Journal of Horticulture and Plant Research
ISSN: 2624-814X, Vol. 1, pp 26-34
doi:10.18052/www.scipress.com/JHPR.1.26
2018 SciPress Ltd, Switzerland

Submitted: 2017-12-09
Revised: 2018-01-15
Accepted: 2018-01-29
Online: 2018-03-12

Antioxidant and Allelopathic Activities of Rice (*Oryza sativa* L.) Bran

Roni Yulianto[1,a] and Tran Dang Xuan[1,b*]

[1]Graduate School for International Development and Cooperation (IDEC), Hiroshima University, 1-5-1 Kagamiyama, Higashi-Hiroshima, Hiroshima, 739-8529, Japan

[a]roniyulianto99@gmail.com, [b]tdxuan@hiroshima-u.ac.jp

*Corresponding author: tdxuan@hiroshima-u.ac.jp

Keywords: rice bran, chemical compound, allelopathic activity, antioxidant activity, GC-MS

Abstract. Rice by-products had higher amount of nutrients when compared to the polished rice. In this study, rice bran was investigated for antioxidant and allelopathic activities as well as identified its potent phytochemicals. The methanol (MeOH) extract from 8.9 kg rice bran was dissolved in water and successfully extracted using hexane and ethyl acetate, then ethyl acetate crude extract was subjected to normal phase column chromatography using the eluent of n-chloroform:methanol. Of which, ten fractions was collected, among them the fraction 5 (Fr5), showed maximum antioxidant activity, followed by the Frs 6, 1, and 10. Accordingly, the Fr5 showed the greatest inhibitory on germination and elongation of roots and shoots of radish (*Raphanus sativus*). There were 10 phytochemicals have been identified in the Fr5. The utilization of the identified constituents should be further investigated.

Introduction

Rice bran is one of the most abundant by-products in rice milling industry [1]. This by-product has been recognized as a source of protein, carbohydrate, dietary fibers, ash, fat, minerals, vitamin and natural antioxidant compounds [2–4]. However, it has underutilized as human food and traditionally has been used as primarily for livestock feed. Thus, it has a huge potential to be exploited as bioactive sources, such as antioxidant and allelopathic agents. Natural antioxidants become important concerning of synthetic antioxidants may have toxic, carcinogenic and abnormal effect on humans [5]. The use of antioxidant constituents may help to prevent damage by interfering with free radical propagation cascades before they attack biological targets in the cell [6].

Allelopathic activities of the plants may help the donor plant protect from microorganisms, viruses, insects and other pathogens or predators, even inhibitneighboring plant's growth or stimulate the growth of the seeds [7–10]. In recent decades, it has been reported that secondary metabolites from plants with allelopathic activity showed either inhibitory or stimulatory effects on the weeds [11–13]. These compounds were suggested to be useful for biological control of weeds and pathogens [14-16]. Allelochemicals released from plants can occur by various mechanisms such as volatile emission or leaching from leaves, or exudation from roots [10, 17]. Since released to the environment, allelochemicals can affect the growth of other coexisting plants [18]. The important allelochemicals include alkaloids, terpenoids, flavonoids, steroids, tannins, and phenolic compounds [16, 19, 20]. The allelochemicals affect plant growth and development and can be employed successfully against pathogens for weed reduction and enhancement of yield in crops [21].

Further studies reported that rice bran contains higher iron, polyphenol, and antioxidant properties. It has also been reported that rice bran has scavenging activities and antioxidant activities [22]. However, based on the best our knowledge, there has been no previous study reported the biological activities to reduce weed emergence by using isolated compounds from rice bran. Therefore, the objective of this study was to investigate the antioxidant and allelopathic activities of isolated compounds from rice bran and identify potent phytochemicals.

Materials and Methods

Preparation of rice bran extract

Rice bran (8.9 kg) (var. Koshihikari), Hiroshima, Japan, was immersed in MeOH 20 L for one week. The filtrate was then evaporated using rotary evaporator (SB-350-EYELA, Tokyo Rikakikai Co., Ltd, Tokyo, Japan) to produce 1.2 kg of MeOH crude extract. The crude extract was then suspended in distilled water and subsequently extracted using hexane and ethyl acetate to yield 886.46 g of hexane, 46.45 g of ethyl acetate, and 250.79 g of distilled water. Each of the crude extract was then diluted using chloroform and then isolated by column chromatography.

Fractionation of the ethyl acetate extract

The ethyl acetate extract (46.45 g) was subjected to normal-phase column chromatography (40 mm diameter × 600 mm height, Climbing G2, Tokyo, Japan) over silica gel (size Å 60, 200-400 mesh particle size, Sigma-Aldrich) and yielded 42 fractions with following eluents: chloroform fraction consists of fractions 1-4 (Fr1), fractions 5-9 (Fr2), fractions 10-11 (Fr3), fractions 12-13 (Fr4) and fractions 14-21 (Fr5). Chloroform:methanol (9.8:0.2) consist of fractions 22 (Fr6), fractions 23 (Fr7) and fractions 24-34 (Fr8). In the chloroform:methanol (9.5:0.5) eluent, it consisted of fractions 35 (Fr9), and fractions 36-42 (Fr10).

Thin-layer chromatography (TLC), used to control column chromatography result, was carried out on pre-coated silica gel plate (Merck) with a layer thickness of 0.5 mm. Spots were detected under UV light (254-366 nm) before and after the plates were dipped in a chamber containing 1% vanillin-sulfuric acid (ethanol solution).

DPPH radical scavenging activity

DPPH radical-scavenging activity of fraction from ethyl acetate extract was determined according to the method reported by Elzaawely et al. [23]. The mixture consisted of 0.5 ml sample extract, 0.25 ml DPPH (0.5 mM) and 0.5 ml of 0.1 M acetate buffer (pH 5.5). The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm against a blank, using a UV–Vis spectrophotometer (HACH DR/4000U-USA). The percentage of scavenging activity DPPH was calculated using the formula as follows:

$$\% \text{ radical scavenging} = [(abs_{control} - abs_{sample}]/abs_{control}] \times 100$$

The $abs_{control}$ is the absorbance of the DPPH solution without extract, and abs_{sample} is the absorbance of a sample with DPPH solution. The DPPH radical-scavenging activity was presented by IC_{50} values. IC_{50} the concentration of the sample required to scavenge 50% of DPPH. Therefore, a lower IC_{50} value reflects a higher DPPH radical scavenging activity.

Germination and growth bioassay

The germination and growth assay was conducted using radish (*R. sativus*) as an indicator plant. Different fractions (Fr1 to Fr10) and concentrations (500, 250, and 50 ppm) were applied to this assay. A volume of 300 µL of test solution was added into 12-well plate lined by filter papers. After methanol was evaporated (6 hours), a volume of 300 µL of distilled water then added to each well. Ten seeds of radish were then put into the well. The photoperiodic control was set up at day/night 12/12 with temperature 25/23^{o}C in a growth chamber. A volume of 100 µL water was added every day for five days and then data were recorded (germination, roots length, and shoot height). The data calculation based on a formula [23] as follows: germination of percentage (%) = number of germinated seeds/total number of seeds x 100; germination inhibition (%) = control - number of germination seeds/control x 100; percentage inhibition (%) root and shoot length = control- inhibition (cm)/control x 100.

Chemical constituent identification from rice bran fractions using GC-MS (gas chromatography-mass spectrometry)

Chemical components of Fr5 fraction of ethyl acetate extract of rice bran were determined by GC-MS (JEOL JMS-T100GCV). A DB-5MS column (30 mm x 0.25 mm x 0.25 mm) was used. These columns used helium as the carrier gas and adjusted to 1.5 ml/min of flow rate. The split ratio was 5:1. The initial temperature was 50 °C and increased up to 300 °C with a rate of 10 °C/min, then maintained at the temperature for 20 min. The injection volume was one µL, and methanol was used as the injection solvent. Identification chemical constituent was performed by comparing the spectrum of the sample with the NIST08 mass spectrum library. The relative concentration was calculated using peak area normalization method.

Statistical analysis

The result of all data was expressed as means ± standard deviation (SD). Data were analyzed by the Minitab 16 software using one-way analysis of variance (ANOVA) to determine whether significant differences among treatments at a confidence level 95 % ($p<0.05$) by Fisher's test.

Results and Discussion

DPPH free radical scavenging activity

The antioxidant activity of different fractions separated from the ethyl acetate extract was determined by DPPH free radical scavenging assay. The antioxidant activity (IC_{50} values) of each fraction are shown in Table 1. Statistically, the IC_{50} value of the Fr5 was similar with that Fr1, Fr2, Fr3, Fr8, and Fr10, but in value, it showed the strongest activity (IC_{50} value). The antioxidant activity of the Fr5 (IC_{50}) was 0.012 ± 0.004 mg/ml.

The antioxidant activities of natural compounds may contribute as an alternative product to the synthesis of novel and safe antioxidants which can be used in the treatment of oxidative stress and damages in food production [20]. Besides, antioxidant compounds were reported potent for their medicinal and pharmaceutical properties [25].

Table 1. Inhibitory value (IC_{50}) of DPPH radical scavenging activity of fractions isolated from rice bran

Treatment code	IC_{50} (mg/ml)
Fr1	0.014 ± 0.004 d
Fr2	0.034 ± 0.010 d
Fr3	0.026 ± 0.002 d
Fr4	0.099 ± 0.009 c
Fr5	0.012 ± 0.004 d
Fr6	0.273 ± 0.100 a
Fr7	0.206 ± 0.007 b
Fr8	0.016 ± 0.006 d
Fr9	0.269 ± 0.017 a
Fr10	0.018 ± 0.002 d

Values represent mean ± standard deviation (n=3). The values in column with similar letters are not significantly different ($p<0.05$)

Germination bioassay

Table 2 showed that the germination of radish in the Fr5 bioassay were the lowest as compared with other fractions. It is suggested that potent allelochemicals may be present in the Fr5 that

caused stronger inhibition than other fractions. Simultaneously, the Fr5 gave the maximum inhibition on germination of radish, as compared to other fractions. The values of germination inhibition of Fr5 was subsequently 26.67 ± 5.77% (50 ppm), 27.15 ± 10.17% (250 ppm), and 30.00 ± 10.00% (500 ppm). Thus, this fraction was used for further analysis.

Table 2. Inhibitory effects of fractions isolated from ethyl acetate extracts of rice bran on germination of radish

Treatment code	Concentration (ppm)	Germination percentage (%) ± SD	Germination inhibition (%) ± SD
Fr1	500	83.33 ± 5.77 c	12.55 ± 5.90 efgh
	250	86.67 ± 5.77 c	10.20 ± 5.87 fgh
	50	83.33 ± 5.77a	20.00 ±16.67 bcdefgh
Fr2	500	90.00 ± 0.00 c	5.73 ± 0.00 h
	250	80.00 ± 10.00 c	16.98 ± 10.17 bcdefgh
	50	80.00 ± 10.00 b	20.00 ±10.00 abcdefg
Fr3	500	70.00 ± 0.00 c	26.19 ± 0.00 abcd
	250	73.33 ±15.28 c	23.76 ± 15.53 abcde
	50	73.33 ±15.28 c	26.67 ± 15.28 abcd
Fr4	500	86.67 ± 5.77 c	9.14 ± 5.90 gh
	250	73.33 ± 5.77 c	23.76 ± 5.87 abcde
	50	70.00 ± 10.00 c	30.00 ± 10.00 ab
Fr5	500	63.33 ± 5.77 c	33.00 ± 5.90 a
	250	70.00 ±10.00 c	27.15 ± 10.17 abc
	50	73.33 ± 5.77 c	26.67 ± 5.77 abcd
Fr6	500	76.67 ± 5.77 c	20.37 ± 5.87 ab
	250	76.67 ± 5.77 c	23.33 ± 5.77 abcdefg
	50	76.67 ± 5.77 c	23.33 ± 5.77 abcdef
Fr7	500	83.33 ± 11.55 c	12.55 ± 11.80 efgh
	250	83.33 ± 5.77 c	13.59 ± 5.87 defgh
	50	76.67 ± 5.77 c	23.33 ± 5.77 abcdef
Fr8	500	73.33 ± 5.77 c	22.77 ± 5.90 abcdef
	250	76.67 ± 5.77 c	20.37 ± 5.87 abcdefg
	50	80.00 ± 10.00 c	20.00 ± 10.00 abcdefg
Fr9	500	80.00 ± 10.00 c	15.95 ± 10.22 cdefgh
	250	76.67 ± 5.77 c	20.37 ± 5.87 abcdefg
	50	76.67 ± 5.77 c	23.33 ± 5.77 abcdef
Fr10	500	70.00 ± 0.00 c	26.19 ± 0.00 abcd
	250	76.67 ± 5.77 c	20.37 ± 5.87 abcdefg
	50	80.00 ± 0.00 c	20.00 ± 0.00 abcdefg

Values represent means ± standard deviation (SD) (n=3). Values in a column with similar letters are not significantly different (p<0.05). SD: standard deviation

Growth bioassay

Table 3 showed the emergence of radish, including root and shoot length. It was observed that the inhibitory levels were proportional to the applied doses. Similar to Table 2, the Fr5 exhibited potent inhibition on emergence of radish, as compared with that of other fractions. It is suggested that rice bran may possess allelochemicals that exerted inhibition on germination and growth of radish. In plants, hydroxycinnamic acid, benzoic acid, and flavonoids not only were reported to have antioxidant activity but also to have exhibit allelopathic activity [26]. Rutin inhibited germination, root elongation and epicotyls growth of *Raphanus sativus* seeds [27].

Table 3. Inhibition effects of various fractions from ethyl acetate extract from rice bran on seedling growth of radish (*R. sativus*)

Fractions	Conc. (ppm)	Root length (cm)	Shoot height (cm)	Growth inhibition (%)	
				Roots length (%)	Shoots height (%)
Fr1	500	0.55 ± 0.14 b	0.83 ± 0.32 b	42.63 ± 14.21 cdefghijk	26.53 ± 29.90 abcde
	250	0.62 ± 0.22 b	0.69 ± 0.16 b	43.53 ± 20.39 ab	44.08 ± 13.88 abcde
	50	0.75 ± 0.28 b	0.67 ± 0.25 b	34.77 ± 23.63 a	47.05 ± 20.82 defgh
Fr2	500	0.54 ± 0.12 b	1.03 ± 0.48 b	43.78 ± 14.56 l	9.52 ± 41.99 abcde
	250	0.69 ± 0.24 b	0.98 ± 0.35 b	37.70 ± 23.35 fghijkl	21.24 ± 28.71 bcdefgh
	50	0.75 ± 0.28 b	0.67 ± 0.25 b	29.17 ± 17.89 defghijk	25.00 ± 26.76 fgh
Fr3	500	0.52 ± 0.14 b	0.80 ± 0.34 b	45.16 ± 14.39 bcdefghij	29.70 ± 28.61 abcd
	250	0.72 ± 0.21 b	0.93 ± 0.29 b	36.15 ± 19.67 cdefghijk	24.36 ± 24.24 defgh
	50	0.65 ± 0.22 b	1.04 ± 0.27 b	28.67 ± 22.11 ghijkl	19.77 ± 21.63 gh
Fr4	500	0.58 ± 0.11 b	0.99 ± 0.34 b	38.79 ± 12.46 kl	12.80 ± 30.03 bcdefg
	250	0.67 ± 0.14 b	1.09 ± 0.31 b	39.86 ± 13.57 kl	11.51 ± 28.58 abcdefg
	50	0.75 ± 0.21 b	1.09 ± 1.30 b	42.49 ± 17.31 efghijkl	21.09 ± 24.69 abcde
Fr5	500	0.47 ± 0.23 b	0.64 ± 0.29 b	52.06 ± 27.33 ab	44.38 ± 28.51 a
	250	0.60 ± 0.31 b	0.76 ± 0.31 b	45.63 ± 27.64 abcd	38.45 ± 24.23 abcd
	50	0.79 ± 0.29 b	0.89 ± 0.42 b	36.55 ± 24.07 abcde	36.82 ± 27.43 bcdefgh
Fr6	500	0.57 ± 0.17 b	0.76 ± 0.36 b	38.75 ± .8.38 abcdef	35.85 ± 30.05 abcdefg
	250	0.71 ± 0.16 b	0.76 ± 0.27 b	35.77 ± 14.14 abcd	38.54 ± 22.31 cdefgh
	50	0.81 ± 0.29 b	0.81 ± 0.30 b	29.85 ± 25.29 abcdef	36.32 ± 22.07 fgh
Fr7	500	0.50 ± 0.14 b	0.76 ± 0.23 b	47.23 ± .16.13 abcdefgh	33.92 ± 19.34 abc
	250	0.59 ± 0.15 b	0.83 ± 0.25 b	46.35 ± 14.07 abcdefgh	33.11 ± 20.25 abcd
	50	0.70 ± 0.22 b	0.84 ± 0.27 b	38.95 ± 21.04 abcdefgh	33.93 ± 20.38 bcdefg
Fr8	500	0.50 ± 0.14 b	0.76 ± 0.23 b	48.61 ± 20.66 abc	40.08 ± 30.24 ab
	250	0.59 ± 0.15 b	0.83 ± 0.25 b	32.23 ± 17.68 hijkl	19.57 ± 30.55 efgh
	50	0.70 ± 0.22 b	0.84 ± 0.27 b	41.03 ± 17.08 ijkl	14.88 ± 29.73 abcdef
Fr9	500	0.69 ± 0.17 a	0.96 ± 0.33 a	28.39 ± 18.66 ijkl	15.42 ± 30.42 fgh
	250	0.71 ± 0.22 a	0.97 ± 0.43 a	36.17 ± 20.90 fghijkl	20.98 ± 33.94 cdefgh
	50	0.85 ± 0.27 a	1.09 ± 0.36 a	25.68 ± 22.25 jkl	14.86 ± 28.13 h
Fr10	500	0.53 ± 0.19 a	0.71 ± 0.33 a	44.16 ± 25.07 abcdefg	37.96 ± 29.41 abcde
	250	0.62 ± 0.34 a	0.80 ± 0.32 a	43.65 ± 31.15abcdefg	35.38 ± 25.21 abcde
	50	0.68 ± 0.33 a	0.88 ± 0.30 a	40.98 ± 28.43 bcdefghi	29.99 ± 22.34 abcdefg

Values represent means ± standard deviation (SD) (n=3). Values in a column with similar letters are not significantly different (p<0.05). SD: standard deviation

Comparison of the IC_{50} values of the Fr5 fraction on germination and growth of radish

Fig. 1 showed the IC_{50} value of the Fr5 fraction on germination and root and shoot length of radish. It was observed that the inhibitory on root length was the greatest (1.93, 2.56, and 3.79 mg/mL), followed by shoot length (2.42, 3.57, and 5.56 mg/mL), whilst germination was the lowest (3.79, 4.25, and 6.67 mg/mL) for applied doses of 500, 250, and 50 ppm, respectively.

The seed germination is known as a physiological process beginning with water imbibition by seeds and culminating on the emergence of the rootlets [28]. The Fr5 fraction showed potent inhibition on germination and shoot and root length of radish. The phytotoxicity of rice bran is potential for weed management [29]. Like other natural products, rice bran can be extensively applied for weed management because of their sensitivity, simplicity, and low-cost [30, 31].

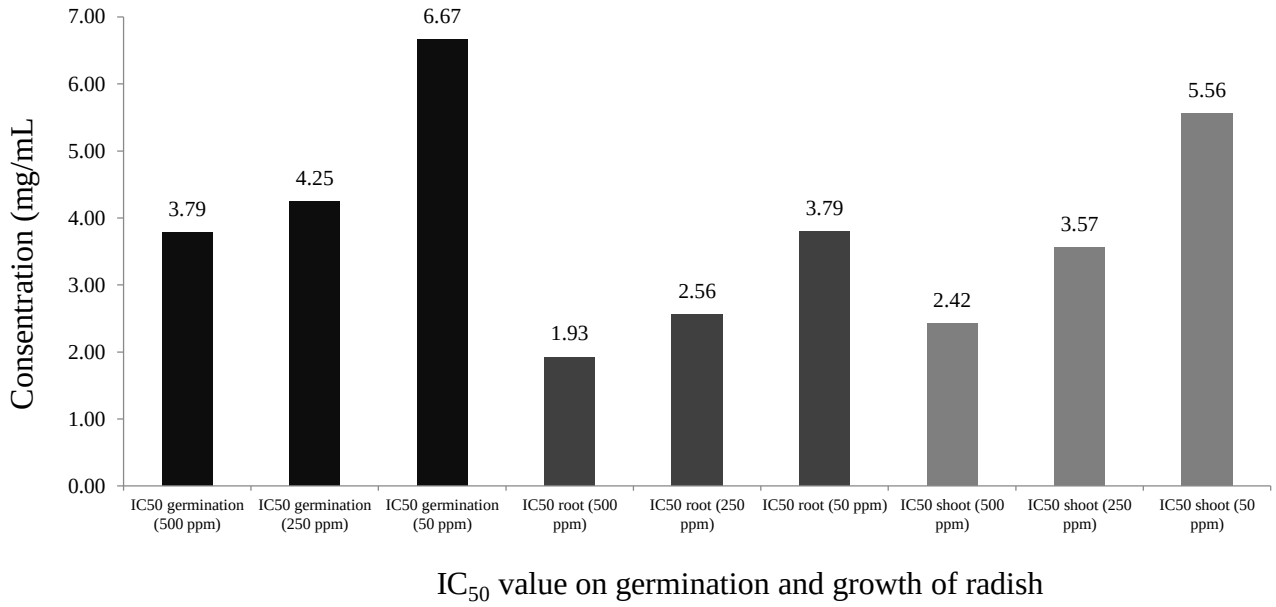

Figure 1. The IC_{50} value of the Fr5 fraction on germination and growth of radish

Gas chromatography and mass spectrometry (GC-MS)

GC-MS is the ideal analytical instrument to identify bioactive constituents of long chain hydrocarbons, alcohols, acids, alkaloids, steroids, amino, and the nitrogen compound [33]. The best fraction was the Rf5, that consisted of ten mixture compounds identified (Table 4). The GC-MS analysis of the Fr5 fraction showed that existence of the compound on percent of peak area consist of: hydrazine (9.85%), 3-acetylthymine (24.81%), 2-formyl-9-[β-d-ribofuranosyl]hypoxanthine (30.12%), carbohydrazide (16.53%), octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- (8.61%), methylamine (59.45%), silicic acid, diethyl bis(trimethylsilyl) ester (19.27%), heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- (74.44%), hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl- (29.15%), 1,1,1,3,5,5,5-heptamethyltrisiloxane (50.51%) were present. Many medicals plant are rich source of secondary metabolites such as alkaloids, phenol, glycosides, flavonoids, tannins, and terpenoids, which can be determined by GC-MS [32, 33].

The plants contain primary and secondary metabolites exert a range of biological activities on physiological systems [34]. Flavonoids, palmitic acid (hexadecanoic acid, ethyl ester and n-hexadecanoic acid), fatty acid and linolenic (docosatetraenoic acid and octadecatrienoic acid) showed potent antimicrobial, antibacterial, anti-inflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistaminic, antieczemic and anti-coronary activities[35]. The crude extracts could give the contribution to medicinal are more biologically active if compared than a single isolated compounds due to their synergistic and interactive effects [36].

Table 4. Gas chromatography and mass spectrometry (GC-MS) analysis of the rice bran of Fr5 fraction from rice bran

No	Retention time	Compounds	% of peak area	Molecular weight
1	9.97	2-Formyl-9-[β-d-ribofuranosyl]hypoxanthine	30.12	296
2	2.62	Hydrazine	9.85	32
3	24.84	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	8.61	579
4	4.97	3-Acetylthymine	24.81	168
5	12.23	Carbohydrazide	16.53	90
6	26.54	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	74.44	505
7	26.07	Silicic acid, diethyl bis(trimethylsilyl) ester	19.27	296
8	27.61	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl	29.15	430
9	25.86	Methylamine	59.45	31
10	29.31	1,1,1,3,5,5,5-Heptamethyltrisiloxane	50.51	222

Conclusions

The isolated Fr5 fraction from rice bran gave the highest inhibitory activity on germination and growth of radish seed (*Raphanus sativus*) than other fractions. The Fr5 fraction at 500 ppm provided promising inhibition on root growth by 52.06%, whereas shoots growth was 44.38%, and germination inhibition achieved 33.00%. The Fr5 fraction also exerted the strongest DPPH radical scavenging activity (IC_{50} = 0.012 mg/ml). The IC_{50} value of the Fr5 fraction for roots, shoots, and germination were 0.42, 0.86, and 1.73 mg/ml, respectively. The exploitation of chemicals from this fraction should be further investigated.

Conflict of Interest

The authors declare no conflict of interests.

Acknowledgments

The authors thank Indonesia Endowment Fund for Education (LPDP), Ministry of Finance Republic Indonesia for the financial support to Roni Yulianto. Thanks are due to Vietnam National Foundation for Science and Technology Development (NAFOSTED) [code:106-NN.03-2013.51]. Yusuf Andriana and Truong Thi Tu Anh are appreciated for assistance to this manuscript.

References

[1] FAOSTAT, FAO Statistics Division 2013, (2013). [Online]: http://fao.org

[2] C.R. Chen et al., Supercritical-carbon dioxide extraction and deacidification of rice bran oil, J. Sup. Flu. 45 (2008) 322-331.

[3] K.A. Moldenhauer et al., Functional foods, Technomic Publishing Co. Inc. Lancanster, Basel, Switzerland, 2003.

[4] C. Saenjum et al., Antioxidant and anti-inflammatory activities of gamma-oryzanol rich extracts from Thai purple rice bran, J. Med. Plan. Res. 6 (2012) 1070-1077.

[5] N.G. Baydar, G. Ozkanb, S. Yasar, Evaluation of the antiradical and antioxidant potential of grape extracts, Food Control. 18 (2007) 1131-1136.

[6] A. Matkowki, Plant *in vitro* culture for the production of antioxidant-a review, Biotech. Adv. 26 (2008) 548-560.

[7] F.A. Einhelling, Interactions involving allelopathy in cropping systems, Agr. J. 88 (1996) 886–893.

[8] D.S. Seigler, Chemistry and mechanisms of allelopathic interactions, Agr. J. 88 (1996) 876–885.

[9] F.E. Dayan, J.G. Romagni, S.O. Duke, Investigating the mode of action of natural phytotoxins, J. Chem. Eco. 26 (2000) 2079-2094.

[10] Inderjit, J. Weiner, Plant allelochemical interference or soil chemical ecology?, Per. Plant Eco. Evo. Sys. 4 (2001) 3–12.

[11] J. Mizutani, Selected allelochemicals, Cri. Re. Plant Sci. 18 (1999) 653–671.

[12] J.R. Vyvyan, Allelochemicals for new herbicides and agrochemicals, Tetrahedron. 58 (2002) 1631-1646.

[13] F.A. Macias et al., Bioactive steroids from *Oryza sativa* L. Steroids. 71 (2006) 603–608.

[14] V. Rowshan, F. Farhadi, S. Najafian, The essential oil of *Dodonaea viscosa* leaves is allelopathic to rosemary (*Rosmarinus officinalis* L.), Ind. Crops Pro. 56 (2014) 241-245.

[15] M. Scognamiglio et al , Allelopathic potential of alkylphenols from *Dactylis glomerata* subsp. hispanica (Roth) Nyman, Phyto. Let. 5 (2012) 206–210.

[16] L.R.Scrivanti, Allelopathic potential of *Bothriochloa laguroides* var. laguroides (DC) Herter (Poaceae: Andropogoneae), Flora. 205 (2010). 302–305.

[17] T.L. Weir, S.W. Park, J.M. Vivanco, Biochemical and physiological mechanisms mediated by allelochemicals, Cur. O. Plant Bio. 7 (2004). 472-479.

[18] H.J. Bouwmeester et al., Secondary metabolites signalling in host–parasitic plant interactions, Cur. O. Plant Bio. 6 (2003) 358–364.

[19] A. Fiorentino et al., Potential allelopathic effects of stilbenoids and flavonoids from leaves of *Carex distachya* Desf, Bio. Sys. Eco. 36 (2008) 691–698.

[20] V.A. Areco et al, Effect of pinene isomers on germination and growth ofmaize, Bio. Sys. Eco. 55 (2014) 27–33.

[21] T.D. Xuan et al., Biological control of weeds and plant pathogens in paddy rice by exploiting plant allelopathy: an overview, Crop Prot. 24 (2005) 197-206.

[22] S. Yodmanee, T.T. Karrila, P. Pakdeechanuan, Physical, chemical and antioxidant properties of pigmented rice grown in Southern Thailand, Int. Food Res. J. 18 (2011) 901-906.

[23] A.A. Elzaawely, T.D. Xuan, S. Tawata, Antioxidant and antibacteria activity of *Rumex japonicas* Houtt, Biol. Pharm. Bull. 28 (2005) 2225-2230.

[24] R. Touati et al., The potential of cork from *Quercus suber* L grown in Algeria as a source of bioactive lipophilic and phenolic compounds, Ind. Crops Prod. 76 (2015) 936-945.

[25] N. Dolai et al, Free radical scavenging activity of Castanopsis indica in mediating hepatoprotective activity of leaves activity of carbon tetrachloride intoxicated rats, Asian Pac. J. Trop. Biomed. 5 (2012) 242-251.

[26] C.B. Silva et al, Allelopathic and antioxidant activity and total phenolic contents of *Hydrocotyle bonariensis* Lam. (Araliaceae), Acta Scientiarum Tech. 32 (2010) 413–420.

[27] A. Basile et al, Antibacterial and allelophathic activity of extract from *Castanea sati* leaves, Fitoterapia. 71 (2000) S1–S140.

[28] H.A. Kordan, Seed viability and germination: a multi-purpose experimental system, J. Biol. Educ. 26 (1992) 247-251.

[29] U. S. Environmental Protection Agency, Nanotechnology White Paper External Review Draft, (2005), [Online]: http://www.epa.gov/osa/pdfs/EPA_nanotechnology_white_paper_external review draft_12-02-2005.pdf.

[30] O. Munzuroglu, H. Geckil, Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*, Arch. Environ. Contam. Toxicol. 43 (2002) 203-213.

[31] X.D. Wang et al., Validation of germination rate and root elongation as indicator to assess phytotoxicity with *Cucumis sativus*, Chemosphere. 44 (2001) 1711-1721.

[32] P. Saravanan et al., GC-MS analysis of phytochemical constituent in ethanolic bark extract of *Ficus religiosa* Linn., Int. J. Phar. Phar. Sci. 6 (2014) 457-460.

[33] K. Lewis, F.M. Ausubel, Prospects for plant-derived antibacterial, Nat Biotech. 24 (2006) 1504-1507.

[34] A.S. Adekunle, Preliminary assessment of antimicrobial properties of aqueous extract of plants against infectious diseases, Biol Med. 1 (2009) 20-24.

[35] J.A. Olagunju et al., Effects of an ethanolic root extract of *Plumbago zeylanica* L on some serum parameters of the rats, RPMP-Drug Dev Mol. 11 (2006) 268-276.

[36] P.P. Kumar, S. Kumaravel, C. Lalitha, Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*, Afr. J. Biochem. 4 (2010) 191-195.

[37] S. Jana, G.S. Shekhawat, Phytochemical analysis and antibacterial screening of *in vivo* and *in vitro* extracts of Indian medicinal herb: *Anethum graveolens*, Res. J. Med. Plant. 4 (2010) 206-212.