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Antioxidant and antimicrobial activities of crude sorghum extract

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ABSTRACT

Here, we report the antioxidant and antimicrobial activities of sorghum (Sorghum bicolor Moench) extracts prepared from 25 cultivars from South Korea. Four cultivars of sorghum were extracted with methanol, then further fractioned with *n*-hexane, ethyl acetate, *n*-butanol, and water. The RC₅₀ (the concentration of antioxidant required to achieve absorbance equal to 50% that of a control containing no antioxidants) value of the DPPH method and reducing power showed higher efficiency in the BuOH layer of all selected cultivars except Neulsusu. The various fractions were then examined for antimicrobial activity by a serial two fold dilution assays using the paper disc method. The methanol extracts showed higher levels of antimicrobial activity than the other fractions. Our results indicate that sorghum extracts could be used as a source of antioxidant and antimicrobial ingredients in the food industry.

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1. Introduction

Sorghum (Sorghum bicolor Moench) is a major cereal food crop in many parts of the world; however, it is particularly important as a human food resource and folk medicine in Asia and Africa ([Ryu, Kim, & Kim, 2006](#page-5-0)). Sorghum is rich in phytochemicals known to significantly affect human health, such as tannins, phenolic acids, anthocyanins, phytosterols, and policosanols ([Awika & Roo](#page-4-0)[ney, 2004\)](#page-4-0). Recent studies have shown that sorghum has antioxidant activity ([Choi, Jeong, & Lee, 2006\)](#page-5-0), anticarcinogenic effects ([Kwak, Lim, Kim, Park, & Lee, 2004](#page-5-0)), and cholesterol-lowering effects [\(Ha, Cho, & Lee, 1998\)](#page-5-0), and can reduce the risk of cardiovascular disease [\(Cho, Choi, & Ha, 2000](#page-5-0)). Furthermore, sorghum has been shown to possess DPPH radical-scavenging activity and direct antimutagenic effects ([Kwak et al., 2004](#page-5-0)). HMG-CoA reductase inhibitory activity has also been detected in methanol extracts of sorghum ([Ha et al., 1998](#page-5-0)). However, little information is available concerning the antimicrobial effects of sorghum.

Free radicals, chemical reactions, and several redox reactions of various compounds may cause protein oxidation, DNA damage,

and lipid peroxidation in living cells [\(Morrissey & O'Brien, 1998\)](#page-5-0). Increased consumption of whole grains, fruits, and vegetables is related to a reduced risk of chronic diseases [\(Hu, 2002\)](#page-5-0). Antioxidants, including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butyl hydroquinone (TBHQ), are added to stabilise lipids against oxidation ([Moure et al., 2001](#page-5-0)). Chelating agents such as citric acid can be used to prevent their catalytic effects on oxidation ([O'Brien, 2004\)](#page-5-0).

Food spoilage is one of the most important issues facing the food industry ([Sokmen et al., 2004](#page-5-0)). In fact, food-borne illness is a global problem, even in developed countries. Food spoilage or deterioration is predominantly caused by the growth of microorganisms. Many pathogenic microorganisms, including Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Listeria monocytogenes, Campylobacter jejuni, Candida spp., Zygosaccharomyces spp., Fusarium spp., Aspergillus spp., Rhizopus spp., Penicillium spp., and Salmonella spp. have been identified as the causal agents of foodborne diseases or food spoilage ([Betts, Linton, & Betteridge, 1999;](#page-4-0) [Walker, 1988\)](#page-4-0). S. aureus is an extracellular, pyrogenic pathogen that can cause localised infections or life-threatening systemic diseases through its ability to destroy tissue and protect bacteria from the host immune response ([Lowy, 2000\)](#page-5-0). Candida albicans, which exists as a commensal organism in the mucocutaneous cavities of the skin, vagina, and intestine in humans ([Kaufman, 1997](#page-5-0)), can cause infections under altered physiological and pathological conditions, such as infancy, pregnancy, diabetes, prolonged broadspectrum antibiotic treatment, steroidal chemotherapy and AIDS

Abbreviations: BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DPPH, diphenyl-2-picrylhydrazyl.

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([Friedman, Richardson, Jacobs, & O'Brien, 2000; Kennedy, Laurier,](#page-5-0) [Gautrin, Ghezzo, & Pare, 2000](#page-5-0)).

Therefore, to screen for reducing power and free radical-scavenging capacity, a serial twofold dilution assay and paper disc diffusion assay were performed to check for in vitro antioxidant and antimicrobial activities of sorghum cultivars.

2. Materials and methods

2.1. Chemicals

All solvents used were of analytical grade. Methanol was obtained from Baker (Phillipsburg, NJ). Trichloroacetic acid, potassium ferricyanide, potassium phosphate, ferric chloride, and sodium carbonate were from Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), a-tocopherol, BHA, BHT, and tetracycline were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Plant material and extraction

Sorghum cultivars were provided by the Sillim Agricultural Cooperative in Kangwon province, South Korea. Voucher herbarium specimens were deposited with the reference number 2865 in the Herbarium of the Universidad de Talca. The seeds were stored at 4° C. Susu cultivars used in this study were Gumeunchalsusu, Ginjangmoksusu, Kkachisusu, Kkachisusu (daerip), Kkomadansusu, Neulsusu, Mesusu, Moktaksusu, Mongdangsusu, Bulkeunsaeksusu, Bulkeunjangmoksusu, Bulkeunjangsususu, Bulkeunchalsusu, Bitjarususu, Susongsaengi, Sikyungsusu, Ilbanchalsusu, Jangmoksusu, Jangsusu, Jaeraejongsusu, Joburangsusu, Chalsusu (RDA), Chalsusu (2), Heuinsusu and Heuinjangmoksusu. Samples of approximately 2 g of seeds from each cultivar were finely ground using a homogeniser and extracted with 100% methanol at room temperature for 24 h. Each mixture was then filtered through Whatman No. 42 filter paper to remove the debris, and the extracts were then evaporated at 40 \degree C using a rotary evaporator. The crude extracts were suspended in water and partitioned successively with hexane, ethyl acetate, n-butanol, and water.

2.3. DPPH assay

The effects of herbal product extracts on DPPH radicals were studied using the modified method of [Shimada, Fujikawa, Yahara,](#page-5-0) [and Nakamura \(1992\)](#page-5-0). Briefly, 0.15μ M DPPH in 4 ml of methanol was prepared and 1.0 ml of this solution was added to the test sample. The reaction mixture was shaken well and incubated for 30 min at room temperature. The absorbance of the resulting solution was read at 517 nm against a blank. The inhibitory percentage of DPPH was calculated according to the following equation:

Scavenging activity (%)=(1—absorbance_{sample}/absorbance_{control})× 100.

The EC_{50} value (mg/ml) is the concentration at which the scavenging activity is 50%.

2.4. Reducing power

The reducing power was determined according to the method of [Oyaizu \(1986\).](#page-5-0) A concentration of sorghum methanolic extract $(200 \mu l)$ was mixed with 500 μl of 0.2 M sodium phosphate buffer (pH 6.6) and 500 µl of 1% potassium ferricyanide, and the resultant mixture was incubated at 50 °C for 20 min. After addition of 2.5 ml of 10% trichloroacetic acid (w/v), the mixture was centrifuged at 650 rpm for 10 min. The upper layer (500 μ l) was mixed with 500 µl of deionised water and 100 µl of 0.1% ferric chloride, and the absorbance was measured at 700 nm: higher absorbance indicates higher reducing power. The assays were carried out in triplicate and the results are expressed as means ± standard deviation. The extract concentration providing absorbance of 0.5 (EC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration. a-Tocopherol, BHA, and ascorbic acid were used as standards.

2.5. Two fold dilution assay

Five bacterial strains and one fungal strain were used in this study: Bacillus subtilis, S. aureus, E. coli, Salmonella typhimurium, K. pneumoniae and C. albicans. All strains were obtained from the Bioherb Research Institute at Kangwon National University, South Korea. The bacterial cultures were grown in liquid medium at a suitable temperature. The bacterial strains were incubated on micrococcus, nutrient, and YM media then cultured with shaking for 12 h at 37 or 30 °C.

Twenty-five sorghum cultivars were screened for antimicrobial activity using a serial twofold dilution assay. Inocula were prepared from 12 h broth cultures of the bacterial strains, then diluted 100-fold with nutrient broth [\(Kobayashi, Koguchi, Takahashi, Kan](#page-5-0)[zaki, & Kawazu, 1993](#page-5-0)). The extracts were then diluted to the highest concentration $(500 \mu g/ml)$ for testing and serial twofold dilutions were made over the concentration range of $7.8-500 \mu g$ ml. Briefly, 96-well plates were prepared by dispensing $180 \mu l$ of the diluted inocula into each well. Aliquots of $20 \mu l$ of the stock solution for each extract (prepared at a concentration of 500 μ g/ ml) were added to the first series of wells. Next, 100 µl from each serial dilution was dispensed into six consecutive wells. The final volume in each well was 100 μ l. The contents of each well were mixed on a plate shaker at 300 rpm for 20 s, and then incubated at the appropriate temperature for 24 h. The minimum inhibitory concentration (MIC) of each compound was defined as the lowest concentration that inhibited microorganism growth. Bacterial growth was evaluated visually based on the degree of turbidity. Tetracycline and ketoconazole were used as standard antibiotics.

2.6. Paper disc diffusion assay

The antimicrobial activities of the plant extracts and fractions were determined by paper disc diffusion assay. The bacterial pathogens and fungal strain were grown in liquid medium for 20 h to yield a final concentration of 10^6 – 10^7 CFU/ml. Next, aliquots of 0.1 ml of the test microorganisms were spread over the surface of agar plates. Sterilised filter paper discs were saturated with 50 μ l of the methanol extract and the various fractions at 10,000 ppm. The soaked discs were then placed in the middle of the plates and incubated for 24 h, after which the diameter of each inhibitory zone was measured (in mm). Negative controls were prepared using the same solvents employed to dissolve the plant extracts.

2.7. Statistical analysis

To determine whether there were any differences between activity and concentration of samples, one-way analysis of variance (ANOVA) was applied and $P < 0.05$ was considered to indicate statistical significance.

3. Results and discussion

3.1. Antioxidant activity

3.1.1. Scavenging of DPPH radicals

DPPH is a free radical compound that has been widely used to determine free radical-scavenging ability [\(Amarowicz, Pegg,](#page-4-0)

[Rahimi-Moghaddam, Barl, & Weil, 2004\)](#page-4-0). The DPPH free radicalscavenging activities of sorghum product extracts, α -tocopherol, BHA, BHT, and ascorbic acid are presented in Tables 1 and 2. A solu-

Table 1

DPPH^a free radical-scavenging activity in accessions of Sorghum.

^a DPPH: 1,1-diphenyl-2-picryl-hydrazyl.

b RC50(µg/µl): Amount required for 50% reduction of DPPH after 30 min. Each value is mean±standard deviation of three replicate tests.

^c BHA: Butylated Hydroxyanisole.

^d BHT: Butylated Hydroxytoluene.

Table 2

DPPH^a free radical-scavenging activity of fractions.

^a DPPH: 1,1-diphenyl-2-picryl-hydrazyl
^b PC50(ug/ul): Amount required for 50

 $RC50(\mu g/\mu)$: Amount required for 50% reduction of DPPH after 30 min. Each value is mean ± standard deviation of three replicate tests.

^c BHT: Butylated Hydroxytoluene.

^d BHA: Butylated Hydroxyanisole.

tion of each extract at a concentration of 1.0 mg/ml was prepared. The activities of sample extracts were between 4.0 and 129 μ g/ μ l at 1.0 mg/ml. Most of the samples showed high antioxidant activity using DPPH in sorghum. With regard to RC_{50} values (the concentration of antioxidant required to achieve absorbance equal to 50% that of a control containing no antioxidants), Gumeunchalsusu $(RC_{50} = 4.0 \pm 0.0)$ and Jangsususu $(RC_{50} = 4.6 \pm 1.1)$ had the highest radical-scavenging abilities, whereas Kkachisusu (RC_{50} = 129.0 ± 1.7), Kkachisusu-daerip (RC50 = 60.6 ± 1.5), and Bitjarususu $(RC_{50} = 23.3 \pm 1.1)$ had the lowest radical-scavenging abilities. Exposure to proton radical scavengers is known to significantly decrease the level of DPPH [\(Yamaguchi, Takamura, Matoba, &](#page-5-0) [Terao, 1998\)](#page-5-0). Therefore, free radical-scavenging activity has a marked impact on the phenolic composition of the sample.

3.1.2. Reducing power activity

Fig. 1 shows the reducing power of sorghum species methanolic extracts and fractions as a function of their concentration. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound. The presence of reducers in the test solution results in reduction of the $Fe^{3+}/$ ferricyanide complex to the ferrous form. The reducing power of both species was excellent; at 1 mg/ml the reducing power was higher than BHA in the BuOH fraction except for Neulsusu. At 1 mg/ml, the reducing power of the EtOAc fraction from Neulsusu was 0.44. The reducing powers of α -tocopherol at 1 mg/ml, BHA at 1 mg/ml, and ascorbic acid at 1 mg/ml were 0.73, 0.58, and 0.82, respectively. It was reported previously that the reducing power of mushrooms was generally increased with increasing sample concentration ([Gordon, 1990](#page-5-0)).

3.2. Antimicrobial activities of the selected cultivars

The antimicrobial activities in various fractions of the sorghum extracts were assessed by a serial twofold dilution assay and paper disc diffusion assay. The results indicated variation in the antimicrobial properties of the plant extracts and fractions [\(Table 3](#page-3-0)). In general, the methanol extract was more effective than the fractions. The most effective extracts were from Neulsusu (methanol extract and ethyl acetate layer) and Bulkeunchalsusu (methanol extract), which had MICs of 250 and 500 μ g/ml, respectively. Neulsusu showed the highest level of antimicrobial activity, especially against S. aureus. Extracts from Dryopteris crassirhizoma, Sophora

MeOH extracts and fractions

Fig. 1. Reducing power of extract and fractions from Sorghum.

Table 3

S.a, Staphylococcus aureus; B.s, Bacillus subtilis; S.t, Salmonella typhimurium; K.p, Klebsiella pneumonia; E.c, Escherichia coli; C.a, Candida albicans.

The MIC value against bacteria and yeast were determined by the serial 2-fold dilution method.

Table 4

Inhibition effect of the methanol extracts and subfractions from Sorghum against the microorganisms.

a S.a, Staphylococcus aureus; B.s, Bacillus subtilis; S.t, Salmonella typhimurium; K.p, Klebsiella pneumonia; E.c, Escherichia coli; C.a, Candida albicans. -: No inhibition.

flavescens, Pinus densiflora and Glycyrrhiza uralensis have been suggested as potential sources of antimicrobial agents against methicillin-resistant S. aureus ([Eum & Park, 2007](#page-5-0)). Ethyl acetate fractions purified from the whole-herb extracts of species of Mongolian flora exhibited particularly potent antimicrobial effects, especially against S. aureus [\(Gonchig et al., 2008](#page-5-0)).

A comparison of the sensitivities of the bacterial strains to the sorghum extracts and fractions indicated that the strongest inhibitory effect was against E. coli. However, none of the cultivars showed antifungal or anticandidal activity. More precise data concerning the antimicrobial properties of the extracts were obtained by paper disc diffusion assay (Table 4 and [Fig. 2](#page-4-0)). The maximal inhibitory zones for each of the microorganisms that were sensitive to the extracts and fractions of Neulsusu, Gumeunchalsusu, Jangsususu, and Bulkeunchalsusu, were in the range of 2.0–10.5, 5.0–9.0, 2.5–11.0 and 6.0–11.5 mm, respectively (Table 4 and [Fig. 2](#page-4-0)). Greater inhibition was observed in the case of the methanol extracts than the other fractions. Among the four cultivars, the methanol extract of Bulkeunchalsusu had the highest level of antimicrobial activity against all of the microorganisms tested. Similarly, the methanol extracts of Jangsususu and Neulsusu showed significant antimicrobial or antifungal activities against all of the

Fig. 2. Inhibition effect of the methanol extracts and subfractions from Sorghum against the microorganisms in paper disc diffusion assay. (A) Staphylococcus aureus, (B) Escherichia coli., (C) Salmonella typhimurium, (D) Klebsiella pneumonia, (E) Candida albicans, (F) Bacillus subtilis, 1, negative control, 2, positive control, A3, Gumeunchalsusu EtoAc, A4, Jangsususu Hexane, B3, Jangsususu MeOH, B4, Neulsusu MeOH, C3: Bulkeunchalsusu MeOH, C4 Bulkeunchalsusu water, D3, Neulsusu MeOH, D4, Bulkeunchalsusu MeOH, E3, Neulsusu MeOH, E4, Neulsusu water, F3, Bulkeunchalsusu MeOH.

microorganisms tested except B. subtilis. In contrast, the methanol extract of Gumeunchalsusu was active only against C. albicans. Ethanolic and aqueous extracts isolated from ten of 20 Palestinian plant species used in folk medicine were active against C. albicans (Ali-Shtayeh, Yaghmour, Faidi, Salem, & Al-Nuri, 1998). The antifungal properties of origanum oil were examined both in vitro and in vivo using C. albicans ([Manohar et al., 2001](#page-5-0)); their results indicated that origanum oil at 0.25 mg/ml completely inhibited the growth of C. albicans in culture.

Among the organic solvent fractions, the hexane layer of Jangsususu showed significant antimicrobial activity against S. aureus. In comparison, the ethyl acetate and butanol layers of Gumeunchalsusu showed significant antimicrobial activity against S. aureus and S. typhimurium, respectively. However, an n-butanol-purified saponin extract of S. bicolor was reported to show antimicrobial activity against S. aureus [\(Soetan, Oyekunle, Aiyelaagbe, & Fafunso,](#page-5-0) [2006\)](#page-5-0). Antimicrobial activity in plant extracts depends not only on the presence of phenolic compounds but also on the presence of various secondary metabolites ([Gordana et al., 2007\)](#page-5-0). It was reported that sorghum had the highest level of activity against E. coli but that tannic acid was also effective [\(Lee, Son, Maeng,](#page-5-0) [Chang, & Ju, 1994](#page-5-0)). These observations suggest that the antimicrobial activity of sorghum may be due to the presence of tannic acid. Other phenolic acid-like phenols are thought to contribute to plant defenses against pests and pathogens (Awika & Rooney, 2004).

4. Conclusion

In summary, the results of the present study conclusively demonstrated the antioxidant and antimicrobial activities of S. bicolor, a popular cereal that is consumed worldwide. The effects of various fractions from different cultivars of sorghum were examined for radical-scavenging activity by DPPH methods and for antioxidant activity by determining reducing power. With the exception of Kkachisusu, Kkachisusu (daerip), and Bitjarususu, all sorghum cultivars examined showed high antioxidant activity. The RC_{50} of DPPH and reducing power showed higher efficiency in the BuOH layer of 3 selected cultivars other than Neulsusu. The ethyl acetate layer of the Neulsusu cultivar showed the highest efficiency RC_{50} value of DPPH and reducing power. The results also indicated that crude extracts and fractions of S. bicolor have useful antimicrobial properties. A methanol extract of S. bicolor was found to be more effective at inhibiting microorganism growth than the other fractions. Of the four cultivars tested, Neulsusu showed the highest level of antimicrobial activity. The results of the paper disc diffusion assay in the present study indicated that the methanol extract of Bulkeunchalsusu exhibited the highest level of antimicrobial activity against all of the microorganisms tested. The results presented here suggest that Sorghum could be used as a natural ingredient with biological function for its antioxidant and antimicrobial properties in the natural pigment industry.

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