Resistant S Aureus* (VRSA, ATCC 29213), were used as test organisms. These test organisms were grown on tryptic soy agar plates at 37°C. Whatman filter paper discs (8 mm diameter) were impregnated with 20 ml of extracts at various concentrations (0.125, 0.25, 0.5, 1.0 mg/ml) and placed on the surface of agar plates seeded with bacteria (20 µl/ml). The plates were incubated for 24 h at 37°C to obtain maximum growth in the culture media. For each extract, the diameter of the inhibition zone of growth minus the diameter of the disc was measured to estimate the degree of antibacterial activity. Antibiotic discs of ampicillin (30 µg) for *E. coli* and *S. flexneri*, and gentamicin (30 µg) for *L. monocytogenes*, *B. coagulans*, MRSA, and VRSA were used as positive controls, respectively. Analyses were performed a total of three times per strain.

DPPH radical scavenging activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured by using the method described by Nanjo et al. (1996). Briefly, 60 µl of each extract at each concentration was added to 60 µl of DPPH (60 µM) in a methanol solution. After the solution was mixed vigorously for 10 sec, it was transferred into a 100 µl Teflon capillary tube, and the scavenging activity with regard to DPPH radicals was measured with an ESR spectrometer (JEOL Ltd., Tokyo, Japan). A spin adduct was measured on an ESR spectrometer exactly 2 min later. Experimental conditions were as follows: central field, 3475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 5 mW; gain, 6.3 × 10^5; and temperature, 298 K.

Statistical analysis

All data are presented as means ± standard deviation, and statistical analyses were performed using Statistical Analysis System version 8.0 (SAS Institute, Cary, NC, USA). The differences between means were assessed by the Duncan's multiple range tests and results with values of $p<0.05$ were considered statistical significant.

Table 1. Tannin concentration (%) of agricultural by-products.

Item	Distilled water extracts	Ethanol extracts	Acetone extracts
Green tea waste	3.34 ± 0.78^{bC}	11.90 ± 2.07^{aA}	5.27 ± 0.26^{bB}
Acorn hull	0.60 ± 0.13^{bD}	0.45 ± 0.00^{bC}	1.28 ± 0.18^{aC}
Chestnut hull	8.92 ± 1.53^{cB}	13.35 ± 0.82^{bA}	26.03 ± 1.58^{aA}
Persimmon hull	13.60 ± 0.50^{bA}	5.84 ± 0.57^{cB}	24.47 ± 1.11^{aA}

$^{a, b, c}$Means with different superscript in the treatment rows are significantly different (p<0.05). $^{A, B, C, D}$ Means with different superscript in the treatment columns are significantly different (p<0.05).

RESULTS

Tannin concentrations of several agricultural by-products

Total tannin concentrations in the distilled water, ethanol and acetone extracts of green tea waste and acorn, chestnut, and persimmon hulls are shown in Table 1. Tannin concentrations were highest when by-products except for green tea waste, were extracted by acetone (p<0.05). For green tea waste, the tannin concentration was the highest in ethanol extracts. Especially tannin concentrations of ethanol and acetone extract in chestnut hull were the highest compared to that of green tea waste, acorn hull and persimmon hull (p<0.05). However, among distilled water extracts, the tannin concentration was significantly higher (p<0.05) from persimmon hull than from green tea waste, acorn hull, or chestnut hull.

Antibacterial activity of tannins extracted from agricultural by-products with several solvents

Antibacterial activities of tannins extracted by distilled water from agricultural by-products are shown in Table 2. The tannin extract of distilled water from green tea waste exhibited strong antibacterial activity with almost all the strains tested. The maximum inhibition zone (2.25 mm) was observed with *B. coagulans* in the distilled water extract of green tea waste, and the minimum inhibition zone (0.5 mm) occurred with *E. coli*. The distilled water extract of acorn hull exhibited strong antibacterial activity against VRSA (inhibition zone, 2.0 mm) and *B. coagulans* (inhibition zone, 1.75 mm). The tannin extract of distilled water from chestnut hull exhibited maximum antibacterial activity against *S. flexneri*, followed by *B. coagulans*. On the other hand, the tannin extract of distilled water from persimmon hull showed antibacterial activity against only *S. flexneri* and *B. coagulans*.

Antibacterial activities of tannins extracted by ethanol from agricultural by-products are shown in Table 3. With the ethanol extract of green tea waste, the maximum inhibitory zone (6.0 mm) was observed against MRSA (6.0 mm) and the minimum inhibition zone occurred with *L. monocytogenes* (1.75 mm). The ethanol extracts from acorn hull and chestnut hull also showed a range of bacterial inhibition that varied from 2 to 7 mm above an average of 2 mm against VRSA, MRSA and *S. flexneri*. However, the ethanol extract of persimmon hull exhibited low inhibitory activity against *E. coli* (0.25 mm) only, and whereas no activity was observed with the remaining five strains. Table 4 shows the antibacterial activities of tannins extracted by acetone from agricultural by-products. In the present study, the acetone extract of green tea waste exhibited the maximum inhibitory zone (3.75 mm) against MRSA, followed by VRSA, *S. flexneri*, *B. coagulans*, and *E. coli*, and the minimum was observed with *L. monocytogens,* with an average inhibitory zone of 1.75 mm. The acetone extract of acorn hull exhibited a maximum antibacterial activity against MRSA, followed by *S. flexneri*, VRSA, *E. coli*, and *L. monocytogenes, with* minimum activity against *B. coagulans*. The acetone extract from chestnut hull exhibited antibacterial activity against almost all the strains tested, although the activity was lower than that of the extracts from green tea waste and acorn hull. The acetone extract of persimmon hull exhibited low inhibitory activity against MRSA and *E. coli*, and no activity was observed in the remaining four strains.

Free radical scavenging activity of tannins extracted by several solvents from agricultural by-products

The antioxidant activity of tannins extracted from agricultural by-products was investigated by measuring DPPH radical scavenging with an ESR spectrometer. DPPH is a stable radical that is used to screen the free radical-scavenging ability of compounds or the antioxidant activity of plant extracts. The effects of tannin extracted from agricultural by-products, including green tea, waste and acorn, chestnut and persimmon hulls on DPPH radical scavenging activity is shown in Table 5, the lower the concentration inhibiting 50% of the free radical generation (IC_{50}), the greater the antioxidant activity. DPPH radical scavenging activities at the IC_{50} of distilled water extracts from green tea waste and acorn hull had significantly higher activity than those of extracts from chestnut and persimmon hulls (p<0.05). On the other hand, ethanol and acetone extracts from green tea waste

Table 2. The antibacterial activity of tannins extracted by distilled water from agricultural by-products.

Variable	mg/disc	*VRSA*[1]	*MRSA*[2]	*B. coagulans*	*L. monocytogenes*	*E. coli*	*S. flexneri*
Green tea by-product	2	+	-	++	+	-	+
	3	++	+	++	+	-	+
	4	++	+	++	++	+	++
	5	++	++	+++	++	+	++
	antibiotic[3]	+++	+++	++++	++	+++	++++
Acorn hull	2	++	-	+	+	-	-
	3	++	+	++	+	-	+
	4	++	+	++	+	-	+
	5	++	+	++	+	-	+
	antibiotic	+++	+++	++++	++	+++	++++
Chestnut hull	2	-	-	+	-	-	++
	3	-	-	+	-	-	++
	4	-	-	+	+	-	++
	5	+	+	+	+	-	++
	antibiotic	+++	+++	++++	++	+++	++++
Persimmon hull	2	-	-	-	-	-	+
	3	-	-	-	-	-	+
	4	-	-	+	-	-	+
	5	-	-	+	-	-	++
	antibiotic	+++	+++	++++	++	+++	++++

Inhibitory zone diameter: >8.0 mm, ++++; 5.0–7.0 mm, +++; 2.0–4.5 mm, ++; 0.2–1.5 mm, +; and <0.1 mm, –; [1]VRSA: Vancomycin-Resistant *Staphylococcus aureus;* [2]MRAS: Methicillin-Resistant *S. aureus;* [3]Antibiotic discs of ampicillin for *E. coli* and *S. flexneri*, and gentamicin for *L. monocytogenes*, *B. coagulans*, methicillin-resistant *S. aureus* and vancomycin-resistant *S. aureus* were used as positive controls, respectively.

exhibited stronger DPPH radical scavenging activity than extracts from the others by-product (p<0.05). In particular, the IC_{50} value for DPPH scavenging activity of the ethanol extract from green tea waste was greater than that of vitamin C (green tea waste at 0.0022 mg/ml vs. vitamin C at 0.0031 mg/ml) (data not shown). The antioxidant activity of green tea waste was also higher in ethanol extracts than in distilled water and acetone extracts (p<0.05).

DISCUSSION

Many previous studies have reported that polyphenols of various plants and herbs are beneficial to animal and human health. Some bioactive substances in polyphenol-rich plants, such as green tea, berries, canola, and rapeseed, have been made into functional foods or supplements (Amarowicz et al., 2000; Puupponen-Pimiä et al., 2005; Taguri et al., 2004). Tannins are complex polyphenolic compounds widely found in higher plants. There are two chemically different types of tannins: hydrolysable and condensed tannins (Haslam, 1979). Similar to many polyphenols, tannin has been shown to possess antioxidant (Amarowicz et al., 2000; Tebib et al., 1997) and antibacterial activities (Akiyama et al., 2001; Funatogawa et al., 2004; Taguri et al., 2004). However, high concentrations of tannin may decrease the nutrient value of food and feed products (Deshpande et al., 1986). Our present study was conducted to evaluate antibacterial and antioxidant activities of tannins extracted from agricultural by-products, including green tea waste and acorn, chestnut, and persimmon hulls, which are largely produced in agricultural field of Korea. It has been reported that tannins are commonly extracted from plants by different solvents such as boiling aqueous methanol, acetone, or acidic methanol (Hagerman, 1988). In the present study, we extracted tannin from green tea waste and acorn, chestnut, and persimmon hulls with distilled water, ethanol, and acetone. Tannin concentration in the extracts was the highest when extracted by acetone, except for the green tea waste. Similar to our results, findings by Majorie (1999) showed that some solvents, especially acetone, are selective for tannins. On the other

Table 3. The antibacterial activity of tannins extracted by ethanol from agricultural by-products.

	mg/disc	*VRSA*[1]	*MRSA*[2]	*B. coagulans*	*L. monocytogenes*	*E. coli*	*S. flexneri*
Green tea by-product	2	++	++++	++	+	++	++
	3	++	++++	++	++	++	++
	4	+++	++++	+++	++	++	+++
	5	+++	++++	+++	++	++	+++
	antibiotic[3]	+++	++	++++	++	++++	++++
Acorn hull	2	++	++	-	+	+	++
	3	++	++	-	+	+	++
	4	++	++	-	+	+	++
	5	++	++	+	++	+	+++
	antibiotic	+++	++	++++	++	++++	++++
Chestnut hull	2	+	++	-	+	-	++
	3	+	++	-	++	-	++
	4	++	++	+	++	-	++
	5	++	+++	+	++	+	++
	antibiotic	+++	++	++++	++	++++	++++
Persimmon hull	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
	5	-	-	-	-	+	-
	antibiotic	+++	++	++++	++	++++	++++

Inhibitory zone diameter: >8.0 mm, ++++; 5.0–7.0 mm, +++; 2.0–4.5 mm, ++; 0.2–1.5 mm, +; and <0.1 mm, −; [1]VRSA: Vancomycin-Resistant *Staphylococcus aureus;* [2]MRAS: Methicillin-Resistant *S. aureus;* [3]Antibiotic discs of ampicillin for *E. coli* and *S. flexneri*, and gentamicin for *L. monocytogenes*, *B. coagulans*, methicillin-resistant *S. aureus* and vancomycin-resistant *S. aureus* were used as positive controls, respectively.

hand, water is generally used in traditional settings to prepare plant decoctions for health remedies (Majorie, 1999). In the present study, for distilled water extracts persimmon hull had the highest tannin concentration, followed by chestnut hull, green tea waste, and acorn hull. However, tannin concentrations in ethanol and acetone extract from chestnut hull were the highest among all the samples.

It is well demonstrated in the literature that compounds found in plant extracts, such as tannin, gallic acid, ellagic acid and catechin have antimicrobial effects (Akiyama et al., 2001; Funatogawa et al., 2004; Nascimento et al., 2000). In particular, tannins inhibit bacterial growth and protease activity by damaging the cell wall and cytoplasm, causing rapid structural destruction (Andrade et al., 2006; Cowan, 1999). In our present study, antibacterial activity was higher with tannins extracted from green waste than with those extracted from acorn, chestnut, and persimmon hulls using distilled water, ethanol and acetone. Tannin extracts of distilled water, ethanol, and acetone from green tea waste exhibited strong antibacterial activity against all bacterial strains tested in this study, including VRSA, MRSA, *B. coagulans*, *L. monocytogenes*, *E. coli,* and *S. flexneri*. In contrast, the tannin extracts of distilled water, ethanol, and acetone from persimmon hull exhibited no inhibitory activity against almost all strains, with the exceptions of *E. coli* and *S. flexneri*. From a clinical point of view, *E. coli* can infect the gall bladder, surgical wounds, skin lesions, and the lungs, especially in debilitated and immunodeficient patients (Black, 1996). Further, vancomycin-resistant enterococci have emerged as important nosocomial pathogens during the past decade. These bacteria are also known to have intrinsic resistance to commonly used antimicrobial agents (Lee and Rhee, 2011; Livornese et al., 1997). Chandrappa et al. (2011) reported that acetone and ethyl acetate extracts of *Adiantum pedatum*, which are rich in steroids, glycosides, and terpenoids as well as phenols, showed inhibitory activity against clinically important standard reference bacterial strains including *S. aureus*, *Klebsiella pneumoniae*, *P. aeruginosa,* and *E. coli*. Similarly, it was reported that the condensed tannin extracts from Shinnery and Post oaks had strong inhibitory activity against *S*. *aureus*, having growth inhibition zones exceeding 23 mm at 8 mg tannin extract/mL (Min et al., 2008). In another previous study, the minimum inhibitory concentration of tannins for *P. fluorescens*, *S. aureus*, *E. coli*, *K. pneumoniaw*, and other pathogenic bacteria was 5 mg and the proportional range was between 5 and 25 mg/mL (Chung et al., 1993). These findings agree with our results for the presence of tannins extracted from agricultural by-products, except for

Table 4. The antibacterial activity of tannins extracted by acetone from agricultural by-products.

	mg/disc	*VRSA*[1]	*MRSA*[2]	*B. coagulans*	*L. monocytogenes*	*E. coli*	*S. flexneri*
Green tea by-product	2	++	+++	++	+	++	++
	3	+++	+++	++	++	++	+++
	4	+++	+++	+++	++	++	+++
	5	+++	++++	+++	++	++	+++
	antibiotic[3]	++++	++	++++	++	++++	++++
Acorn hull	2	+	+++	-	+	+	++
	3	+	+++	-	+	+	++
	4	+	+++	-	+	+	++
	5	+	+++	+	++	+	++
	antibiotic	++++	++	++++	++	++++	++++
Chestnut hull	2	+	+	-	+	-	+
	3	+	++	-	++	-	+
	4	+	++	+	++	-	++
	5	+	++	+	++	+	++
	antibiotic	++++	++	++++	++	++++	++++
Persimmon hull	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
	5	-	+	-	-	+	-
	antibiotic	++++	++	++++	++	++++	++++

Inhibitory zone diameter :>8.0 mm, ++++; 5.0–7.0 mm, +++; 2.0–4.5 mm, ++; 0.2–1.5 mm, +; and <0.1 mm, −; [1]VRSA: Vancomycin-Resistant *Staphylococcus aureus;* [2]MRAS: Methicillin-Resistant *S.aureus;* [3]Antibiotic discs of ampicillin for *E. coli* and *S. flexneri*, and gentamicin for *L. monocytogenes*, *B. coagulans*, methicillin-resistant *S. aureus* and vancomycin-resistant *S. aureus* were used as positive controls, respectively.

Table 5. DPPH radical scavenging activity expressed by IC_{50} values (mg/ml) of tannins extracted from agricultural by-products.

Item	Distilled water extracts	Ethanol extracts	Acetone extracts
Green tea waste	0.020 ± 0.008^{aC}	0.002 ± 0.000^{bC}	0.021 ± 0.001^{aC}
Acorn hull	0.011 ± 0.001^{bC}	0.011 ± 0.002^{bB}	0.037 ± 0.002^{aB}
Chestnut hull	0.065 ± 0.002^{aA}	0.008 ± 0.002^{cB}	0.044 ± 0.002^{bB}
Persimmon hull	0.057 ± 0.001^{cAB}	0.576 ± 0.017^{aA}	0.143 ± 0.009^{bA}

Values represent the mean ± SD, *n*=3. IC_{50} values (expressed in mg dry weight of each tannins extracted from agricultural by-products per ml organic solvent) of sample that exhibited DPPH radical scavenging activity of more than 50%. $^{a, b, c}$Means with different superscript in the treatment rows are significantly different ($p<0.05$). $^{A, B, C}$Means with different superscript in the treatment columns are significantly different ($p<0.05$).

persimmon hull extract. Several previous studies have suggested that the antimicrobial effect of tannins may be related to their ability to inactivate microbial adhesions and inhibit hydrolytic enzymes such as proteases and carbohydrolases and cell envelope transport proteins (Min et al., 2003; Scalbert, 1991).

The biological activities of polyphenols have recently drawn increased attention from researchers and industries, as well as consumers. It is well known that polyphenolic compounds act as scavengers of reactive oxygen species, peroxide decomposers, quenchers of singlet oxygen, electron donors, and inhibitors of lipoxygenase (Porter, 1980). Numerous studies have suggested that oxidative stress may lead to pathological conditions such as aging, cancer, liver damage, heart disease, and Alzheimer's disease (Kohen and Nyska, 2002; Osawa et al., 1990). Tannins are water-soluble polyphenol that are present in the bark and fruits of many plants, especially bananas, grapes, sorghum, spinach, red wine, persimmons, coffee, chocolate and tea (Chung et al., 1998; Wu et al., 2004). It has also been reported that tannins are antioxidants often characterized by reducing power and scavenging activities (Minussi et al., 2003). In the present study, we used DPPH radical scavenging properties to also examine the antioxidant activity of tannins extracted from agricultural by-products. DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances (Özcelik et al., 2003). With ethanol extraction, the DPPH scavenging activity of tannins extracted from green tea waste was the highest among the agricultural by-products. Moreover, the IC_{50} value for DPPH scavenging activity of the ethanol extract from green tea waste was greater than that of vitamin C (data not shown). The antioxidant activity of green tea waste was also higher in the ethanol extract than in the distilled water and acetone extracts. Consequently, these results indicate that tannin extracted from agricultural by-products, especially green tea waste has effective and powerful antioxidant activity. Green tea, which is a widely consumed drink, has received much attention due to the beneficial biological effects attributable to its strong antioxidant activity (Ho et al., 1992). In a previous study, it was reported that an extract from the leaves of *Camellia. taliensis* with abundant hydrolysable tannins showed remarkable antioxidant activity *in vitro* (Gao et al., 2008). Moon and Park (2000) reported that the chloroform and ethylacetate fractions from persimmon leaves that contained tannin had strong antioxidant activities. In another study, persimmon tannin was found to significantly increase the activity of antioxidant enzyme such as superoxide dismutase and catalase, while decreasing the raised malondialdehyde (MDA) level which is a marker for oxidative stress *in vivo* (Tian et al., 2011). More recently, Zhang and Lin (2009) stated that tannin extracted by organic solvent from *Syzygium cumini* fruit showed strong DPPH scavenging activity and ferric reducing power. In addition, it was reported that crude tannins isolated from canola and rapeseed hulls exhibited strong DPPH radical scavenging activity ranging from 35.2 to 50.5% at a dose of 1 mg (Amarowicz et al., 2000). These results agree with our findings on tannins extracted from agricultural by-products. Therefore, it is strongly expected that tannins extracted from agricultural by-products such as acorn, chestnut, and persimmon hulls as well as green tea waste would be natural source of a substitute for current antioxidants added to foods and pharmaceuticals as well as animal feed and antibiotics because of their potent antibacterial activity and antioxidant capacity.

Conclusion

Our results showed that tannins extracted from agricultural by-products, in particularly green tea waste, exhibited strong antibacterial and antioxidant activity. These findings suggest that the tannin sources and therefore their chemical composition as well as their concentration in agricultural by-products influence antimicrobial and antioxidant activities. It is also expected that tannins extracted from green tea waste may provide alternatives and supplements to conventional anti-bacterial and antioxidant feed additives, although the mechanism for antibacterial and antioxidant activity of tannin extracted from green tea waste are not clear at this time. The potential activity of tannin extracted from green tea waste needs to be further studied to investigate the mode of action.

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REFERENCES

Ahn HS, Jeon TI, Lee JY, Hwang SG, Lim Y, Park DK (2002). Antioxidative activity of persimmon and grape seed extract: in vitro and in vivo. Nutr. Res., 22: 1265–1273.

Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K (2001). Antibacterial action of several tannins against *Staphylococcus aureus*. J. Antimicrob. Chemother, 48: 487–491.

Amarowicz R, Naczk M, Shahidi F (2000). Antioxidant activity of crude tannins of canola and rapeseed hulls. J. Am. Oil Chem. Soc., 77: 957–961.

Andrade RG, Ginani JS, Lopes GKB, Dutra F, Alonso A, Hermes-Lima M (2006). Tannic acid inhibits in vitro iron-dependent free radical formation. Biochimie, 88: 1287–1296.

Barry TN, McNabb WC (1999). The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. Brit. J. Nutr., 81: 263–272.

Black JG (1996). Microbiology: Principles and application. Prentice Hall, New York, pp. 260.

Chandrappa CP, Shilpashree CB, Karthik MR, Govindappa M, Sadananda TS (2011). Antibacterial and antioxidant activities of *Adiantum pedatum* L. J. Phytol., 3: 26–32.

Chung KT, Stevens SE, Lin WF, Wei CI (1993). Growth inhibition of selected food-borne bacteria by tannic acid, propyl gallate and related compounds. Lett. Appl. Microbiol., 17: 29–31.

Chung KT, Wong TY, Wei CI, Huang YW, Lin Y (1998). Tannins and human health: a review. Crit. Rev. Food Sci. Nutr., 38: 421–464.

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564–582.

Deshpande SS, Cheryan M, Salunkhe DK (1986). Tannin analysis of food products. CRC Crit. Rev. Food Sci. Nutr., 24: 401–449.

Doughari JH, El-mahmood AM, Tyoyina I (2008). Antimicrobial activity of leaf extracts of *Senna obtusifolia* (L). Afr. J. Pharm. Pharmacol., 2: 7–13.

Doughari JH, Human IS, Bennade S, Ndakidemi PA (2009). Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. J. Med. Plant Res., 3: 839–848.

Funatogawa K, Hayashi S, Shimomura H, Yoshida T, Hatano T, Ito H, Hirai Y (2004). Antibacterial activity of hydrolysable tannins derived from medicinal plants against Helicobacter pylori. Microbiol. Immunol. , 48: 251–261.

Gao DF, Zhang YJ, Yang CR, Chen KK, Jiang HJ (2008). Phenolic antioxidants from green tea produced from *Camellia taliensis*. J. Agric. Food Chem., 56: 7517–7521.

Gedir JV, Sporns P, Hudson RJ (2005). Extraction of condensed tannins from cervid feed and feces and quantification using a radial diffusion assay. J. Chem. Ecol., 31: 2761–2773.

Hagerman AE (1988). Extraction of tannins from fresh and preserved leaves. J. Chem. Ecol., 40: 453–461.

Haslam E (1979). Vegetable tannins. In Swain et al. (eds) Biochemistry of plant phenolics: Recent Advances in Phytochemistry, Plenum

Press, New York, pp. 475–523.

Ho CT, Chen Q, Shi H, Zhang KQ, Rosen RT (1992). Antioxidative effect of polyphenol extract prepared from various Chinese teas. Prev. Med., 21: 520–525.

Hoskin SO, Barry TN, Wilson PR, Charleston WAG, Hodgson J (1999). Effects of reducing anthelmintic input upon growth and faecal egg and larval counts in young farmed deer grazing chicory (*Chicorium intybus*) and perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture. J. Agric. Sci., 132: 335–345.

Inborr J (2000). Swedish poultry production without in-feed antibiotics - A testing ground or a model for the future? Australian Poult. Sci. Sym., 12: 1–9.

Kaitho RJ, Tegegne A, Umunna NN, Nsahlai IV, Tamminga S, Van Bruchem J, Arts JM (1998). Effect of *Leucaena* and *Sesbania* supplementation on body growth and scrotal circumference of Ethiopian highland sheep and goats fed teff straw basal diet. Lives. Prod. Sci., 54: 173–181.

Kohen R, Nyska A (2002). Oxidation of biological systems: Oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol. Pathol., 30: 620–650.

Lee KH, Rhee KH (2011). Anti-microbial effects of rhizome extracts of *Alpinia officinarum* hance against VRE (vancomycin-resistant enterococci) and other pathogenic microorganisms. Nat. Prod. Sci., 17: 160–164.

Livornese LL, Dias S, Samuel C (1997). Hospital-acquired infection with vancomycin-resistant *Enterococcus faecium* transmitted by electronic ear prove thermometer. Infect. Control Hosp. Epidemiol. 18:771–773.

Lowenthal J (1877). Uber die Bestimmung des Gerbstoffs. Z. Anal. Chem., 16: 33–48.

Majorie MC (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564–582.

Min BR, Barry TN, Attwood GT, McNabb WC (2003). The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Anim. Feed Sci. Technol., 106: 3–19.

Min BR, Pinchak WE, Merkel R, Walker S, Tomita G, Anderson RC (2008). Comparative antimicrobial activity of tannin extracts from perennial plants on mastitis pathogens. Sci. Res. Essays, 3: 66–73.

Minussi RC, Rossi M, Bologna L, Cordi L, Rotilio D, Pastore GM, Duran N (2003). Phenolic compounds and total antioxidant potential of commercial wines. Food Chem., 82: 409–416.

Moon SH, Park KY (2000). Antioxidative effect of persimmon leaves. Korean J. Food Nutr., 13: 53–58.

Nanjo F, Goto K, Seto R, Suzuki M, Sakai M, Hara Y (1996). Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. Free Radic. Biol. Med., 21: 895–902.

Nascimento GGF, Locatelli J, Freitas PC, Silva GL (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Braz. J. Microbiol., 31: 247–256.

Niezen JH, Waghorn TS, Charleston WAG, Waghorn GC (1995). Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. J. Agric. Sci. (Camb.). 125: 281–289.

Osawa T, Namiki M, Kawakishi S (1990). Role of dietary antioxidants in protection against oxidative damage. Basic Life Sci., 52: 139–153.

Özcelik B, Lee JH, Min DB (2003). Effects of light, oxygen and pH on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method to evaluate antioxidants. J. Food Sci., 68: 487–490.

Perez RM (2003). Antiviral activity of compounds isolated from plants. Pharm. Biol., 41: 107–157.

Porter WL (1980). Recent trends in food applications of antioxidants. In Simi and Karel. (eds) Autoxidation in food and biological systems, Plenum Press, New York, pp. 45–59.

Puupponen-Pimiä R, Nohynek L, Alakomi HL, Oksman-Caldentey KM (2005). Bioactive berry compounds-novel tools against human pathogens. Appl. Microbiol. Biotechnol., 67: 8–18.

Robertson HA, Niezen JH, Waghorn GC, Charleston WAG, Jinlong M (1995). The effect of six herbages on liveweight gain, wool growth, and faecal egg count of parasitised ewe lambs. Proc. N. Z. Soc. Anim. Prod., 55: 199–201.

Scalbert A (1991). Antimicrobial properties of tannin. Phytochemistry, 30: 3875–3883.

Taguri T, Tanaka T, Kouno I (2004). Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. Biol. Pharm. Bull., 27: 1965–1969.

Tanner GJ, Moate PJ, Davis LH, Laby RH, Yuguang L, Larkin PJ (1995). Proanthocyanidins (condensed tannins) destabilise plant protein foams in a dose-dependent manner. Aust. J. Agric. Res., 46: 1101–1109.

Taylor RE, Field TG (2001). Reproduction. In: Scientific animal farm production: an introduction to animal science (7th Eds). Prentice Hall, Upper Saddle River, New Jersey, pp. 188–190.

Tebib K, Rouanet JM, Besançon P (1997). Antioxidant effects of dietary polymeric grape seed tannins in tissues of rats fed a high cholesterol-vitamin E-deficient diet. Food Chem., 59: 135–141.

Tian Y, Zou B, Yang L, Xu SF, Yang J, Yao P, Li CM (2011). High molecular weight persimmon tannin ameliorates cognition deficits and attenuates oxidative damage in senescent mice induced by D-galactose. Food Chem. Toxicol., 49: 1728–1736.

Worsley A (2000). Food and consumers: Where are we heading? Asia Pacific J. Clin. Nutr., 9(Suppl.): S103–S107.

Wu LT, Chu CC, Chung JG, Chen CH, Hsu LS, Liu JK, Chen SC (2004). Effects of tannic acid and its related compounds on food mutagens or hydrogen peroxide-induced DNA strands breaks in human lymphocytes. Mutat. Res., 556(1–2): 75–82.

Zargham H, Zargham R (2008). Tannin extracted from Sumac inhibits vascular smooth muscle cell migration. Mcgill J. Med., 11: 119–123.

Zhang LL, Lin YM (2009). Antioxidant tannins from Syzygium cumini fruit. Afr. J. Biotechnol., 8: 2301–2309.