Journal of Food and Drug Analysis

Volume 19 | Issue 4 Article 5

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Journal of Food and Drug Analysis, Vol. 19, No. 4, 2011, Pages 478-485 藥物食品分析 第十九卷 第四期

Antibacterial and Antioxidant Properties of Sorghum Distillery Residue

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(Received: August 11, 2010; Accepted: August 8, 2011)

ABSTRACT

The alcohol extract (AE), cold water extract (CWE) and hot water extract (HWE) of sorghum distillery residues (SDR, also referred to in the literature as sorghum spirits lees, sorghum liquor waste, or grain sorghum dried distillers grains) were found to effectively inhibit the growth of *Bacillus cereus*, *Escherichia coli* O157 : H7, *Salmonella* spp. and *Staphylococcus aureus*. As the concentration of alcohol or water extracts increased to 6 mg/mL, no survival was detected. All extracts of SDR were evaluated for their antibacterial activity, radical-scavenging activity, reducing capacity, ferrous ion-chelating activity, total antioxidant activity and hydrogen peroxide scavenging activity. At a concentration of 75 μg/mL, the reducing capacities of the AE and HWE were not significantly different from BHA or Trolox ($p > 0.05$). The ferrous ion-chelating activities of AE and HWE were higher than that of citrate. Total antioxidant activity increased as concentrations of AE, CWE, and HWE increased, while AE and HWE showed higher total antioxidant activities than CWE. The results indicate that SDR alcohol or water extracts have antibacterial activity and antioxidant properties. They could be used as a source of antioxidant and antimicrobial ingredients in the food industry.

Key words: alcohol extract, antibacterial, antioxidant, sorghum, water extract

INTRODUCTION

In order to inhibit food-borne pathogens and extend shelf life, synthetic chemicals are often used as preservatives in food processing and storage. However, growing consumer awareness over the potential risks of synthetic food additives to human health has renewed the interest in using naturally occurring alternatives instead. Therefore, the use of antimicrobial compounds extracted from plants, as food preservatives, is a subject of growing interest, since plant matrices possess natural antimicrobial products which protect them from infection[(1)]. Plant phenolics appear to possess similar benefits to human health, especially given that the resistance of pathogens to antibiotics is developing faster than ever before. New antimicrobial and antioxidant substances from nature are in great demand. A number of researchers have investigated that various plant extracts appeared to have some effects on against certain pathogens and microorganisms[(2-5)]. Serra *et al.*[(1)] reported on the antimicrobial activities of two extracts derived from the wastes of olive oil and wine production, both rich in polyphenols. The extracts may have important applications in the future as natural antimicrobial agents for the food industry or medical use. Among the pathogens tested, *B. cereus* is one of the most common gram-positive bacteria, often associated with two kinds of food-borne illnesses, diarrhea and emetic symptoms[(6)]. *S. aureus* can cause a wide variety of diseases in humans and other animals through either toxin production or invasion. *Staphylococcal spp.* toxin is a common cause of food poisoning, as it can grow in improperly-stored food. Common symptoms may include nausea, vomiting, and abdominal cramps[(7)]. Gram-negative bacteria such as *E. coli* and *Salmonella spp.* *E. coli* is common in human flora, but there is an enterohaemorrhagic strain that can cause severe diarrhea that is often bloody and accompanied by abdominal cramps. However, in some people, especially young children and the elderly, the illness can progress to haemolytic uraemic syndrome (HUS), a condition that can lead to serious kidney damage and even death[(6)]. *Salmonella* is reported as the most frequent cause of food-borne gastroenteritis outbreaks in the world. Most

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people infected with *Salmonella* develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection[7].

Sorghum distillery residues (SDR) is a cheap and abundant brewery by-product. In the United States, more than 1.3 million metric tons of grain sorghum is used to produce ethanol annually. There are approximately 8.2 kg of dry residue in the form of distillers dried grains with solubles remaining from each 25 kg of grain sorghum used to produce ethanol[8]. In Taiwan, the SDR has been an under-utilized liquor distillation by-product with an estimated production of 250 tons/day in Kinmen. The objective of this study was to explore whether SDR has the potential to be used as an antibacterial and antioxidant agents.

MATERIALS AND METHODS

I. *Chemicals*

Butylated hydroxyanisole (BHA), stable free radical of 1,1-diphenyl-2-picryl-hydrazyl (DPPH•), citric acid, ferrous chloride ($FeCl_2$), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), nicotinamide adenine dinucleotide (NADH), linoleic acid, nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), trichloroacetic acid (TCA), 6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid (Trolox) and polyoxyethylenesorbitan monolaurate (Tween-20) were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Ammonium thiocyanate was purchased from Merck (Merck KGaA, Darmstadt, Germany). All other chemicals were of analytical grade, and also obtained from either Sigma-Aldrich or Merck.

II. *Preparation of Alcohol and Water Extracts*

The SDR was obtained from Kinmen Kaoliang Liquor Inc., Kinmen, Taiwan. For alcohol extraction (AE), cold water extraction (CWE), and hot water extraction (HWE), the SDR was extracted with 50% alcohol at 4°C (1/10, w/v) for 5 h, distilled water at 4°C (1/10, w/v) for 5 h, or autoclaved (1/10, w/v, 121°C, 1 atm) for 12 h, then centrifuged (10,000 ×g, 10 min at 4°C). The supernatants were filtered and freeze-dried to obtain powder form. The powders were then re-dissolved in distilled water according to the specific assay conditions described below.

III. *Antibacterial Assays*

B. cereus (Bioresource Collection and Research Center (BCRC), BCRC 10603), *E. coli* O157 : H7 (ATCC 13099), *S. choleraesuis* (BCRC 10241), *S. enteritidis* (BCRC 10744), *S. typhimurium* (BCRC 12974), and *S. aureus* (BCRC 25923) were used for antibacterial assays. All bacteria were streaked on nutrient agar plates and then incubated at 37°C for 24 h to obtain colonies. A single colony was picked up, seeded in nutrient broth, and then cultivated at 37°C for 12 h. The cultured broth was diluted to the concentration of 10^5 - 10^6 CFU/mL with 0.1% peptone water for the subsequent antibacterial assays. Each 0.5-mL aliquot of the diluted broth (10^5 - 10^6 CFU/mL) was added to 4.5 mL of nutrient broth containing 0, 1, 2, 4 or 6 mg/mL of AE, CWE or HWE, and incubated at 4, 15, or 35°C for 24 h. Samples of 1.0-mL aliquots were spread onto a nutrient agar plate, and incubated at 35°C for 24 h for colony counting. Mean values ± SD of triplicates were calculated.

IV. *DPPH Radical-Scavenging Activity*

One mL of ethanolic DPPH (2,2-diphenyl-1- picrylhydrazyl) solution (0.1 mM) was added to 3 mL of aqueous AE, CWE, and HWE or the ethanolic standard solution of BHA or Trolox at concentrations of 25 to 200 μg/mL[9]. The mixture was shaken vigorously and incubated at room temperature for 30 min. The absorbance at 517 nm was then measured using a spectrophotometer (Model 7800UV/VIS, Jasco, Tokyo, Japan). The free radical-scavenging activity was calculated using the following equation: DPPH-scavenging effects (%) = $(A_0 - A_1) / A_0 \times 100$, where A_0 and A_1 were the absorbance values of the blank and test samples, respectively.

V. *Reducing Capacity*

An 1 mL aliquot of AE, CWE, or HWE (25 - 200 μg/mL) was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution[10]. The mixture was incubated at 50°C for 20 min, 2.5 mL of 10% TCA was added and the mixture was centrifuged for 10 min at 4,000 ×g (Sorvall RC 5C, Dupont, Wilmington, DE) to obtain the upper layer. An aliquot (2.5 mL) of the upper layer was mixed thoroughly with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ solution. The reducing capacity was expressed by the absorbance of the reaction mixture at 700 nm.

VI. *Ferrous Ion-Chelating Activity*

A 0.4 mL aliquot of the test samples (25 - 200 μg/mL) was taken and 0.05 mL of 2 mM $FeCl_2$ solution, 0.2 mL of 5 mM ferrozine, and deionized water were added to make up a total volume of 4 mL[11]. The mixture was shaken vigorously and set aside at room temperature for 10 min. Absorbance was then measured at 562 nm. The ferrous ion-chelating activity was calculated using the following equation: Ferrous ion-chelating activity (%) = $(A_0 - A_1) / A_0 \times 100$, where A_0 and A_1 were the absorbance values of the blank and test samples, respectively.

VII. *Hydrogen Peroxide-Scavenging Activity*

A solution of 40 mM of H_2O_2 in phosphate buffer (pH 7.4) was prepared. A 3.4 mL aliquot of aqueous alcohol or water extract solution (50, 100, or 200 μg/mL), and an ethanolic solution of BHA or Trolox (50 μg/mL) were added to 0.6 mL of H_2O_2 solution[12]. The mixture was allowed to react

for 10 min before the absorbance was measured at 230 nm. The phosphate buffer that contained no H_2O_2 was used as the blank. The scavenging activities were calculated as follows: H_2O_2-scavenging activity (%) = $(A_0 - A_1)/ A_0 \times 100$, where A_0 and A_1 were the absorbance values of the blank and test samples, respectively.

VIII. *Total Antioxidant Activity*

The antioxidant activity was determined according to the ferric thiocyanate method of Mitsuda[13]. Linoleic acid (3.1 μg/mL) was emulsified in 40 mM potassium phosphate buffer (pH 7.0) containing 0.351% Tween-20. A 2.5 mL aliquot of the emulsion was mixed with 2.5 mL of 40 mM potassium phosphate buffer (pH 7.0) that contained AE, CWE, and HWE (50 - 200 μg/mL) or the positive compounds (BHA or Trolox, 50 μg/mL). The mixture (5 mL) was added with 0.1 mL of 20 mM $FeCl_2$ (3.5% HCl) solution and 0.1 mL of 30% ammonium thiocyanate solution, then incubated at 37°C. Samples were taken periodically to measure the maximum absorbance at 500 nm. The inhibition of lipid peroxidation in the linoleic acid emulsion was calculated using the following equation: Inhibition of lipid peroxidation (%) = $100 - (A_1 / A_0) \times 100$, where A_0 and A_1 were the absorbance of the blank and test samples, respectively.

IX. *Determination of Total Phenolics and Flavonoids*

Gallic acid was used as the standard compound. Alcohol or water extracts (0.1 g) were dissolved in 5 mL of 0.3% HCl in methanol/water (60 : 40, v/v). The solution (100 μL) was added to 2% Na_2CO_3 (2.0 mL). After 2 min, 50% Folin-Ciocalteu reagent (100 μL) was added to the mixture, then set aside for 30 min before the absorbance was measured at 750 nm[14].

The total content of flavonoids in alcohol or water extract was determined according to the method of Jia *et al.*[15]. Gallic acid and quercetin were used as the standard compounds. Three mL of extract was placed in a 10-mL volumetric flask and distilled water was added to the flask to make 5 mL, then 0.3 mL of $NaNO_2$ (1 : 20) was added. After 5 min, 3 mL of $AlCl_3$ (1 : 10) was added into the flask. After 6 min, 2 mL of 1 M NaOH was added into the flask, then distilled water was added to make up to a total volume of 10 mL. The solution was thoroughly mixed again, and the absorbance was measured against a blank at 510 nm with a spectrophotometer. The phenolic and flavonoid contents in the test samples were expressed as gallic acid and quercetin equivalents, respectively (mg gallic acid equivalent /g of AE, CWE, or HWE extract powders and mg quercetin equivalent /g of AE, CWE, or HWE extract powders).

X. *Statistical Analysis*

Data were presented as mean ± standard deviation (SD) of three independent experiments. Values were evaluated by one-way ANOVA, followed by Duncan multiple-range tests using Statistical Analysis Software Version 6.11 (SAS Institute, Cary, NC, USA). Control and treatment groups were compared by Student *t*-test. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

I. *Antimicrobial Activities*

The different extracts (AE, CWE, and HWE) inhibited the growth of all bacteria strains in a dose-dependent manner, and the resistance effect was less efficient in *S. aureus* than *E. coli, Salmonella* spp., and *B. cereus* at 4°C, 15°C or 35°C (Tables 1 and 2). In the present study, the minimum inhibitory concentration (MIC) of alcohol or water extract for 24 h incubation against all bacteria was found to be 4 - 6 mg/mL at 35°C. As the concentration of the alcohol or water extracts increased to 6 mg/mL, no survival was detected. The inhibitory effects of the extracts are attributed to their total phenolic concentration and composition. Phenolic compounds are toxic to bacterial cells and inhibit their growth at high concentrations[16-17]. Baydar *et al.*[18] found that grape seed extract exhibited significant antibacterial activity in contrast to grape bagasse extracts, which coincided with its polyphenolic content in the extract. The experimental data suggest the potential use of the alcohol or water extracts of SDR as a natural antibacterial food additives.

II. *DPPH Radical-Scavenging Activity*

Antioxidant properties are very important in counteracting the deleterious role of free radicals in foods or biological systems. Excessive formation of free radicals accelerates the oxidation of lipids in foods, impairs food quality and consumer acceptance[19]. The DPPH radical-scavenging abilities of the SDR alcohol and water extract were in the order of AE (62%) > HWE (55%) > CWE (33%) at a concentration of 75 μg/mL. Gülçin[20] reported that the DPPH radical-scavenging ability of the boiling water extract of black pepper (WEBP) at 75 μg/mL was 55% while the scavenging ability of the ethanol extract (EEBP) was only 48%. Hou *et al.*[21] reported the scavenging abilities of 80% methanolic extracts from SL (small leaf), BL (big leaf), and TL (thin leaf) cultivars of *Liriope spicata* L. (Mai-Men-Dong in Chinese) against DPPH radicals. The DPPH radical-scavenging abilities of the SDR alcohol or water extract in the present study was found to be similar to that of black pepper and lower than those of Mai-Men-Dong extracts.

III. *Reducing Capacity*

Reducing capacities (shown by the absorbance at 700 nm in the ferricyanide reduction test) of all samples in this study increased with increasing concentrations (Figure 2). At the concentration of 25 μg/mL, AE and HWE were inferior to BHA and Trolox in reducing capacity, while at

Table 1. Antibacterial activity of alcohol and water extract of SDR against gram-negative bacteria

Strain	Conc. (mg/mL)	Colony count (log CFU/ mL)								
		CWE (4°C)	HWE (4°C)	AE (4°C)	CWE (15°C)	HWE (15°C)	AE (15°C)	CWE (35°C)	HWE (35°C)	AE (35°C)
Escherichia coli O157:H7 (BCRC 13099)	0	1.15 ± 0.09^{a}	1.23 ± 0.04^{b}	1.16 ± 0.05^{a}	3.38 ± 0.23^{a}	3.48 ± 0.27^{a}	3.55 ± 0.09^{a}	8.31 ± 0.46^{a}	8.55 ± 0.34^{a}	8.22 ± 0.10^{a}
	1	0.74 ± 0.09^{a}	0.49 ± 0.05^{c}	0.61 ± 0.04^{b}	1.82 ± 0.09^{a}	1.69 ± 0.05^{b}	1.80 ± 0.02^{a}	3.12 ± 0.26^{a}	2.78 ± 0.03^{a}	2.77 ± 0.03^{a}
	2	ND	ND	ND	0.26 ± 0.05^{a}	0.20 ± 0.02^{b}	0.25 ± 0.01^{a}	0.58 ± 0.05^{a}	0.50 ± 0.05^{b}	0.56 ± 0.02^{a}
	4	ND	ND	ND	ND	ND	ND	0.22 ± 0.06^{a}	0.11 ± 0.03^{c}	0.18 ± 0.02^{b}
	6	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella choleraesuis (BCRC 10241)	0	1.91 ± 0.29^{a}	1.78 ± 0.03^{b}	1.80 ± 0.02^{a}	3.87 ± 0.38^{a}	3.64 ± 0.04^{b}	4.07 ± 0.08^{a}	7.83 ± 0.20^{a}	7.80 ± 0.02^{a}	7.90 ± 0.18^{a}
	1	0.27 ± 0.04^{a}	0.25 ± 0.01^{a}	0.27 ± 0.01^{a}	2.19 ± 0.24^{a}	1.76 ± 0.11^{c}	1.85 ± 0.03^{b}	3.18 ± 0.17^{a}	3.06 ± 0.07^{b}	3.17 ± 0.05^{a}
	2	ND	ND	0.03 ± 0.00^{a}	0.15 ± 0.04^{b}	0.13 ± 0.01^{c}	0.19 ± 0.01^{a}	0.61 ± 0.04^{a}	0.48 ± 0.03^{c}	0.54 ± 0.02^{b}
	4	ND	ND	ND	ND	ND	ND	0.04 ± 0.01^{a}	0.02 ± 0.01^{b}	0.04 ± 0.01^{a}
	6	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella enteritidis (BCRC 10744)	0	1.06 ± 0.17^{b}	1.15 ± 0.03^{b}	1.25 ± 0.06^{a}	2.79 ± 0.45^{a}	3.11 ± 0.12^{a}	3.08 ± 0.20^{a}	8.02 ± 0.57^{a}	7.91 ± 0.19^{a}	7.78 ± 0.03^{b}
	1	0.34 ± 0.11^{a}	0.18 ± 0.02^{b}	0.23 ± 0.02^{a}	1.51 ± 0.10^{a}	1.26 ± 0.04^{b}	1.29 ± 0.04^{b}	3.57 ± 0.50^{a}	2.72 ± 0.09^{b}	2.82 ± 0.04^{b}
	2	ND	0.01 ± 0.00^{b}	0.08 ± 0.02^{a}	0.30 ± 0.01^{a}	0.25 ± 0.04^{b}	0.26 ± 0.02^{b}	0.41 ± 0.08^{c}	1.85 ± 0.12^{b}	2.02 ± 0.07^{a}
	4	ND	ND	ND	0.07 ± 0.01^{b}	0.14 ± 0.02^{a}	0.16 ± 0.02^{a}	0.57 ± 0.02^{a}	0.20 ± 0.02^{c}	0.26 ± 0.02^{b}
	6	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella typhimurium (BCRC 12974)	0	1.01 ± 0.17^{c}	1.15 ± 0.03^{b}	1.17 ± 0.03^{a}	3.52 ± 0.32^{a}	3.20 ± 0.07^{a}	3.42 ± 0.08^{a}	8.14 ± 0.82^{a}	7.83 ± 0.13^{b}	8.25 ± 0.06^{a}
	1	0.76 ± 0.10^{a}	0.64 ± 0.02^{b}	0.74 ± 0.02^{a}	1.49 ± 0.27^{b}	1.68 ± 0.04^{a}	1.71 ± 0.05^{a}	2.99 ± 0.65^{a}	3.22 ± 0.03^{a}	3.07 ± 0.27^{a}
	2	ND	ND	ND	0.37 ± 0.05^{b}	0.36 ± 0.02^{b}	0.40 ± 0.03^{a}	0.74 ± 0.10^{a}	0.65 ± 0.03^{b}	0.74 ± 0.03^{a}
	4	ND	ND	ND	0.12 ± 0.04^{a}	0.08 ± 0.02^{b}	0.11 ± 0.01^{a}	0.18 ± 0.03^{a}	0.09 ± 0.02^{c}	0.13 ± 0.03^{b}
	6	ND	ND	ND	ND	ND	ND	ND	ND	ND

Means ± SD (n = 3). ND = Not detected. Means in the same row at same temperature that do not share the same lower case alphabetic superscript are significantly different at the 5% level according to Duncan's multiple range test (DMRT).

Table 2. Antibacterial activity of alcohol and water extract of SDR against gram-positive bacteria

Strain	Conc. (mg/mL)	Colony count (log CFU/ mL)								
		CWE (4°C)	HWE (4°C)	AE (4°C)	CWE (15°C)	HWE (15°C)	AE (15°C)	CWE (35°C)	HWE (35°C)	AE (35°C)
Bacillus cereus (BCRC 10603)	0	1.43 ± 0.12^{a}	1.47 ± 0.03^{a}	1.48 ± 0.02^{a}	4.14 ± 0.35^{a}	4.41 ± 0.08^{a}	4.37 ± 0.12^{a}	8.59 ± 0.45^{b}	8.77 ± 0.11^{a}	8.68 ± 0.09^{a}
	1	0.59 ± 0.06^{a}	0.54 ± 0.01^{a}	0.59 ± 0.01^{a}	1.49 ± 0.29^{a}	1.38 ± 0.16^{a}	1.67 ± 0.03^{a}	4.08 ± 0.18^{a}	3.54 ± 0.04^{b}	4.13 ± 0.01^{a}
	2	ND	ND	ND	0.73 ± 0.05^{a}	0.64 ± 0.02^{b}	0.73 ± 0.02^{a}	1.39 ± 0.34^{a}	1.16 ± 0.02^{a}	1.20 ± 0.02^{a}
	4	ND	ND	ND	ND	ND	ND	0.18 ± 0.03^{a}	0.07 ± 0.01^{b}	0.17 ± 0.01^{a}
	6	ND	ND	ND	ND	ND	ND	ND	ND	ND
Staphylococcus aureus (BCRC 25923)	0	2.25 ± 0.15^{a}	2.27 ± 0.05^{a}	2.47 ± 0.02^{a}	4.06 ± 0.21^{a}	4.13 ± 0.15^{a}	4.10 ± 0.02^{a}	7.76 ± 0.46^{b}	8.21 ± 0.04^{a}	8.01 ± 0.17^{b}
	1	0.44 ± 0.09^{a}	0.39 ± 0.02^{a}	0.40 ± 0.02^{a}	2.20 ± 0.20^{a}	1.86 ± 0.02^{b}	2.07 ± 0.06^{a}	4.47 ± 0.19^{a}	4.50 ± 0.02^{a}	4.30 ± 0.07^{b}
	2	0.21 ± 0.03^{a}	0.15 ± 0.01^{b}	0.17 ± 0.02^{b}	1.28 ± 0.26^{a}	0.09 ± 0.02^{b}	1.24 ± 0.02^{a}	1.94 ± 0.19^{a}	1.79 ± 0.04^{a}	2.07 ± 0.08^{a}
	4	ND	ND	ND	0.37 ± 0.06^{b}	0.08 ± 0.01^{c}	0.70 ± 0.02^{a}	0.70 ± 0.05^{a}	0.63 ± 0.01^{b}	0.61 ± 0.03^{b}
	6	ND	ND	ND	ND	ND	ND	ND	ND	ND

Means ± SD (n = 3). ND = Not detected. Means in the same row at same temperature that do not share the same lower case alphabetic superscript are significantly different at the 5% level according to Duncan's multiple range test (DMRT).

the concentration of 75 μg/mL, the reducing capacities of the AE and HWE were not significantly different from BHA or Trolox ($p > 0.05$). Higher absorbance indicated lower reducing capacities. Gülçin[20] showed that the reducing capacities (absorbance) of WEBP and EEBP at 75 μg/mL were 0.67 and 0.86, respectively, while those of AE and HWE at the same concentrations were 0.91 and 1.03, respectively.

Figure 1. Comparison in DPPH radical scavenging activity among AE, CWE, HWE, BHA and Trolox at various concentrations. Data are expressed as means and standard deviations of three independent experiments, each used triplicate samples. Means (n = 3) in the same concentration that don't bear the same superscript (a,b,c,d) were significantly different at 5% level according to Duncan's multiple range test.

Figure 2. Comparison in reducing capacity of citric acid, AE, BHA, CWE, HWE and Trolox at various concentrations. Reducing capacity is shown by absorbance at 700 nm in the ferricyanide reduction test. Data are expressed as means and standard deviations of three independent experiments, each used triplicate samples. Means (n = 3) in the same concentration that don't bear the same superscript (a,b,c,d) were significantly different at 5% level according to Duncan's multiple range test.

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IV. *Ferrous Ion-Chelating Activity*

The ferrous ion-chelating activities of citric acid, EDTA, AE, CWE, and HWE are shown in Figure 3. In this assay, the metal-scavenging effect of those samples decreased in the order of EDTA > AE > HWE > citric acid > CWE. The compounds of AE, CWE, and HWE may have chelated the ferrous ions with hydroxyl groups. It was reported that the compounds with structures containing two or more of the following functional groups: OH, SH, COOH, PO_3H_2, C=O, NR_2, S and O in a favorable structure-function configuration can show the activity of metal chelation[22]. The ferrous ion-chelating activity did not show significant statistical difference between AE and HWE. Besides, the ferrous ion-chelating capacities of 100 μg/mL of AE, citric acid, CWE, EDTA, and HWE were found to be 75, 33, 33, 98, and 73%, respectively. Gülçin *et al.*[20] reported that the ferrous ion-chelating activities of WEBP and EEBP at 75 μg/mL were 84% and 83%, respectively. AE and HWE required a concentration of approximately 100 μg/mL to exhibit the same ferrous ion-chelating activity. Since ferrous ions are the most effective and commonly found pro-oxidant in the food system[23], the ferrous ion-chelating activities of AE and HWE proves their potential for use as a natural antioxidants.

V. *Hydrogen Peroxide-Scavenging Activity*

According to Figure 4, AE and HWE exhibited stronger inhibition effects than CWE. Although the hydroxyl radical-scavenging abilities of the extracts were significantly lower than those of BHA and Trolox, the extracts can serve as free radical inhibitors or scavengers.

VI. *Total Antioxidant Activity*

In Figure 5, the total antioxidant activity rose with the increase in AE, CWE, and HWE concentration. Total antioxidant activities of AE and HWE were not significantly different ($p > 0.05$), and CWE exhibited the lowest activity at various concentrations. Gülçin[20] reported that the total antioxidant activities of WEBP and EEBP at 75 μg/mL were

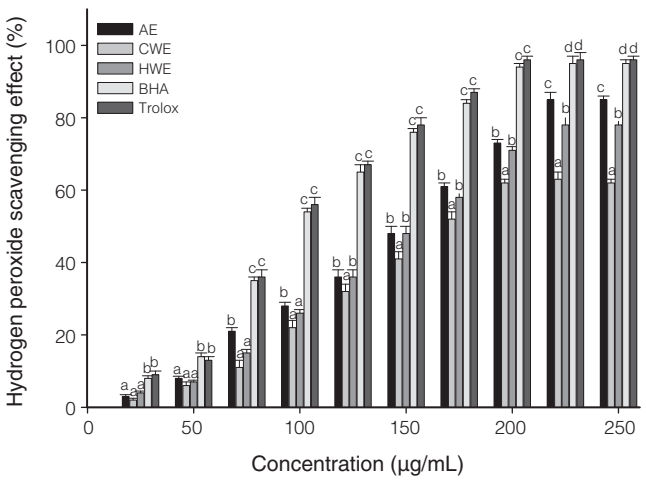

Figure 4. Comparison in hydrogen peroxide scavenging effect among BHA, Trolox, AE, CWE and HWE at various concentrations. Data are expressed as means and standard deviations of three independent experiments, each used triplicate samples. Means (n = 3) in the same concentration that don't bear the same superscript (a,b,c,d) were significantly different at 5% level according to Duncan's multiple range test.

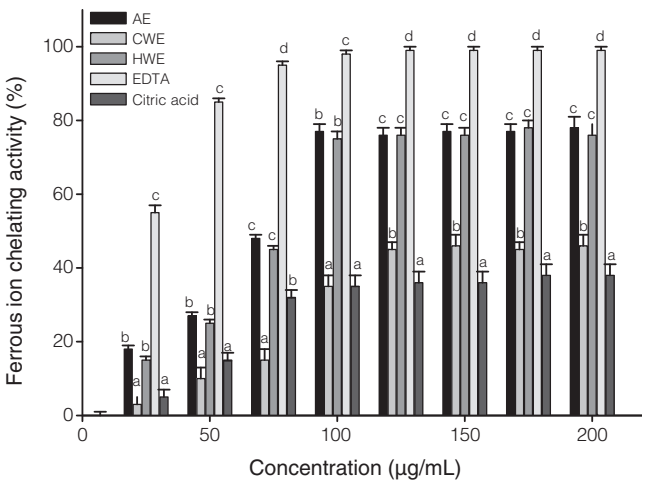

Figure 3. Comparison in ferrous ion-chelating activity among citric acid, EDTA, AE, CWE and HWE at various concentrations. Data are expressed as means and standard deviations of three independent experiments, each used triplicate samples. Means (n = 3) in the same concentration that don't bear the same superscript (a,b,c,d) were significantly different at 5% level according to Duncan's multiple range test.

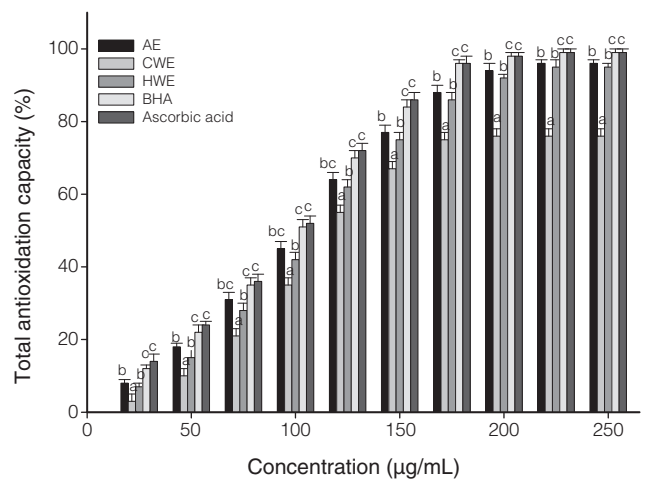

Figure 5. Comparison in total antioxidant activity among BHA, ascorbic acid, AE, CWE and HWE at various concentrations. Data are expressed as means and standard deviations of three independent experiments, each used triplicate samples. Means (n = 3) in the same concentration that don't bear the same superscript (a,b,c,d) were significantly different at 5% level according to Duncan's multiple range test.

Table 3. Total phenolic and flavonoids contents of AE, CWE, and HWE

Components	AE	CWE	HWE
Total phenols (mg gallic acid equivalent/g, of extract powders)	82.4 ± 1.4	58.6 ± 1.1	78.2 ± 1.2
Flavonoids (mg quercetin equivalent/g, of extract powders)	41.3 ± 1.1	24.3 ± 1.0	35.8 ± 0.8

Means ± SD (n = 3).

95% and 93%, respectively. AE and HWE at approximately 200 μg/mL could reach the same total antioxidant activity as WEBP and EEBP.

VII. *Total Phenolic and Flavonoids Content*

Total phenolic content was measured for all the test samples (Table 3). The highest total phenolic and flavonoid contents were observed in the AE (82.4 mg GAE/g, of extract powders and 41.3 mg QE/g, of extract powders, respectively). Many reports suggested that polyphenolic compounds, including flavonoids, possess anti-inflammatory, anti-allergic, antiartherosclerosis, anti-diabetic, and anticarcinogenic properties[24-25]. These biological properties are thought to be related to the antioxidant activity of these compounds[25].

Kil *et al*.[26] reported on the antioxidant and antimicrobial activities of sorghum extracts prepared from 25 cultivars from South Korea. Their results indicated that sorghum extracts could be used as a source of antioxidant and antimicrobial ingredients in the food industry. In our study, the SDR (sorghum distillery residue) which is considered an industrial waste, has the possibility of becoming an inexpensive source of natural food additives. The results of the present study indicate that the alcohol and water extracts of SDR have antibacterial activity and antioxidant properties and phenolic compounds are the active components. Further research on the isolation and identification of the active components of these alcohol or water extracts of SDR and their applications in food systems appears worthwhile.

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