

Evaluation of Biological Activities of Rice Husk Extracts

– Research Note –

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Abstract

This study was conducted to determine the biological activities of 70% ethanol extracts from rice husks of nine rice cultivars in Korea. The relative antioxidant activities of rice husk extracts were evaluated by determining DPPH, ABTS radical scavenging activity and reducing power. The contents of total polyphenol, flavonoid and *r*-oryzanol were measured by spectrophotometric methods. Among the extracts of rice husks, *Nokmi* rice husks tended to have the most effective antioxidant activities compared to other rice husk varieties. *Seolgaeng* rice husk extract showed anti-proliferative activity against cancer cell lines (MCF7 and NCI-H460), and *Hongjinju* rice husk extract significantly exhibited mitogenic activity.

Key words: biological activity, antioxidant activity, rice husk, mitogenic activity, anti-proliferative activity

INTRODUCTION

Rice is one of the most widely grown food grain crops throughout the world and serves as the most important food for the world's human population, especially in Asia. Also, most of the production of rice is used in cooked form for human consumption for the purposes of nutrition and caloric intake. However, there are a number of generally unconsumed portions of rice, such as the husks. The rice husks, which possess approximately 20% dry weight of the harvested rice, have been produced annually at a weight of more than one million tons in South Korea (1,2). However, rice husk, a by-product of rice processing, is mostly wasted because of their low-cost value as feedstock due to low digestibility, peculiar size, low bulk density, high ash/silica contents and abrasive characteristics (3). Generally, the outer layers of plants, such as the peel, shell and husk, play an important role in protecting seeds from oxidative damage and, therefore contain large amounts of strong antioxidants such as flavonoids, hydrocinnamic acid derivatives, isovitexin, phytic acid, anisole, vanillin, syringaldehyde (2,4,5). Thus, rice husks are attractive sources of natural antioxidants because of these unique health benefits (6). In particular, rice husk, a prominent example of this type of residue, contains an antioxidant defense system including polyphenolic compounds to protect the inner materials from oxidative stress (4,7). Recently, Ramarathnam et al. (8) reported that isovitexin

isolated from white rice hull has been shown to exert strong antioxidant activity. Kim et al. (9) showed that momilactone B from rice husks found to have cytotoxic and antitumor activity against human colon cancer cell. Moreover, Jeon et al. (10) demonstrated that phenolic compounds from methanol extracts of rice husk exhibited high antioxidant activity against scavenges of singlet oxygen and inhibited high hydrogen peroxide-induced damage to DNA in human lymphocytes. However, there are few reports published on antioxidant activity by rice husk extracts from several cultivars. In the present investigation, we determined the polyphenolic, flavonoid and *r*-oryzanol contents of rice husk extracts, measured the DPPH, ABTS radical scavenging activities and reducing power of these extracts, determined their anti-proliferative and mitogenic activities, and examined correlations between antioxidant and bioactive compounds.

MATERIALS AND METHODS

Materials

Rice husks from nine rice cultivars (*Oryza sativa* L.), including *Samkwang*, *Seolgaeng*, *Hwaseonchal*, *Keunnun*, *Hongjinju*, *Heugkwang*, *Ilpum*, *Haiami*, and *Nokmi*, were obtained from the National Institute of Crop Science, RDA, Suwon, South Korea during the 2008 growing season. Gallic acid, ascorbic acid, Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis-(3-

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ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium ferricyanide, ferric chloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The human tumor cell lines including MCF7 (breast) and NCI-H460 (lung) were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea). All other reagents and solvents used were of analytical grade.

Sample preparation

Twenty grams of finely ground samples were extracted with 500 mL of 70% ethanol solvent in a shaker (VS-8480SR, Vision Scientific Co., Ltd., Bucheon, Korea) at room temperature (RT) for 24 hr. The extracts were filtered through Whatman No. 2 filter papers and residues were removed. The filtrate was concentrated using a vacuum evaporator (N-1000, EYELA, Tokyo, Japan) under 40°C and then the resulting concentrated slurries were lyophilized and stored at -20°C until analysis.

Determination of antioxidant compounds

Total flavonoid contents in the extracts were determined according to the spectrophotometric assay described by Kong et al. (11) and results expressed as mg catechin equivalents per 1 g of sample. Measurement of the amount of total polyphenolic compounds in the extracts was carried out by spectrophotometric assay according to the Folin-Ciocalteu colorimetric method (12). The results were expressed as mg gallic acid equivalents per 1 g of sample. The content of *r*-oryzanol in rice husk was measured at a wavelength of 326 nm using a method reported by Lilitchan et al. (13). The *r*-oryzanol reagent was used as a reference. All measurements were performed in triplicate.

Determination of antioxidant activities

The ABTS radical scavenging activity of the extracts was determined according to the method of Choi et al. (14) and results expressed as mg of ascorbic acid equivalents (AEAC) per 1 g sample. The scavenging activity of extract on the DPPH radical was measured according to the method of Cheung et al. (15). The reducing power of extract was determined according to the method of Oyaizu (16).

Mitogenic activity

All procedures were conducted under aseptic conditions. The activity was measured according to the procedure of Kim et al. (17). Proliferation was checked by MTT assay method. Specific lymphocyte mitogens, such as lipopolysaccharide (LPS, B-cell mitogen, from *Escherichia coli* serotype, Sigma Chemical Co.), were used as positive control.

Anti-proliferative activity

Both breast (MCF7) and lung (NCI-H460) tumor cells

were grown in RPMI containing 10% fetal bovine serum (FBS), 2 mM glutamine, 100 unit/mL penicillin and 50 µg/mL streptomycin. Anti-proliferative activities of sample extract on tumor cells were measured by evaluating cell viability using the MTT assay (18). The cell viability (%) was obtained by comparing the absorbance between the sample and a negative control.

Statistical analysis

The results were reported as means ± standard deviation (SD) of at least triplicate measurements. The significance of differences among treatment means were calculated using SPSS package for Windows (version 12.0 SPSS Inc., Chicago, IL, USA) with a significance level of 0.05.

RESULTS AND DISCUSSION

Extraction yields

Efficiency of extracts is an important factor for the comparison of antioxidant activity. Water with ethanol was selected as the extraction solvent since both are commonly used by the food industry in a variety of ways and are more highly stable in the human body than any other solvents. Therefore, 70% ethanol solvent was used as the extraction solvent for this study. The yield of 70% ethanol extracts obtained from rice husks of nine rice cultivars is presented in Table 1 and the extraction yield of rice husks varied from 1.20% to 2.46%.

Antioxidant compounds

The role of polyphenolic and flavonoid compounds as natural antioxidants has contributed to our current knowledge of antioxidant activities in the plant kingdom. Increased concentrations of polyphenolic and flavonoid compounds, which are observed in rice husks, reduce reactive oxygen species such as singlet oxygen, superoxide anion radical, hydrogen peroxide, and hydroxyl radicals. Also, there are various phenolic compounds in rice hull including isovitexin, phytic acid, vanillic acid, syringic acid and ferulic acid (1,5) as well as polyphenols, which have an ideal antioxidant structure because of the presence of an aromatic phenolic ring that can stabilize the unpaired electron (19).

Total polyphenolic contents of nine rice husks are shown in Table 1. Among the rice husks tested, the highest total polyphenolic level has been detected at 3.75 mg gallic acid equivalents (GAE)/g sample in *Nokmi* and the lowest at 1.39 mg GAE/g sample in *Hwaseonchal*. A previous study reported that total polyphenolic contents of Thai rice husk fraction had 1.2~2.2 mg GAE/g (4). Therefore, total polyphenolic contents in this study were slightly higher than those of Thai rice husk.

Table 1. Polyphenol, flavonoids, *r*-oryzanol contents and extraction yields of the 70% ethanol extract obtained from rice husk

Cultivar	Yield (%)	Polyphenolics ¹⁾	Flavonoids ²⁾	<i>r</i> -Oryzanol ³⁾	EDA (%)	ABTS radical scavenging activity ⁴⁾
<i>Samkwang</i>	1.65	2.30 ± 0.04 ^{e5)}	251.66 ± 15.26 ^e	52.36 ± 2.11 ^a	35.88 ± 0.59 ^c	1.65 ± 0.06 ^c
<i>Seolgaeng</i>	1.65	1.99 ± 0.05 ^d	243.22 ± 16.97 ^{de}	60.09 ± 1.67 ^{ab}	42.77 ± 1.23 ^e	1.32 ± 0.03 ^d
<i>Hwaseonchal</i>	1.32	1.39 ± 0.04 ^a	153.63 ± 15.92 ^a	85.27 ± 7.67 ^c	33.88 ± 0.94 ^c	0.84 ± 0.03 ^a
<i>Keunnun</i>	2.46	2.21 ± 0.04 ^e	201.58 ± 19.28 ^{bc}	114.08 ± 12.37 ^d	26.81 ± 0.81 ^a	1.35 ± 0.07 ^d
<i>Hongjinju</i>	1.20	1.87 ± 0.04 ^e	235.48 ± 23.56 ^{cde}	69.76 ± 10.94 ^{bc}	44.93 ± 0.99 ^f	1.03 ± 0.03 ^b
<i>Heugkwang</i>	1.42	1.46 ± 0.05 ^a	175.41 ± 13.76 ^{ab}	124.55 ± 3.36 ^d	30.16 ± 1.59 ^b	0.90 ± 0.05 ^a
<i>Ilpum</i>	1.73	2.81 ± 0.09 ^f	340.44 ± 37.33 ^f	46.06 ± 6.41 ^a	38.83 ± 2.63 ^d	1.79 ± 0.07 ^f
<i>Haiami</i>	1.43	1.72 ± 0.02 ^b	208.59 ± 17.22 ^{bcd}	73.18 ± 6.02 ^{bc}	41.29 ± 0.28 ^c	1.12 ± 0.02 ^c
<i>Nokmi</i>	2.10	3.75 ± 0.13 ^g	656.23 ± 27.19 ^g	62.40 ± 7.50 ^{ab}	58.46 ± 0.16 ^g	2.67 ± 0.07 ^g

¹⁾Mean of triplicate determinations expressed as mg gallic acid equivalents per 1 g of sample (wet weight basis).

²⁾Mean of triplicate determinations expressed as µg (+)-catechin equivalents per 1 g of sample (wet weight basis).

³⁾Mean of triplicate determinations expressed as µg per 1 g of sample (wet weight basis).

⁴⁾Results expressed as mg of ascorbic acid equivalents (AEAC) per 1 g sample.

⁵⁾Values are mean ± SD. Different letters in the same column indicate significant difference at $p < 0.05$ by ANOVA and Duncan's test.

Ramarathnam et al. (8) reported that isovitexin, identified as a C-glycosyl flavonoid, was a main antioxidant compound in methanolic rice hull extract. Furthermore, Lee et al. (20) demonstrated that FIR irradiated rice hull using ethylacetate solvent showed the highest total phenolic contents increase of 0.19 from 0.07 mM.

Flavonoids are polyphenols with a diphenylpropane (C6-C3-C6) skeleton. Significant differences in total flavonoid contents were observed among 70% ethanol extracts from rice husks (Table 1). Total flavonoid contents in nine rice husks ranged from 153.63 µg catechin equivalents (µg CE)/g sample to 656.23 µg CE/g sample. The highest flavonoid level was detected in *Nokmi*.

r-Oryzanol is another antioxidant in grains, which has been shown to lower blood cholesterol and to reduce levels of cholesterol in the liver (21). The highest content of *r*-oryzanol was observed in the *Heugkwangbyeo* fraction (124.55 µg/g sample) and the lowest levels of *r*-oryzanol were detected in the *Ilpumbyeo* fraction (46.06 µg/g sample) (Table 1).

Antioxidant activities of rice husk extracts

Free radical scavenging is one of the well-known mechanisms by which antioxidants inhibit lipid oxidation. The methods of DPPH and ABTS free radical scavenging are stable and are widely used to determine the antioxidant activity of specific compounds or extracts simply in a short time (22). The ability to act as donors of hydrogen atoms in the transformation of the DPPH radical to its reduced form (DPPH[•]-H) was investigated for rice husk extracts (4). The DPPH radical scavenging activity of 70% ethanol extracts (1 mg/mL) from nine rice husks is shown in Table 1. Ascorbic acid as positive control showed 85.41% of DPPH radical scavenging activity at concentration of 6.25 µg/mL. *Nokmi* rice husk (58.46%) showed relatively higher DPPH radical scavenging activity

than others (26.81 ~ 44.93%). Park and Lee (1) reported that the highest DPPH values of rice hull extracts after hydrothermal treatment at 120°C for 60 min was 64.77%. Previous studies exhibited good correlation between total phenolic content and antioxidant activity of plant extracts (4,23), whereas no correlation ($R^2=0.4399$) between polyphenolic content and DPPH radical scavenging activity in this study was observed. Also, the correlation coefficient between flavonoid content and DPPH assay was 0.6748 and no correlation ($R^2=0.3735$) between *r*-oryzanol content and DPPH assay was observed (Table 2).

The ABTS method is widely used to evaluate the antioxidant activity of both hydrophilic and lipophilic compounds (4). The stable ABTS radical scavenging activity of 70% ethanol extracts (1 mg/mL) from nine rice husks is presented in Table 1. The highest antioxidant activity (2.67 mg AEAC/g) was found in *Nokmi* rice husk extract and *Hwaseonchal* rice husk extract showed the lowest activity (0.84 mg AEAC/g). In this study, a positive and significant correlation ($R^2=0.9728$) between polyphenolic content and ABTS radical cation scavenging activity was observed, whereas there was no correlation ($R^2=0.2520$) between *r*-oryzanol content and ABTS radical scavenging activity. Also, the significant correlation coefficient

Table 2. Correlation analysis between antioxidant contents and antioxidant activities

	Antioxidant compounds		
	Polyphenolics	Flavonoids	<i>r</i> -Oryzanol
DPPH radical scavenging activity	0.4399 ¹⁾	0.6748	0.3735
ABTS radical cation scavenging activity	0.9728	0.8935	0.2520
Reducing power	0.6961	0.8583	0.2758

¹⁾Correlation coefficient R^2 .

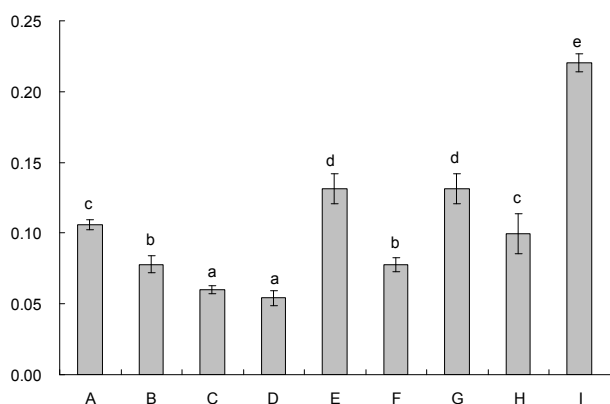


Fig. 1. Reducing power of 70% ethanol extracts obtained from nine rice husks. Each value represents the mean of triplicate measurements of analyzed sample. A, *Samkwang*; B, *Seolgaeng*; C, *Hwaseonchal*; D, *Keunnun*; E, *Hongjinju*; F, *Heugkwang*; G, *Ilpum*; H, *Haiami*; I, *Nokmi*. Values with different letters above bar graphs are significantly different at the 5% level by one-way ANOVA and Duncan's test.

between flavonoid content and ABTS assay was 0.8935 (Table 2). These results show that the polyphenolic compounds may contribute to the major portion of antioxidant activity. The authors (24,25) have reported significant correlation between polyphenolics and antioxidant activities in grains, mushroom and fruits.

The reducing power of rice husk extracts is associated with their potential antioxidant activity, which is shown in Fig. 1. In this method, Fe^{3+} /ferricyanide complex is reduced to the ferrous form depending on the presence of reductants (e.g., antioxidants) (26). *Nokmi* rice husk extract ($A_{700}=0.22$) had relatively higher reducing power than those of other rice husk extracts ($A_{700}=0.05\sim 0.13$). Also, the correlation coefficients between polyphenolic content and reducing power and between flavonoid content and reducing power were 0.6961 and 0.8583, respectively, while showing no correlation with *r*-oryzanol content ($R^2=0.2758$) (Table 2). This indicates that the flavonoid in the 70% ethanol extracts of rice husk may play a role as electron and hydrogen donors.

Mitogenic activities

The mitogenic activities of 70% ethanol extracts obtained from the nine rice husks are shown in Fig. 2(A). The biological response of the rice husk variety extracts in terms of mitogenic activity was not significantly different, except for the *Hongjinju* rice husk extract. Also, mitogenic activity induced by the *Hongjinju* rice husk extract at 500 μ g/mL was higher than that of LPS at 10 μ g/mL. In a previous study, Huang et al. (27) reported that isovitexin, isolated from rice hull of *Oryza sativa*, and had inhibited the release of tumor necrosis factor alpha (TNF- α), prostaglandin E2 (PGE2) and the expression of cyclooxygenase-2 (COX-2) in LPS-activated

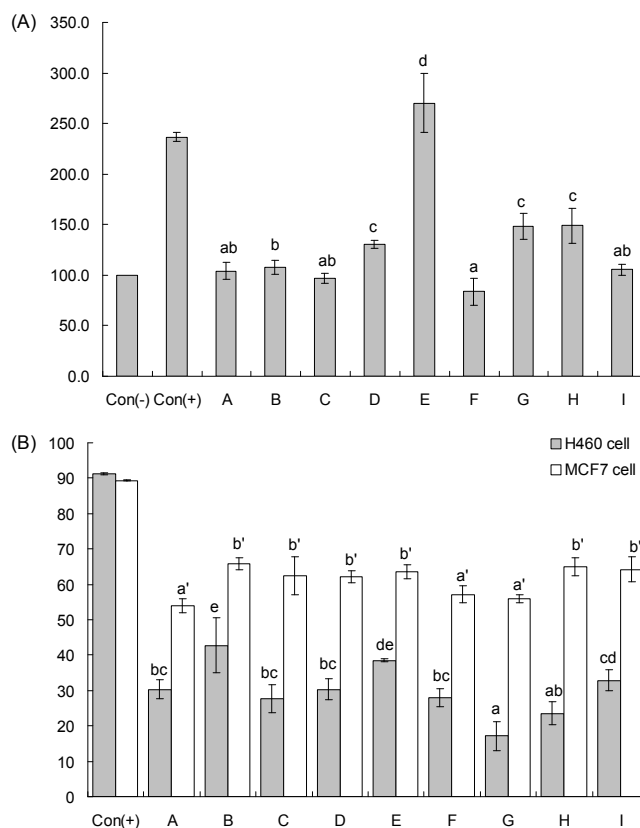


Fig. 2. Mitogenic activity (A) and antiproliferative activity (B) of 70% ethanol extracts obtained from nine rice husks. Each value represents the mean of triplicate measurements of analyzed sample. Negative control (Con(-)); saline, positive control (Con(+)); A, LPS (10 μ g/mL); B, cisplatin (10 μ g/mL); A, *Samkwang*; B, *Seolgaeng*; C, *Hwaseonchal*; D, *Keunnun*; E, *Hongjinju*; F, *Heugkwang*; G, *Ilpum*; H, *Haiami*; I, *Nokmi*. Values with different letters above bar graphs are significantly different at the 5% level by one-way ANOVA and Duncan's test.

RAW 264.7 macrophages. This indicates that isovitexin, as one of the flavonoids in the 70% ethanol extracts of rice husk, may play a role as an immunostimulating compound.

Anti-proliferative activities

The 70% ethanol extracts of rice husk were also assayed for their anti-proliferative effectiveness using human breast cancer cell lines MCF7 and the lung cancer cell line NCI-H460. Anti-proliferative effects were expressed as cytotoxicity (%) in Fig. 2(B). *Seolgaeng* rice husk extract had the highest cytotoxicity at a concentration of 100 μ g/mL against MCF7 (65.82%) and NCI-H460 (42.77%) cells. Other rice husk extracts, including *Hiami* (64.93%) and *Nokmi* (64.20%), expressed greater than fifty percent cytotoxicity against MCF7 cells, whereas 70% ethanol extracts from the nine rice husks against NCI-H460 cancer cells expressed less than fifty percent cytotoxicity. In a previous study, Kim et al. (9) reported that momilactone B, isolated from methanol ex-

tracts of rice hulls, was identified as the active compound having cytotoxic and antitumor activity against human colon cancer cells.

In summary, the entire panel of rice husk extracts tested showed strong antioxidant properties, such as ABTS scavenging activity, DPPH radical inhibition activity, and reducing power. Among the nine rice husk extracts of rice cultivar varieties, the highest antioxidant activities were obtained from *Nokmi* rice husk extracts. The highest mitogenic and anti-proliferative activities were found in *Hongjinju* and *Seolgaeng* rice husk extracts. Therefore, *Nokmi* rice husk extracts tended to have the most effective antioxidant activities compared to other rice husk extracts. It has been found that the antioxidant activities of rice husks extract may play an important role in the amount of antioxidants such as isovitexin, monilactone B in rice husk. Therefore, further identification of specific phytochemicals in rice husks for their antioxidant and antiproliferative activities is worthy of investigation.

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