Agronomic characteristics of 30 promising lines of aromatic, red, and black rice and their antioxidant and cytotoxic effects in some cancer cells

by Enos Tangke Arung

Submission date: 25-Oct-2021 08:48AM (UTC+0700)
Submission ID: 1682972970
File name: Enos_Tangke_Arung-_Biodiversitas_2021.pdf (515.26K)
Word count: 5040
Character count: 25610

BIODIVERSITAS
Volume 22, Number 4, April 2021
Pages: 1695-1700

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220412

Agronomic characteristics of 30 promising lines of aromatic, red, and black rice and their antioxidant and cytotoxic effects in some cancer cells

YUSUF LIMBONGAN[1,♥], RICO RAMADHAN[2,3], KUNIYOSHI SHIMIZU[4,5], ENOS TANGKE ARUNG[6,7,♥♥]

[1]Faculty of Agriculture, Universitas Kristen Indonesia Toraja. Jl. Jenderal Sudirman No. 9, Bombongan, Makale, Tana Toraja 91811, South Sulawesi, Indonesia. Tel.: +62-423-22060, ♥email: yusuflimbongan67@gmail.com

[2]Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Mulyorejo, Surabaya 60115, East Java, Indonesia

[3]Division of Exploration and Synthesis of Bioactive Compounds, Research Center for Bio-Molecule Engineering, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Mulyorejo, Surabaya 60115, East Java, Indonesia

[4]Faculty of Agriculture, Kyushu University. 744 Motooka, Nishi-ku, Fukuoka, 819-0395, Japan

[5]Institute for Asian and Oceanian Studies, Kyushu University. 744 Motooka, Nishi-ku, Fukuoka, 819-0395, Japan

[6]Faculty of Forestry, Universitas Mulawarman. Jl. Penajam, Gunung Kelua, Samarinda 75123, East Kalimantan, Indonesia. Tel./fax.: +62-541-748683, ♥email: tangkearung@yahoo.com

[7]Research Center for Medicine and Cosmetic from Tropical Rainforest Resources, Universitas Mulawarman. Jl. Penajam, Gunung Kelua, Samarinda 75123, East Kalimantan, Indonesia

Manuscript received: 20 November 2020. Revision accepted: 6 March 2021.

Abstract. *Limbongan Y, Ramadhan R, Shimizu K, Arung ET. 2021. Agronomic characteristics of 30 promising lines of aromatic, red, and black rice and their antioxidant and cytotoxic effects in some cancer cells. Biodiversitas 22: 1695-1700.* In our effort to increase rice production, some activities were done such as increased land production, rice planting patterns, and crossbreeding some rice with the special characteristic. We have cross-bred aromatic rice with red rice and black rice. These activities resulted in thirty lines of rice varieties. The thirty lines of rice variation were extracted with ethanol and evaluated the extracts for their phytochemicals, antioxidant activity, and cytotoxicity effect on cancer cell lines (MCF-7, HeLa, and OVK-18). The phytochemicals of thirty lines variation of rice showed variation in their contents. The line of F2BA201 extracts showed the strongest in antioxidant with IC_{50} 2.43 µg/mL while Vitamin C was 32.93 µg/mL. The line of F2LD20 extracts displayed potent inhibition with value of 40.2% against OVK-18 cancer cells while 5FU as positive control revealed percentage inhibition with value of 21.2%. Based on the results, the thirty line rice variations exhibited antioxidant and anti-cancer potential.

Keywords: Antioxidant, cytotoxicity, phytochemicals, rice crossbred

INTRODUCTION

There are various ways to increase land productivity and improve rice planting patterns, but the use of superior varieties or types of rice that produce high yields at an early age is an important part of such efforts. Increasing the production of black rice, brown rice, and aromatic rice to promote food self-sufficiency and improve the welfare of farmers is a very worthwhile goal. Efforts can be made to achieve this goal by increasing the productivity per unit of land area. One such effort involves using technological means to achieve a transformation from one rice harvest to two to three harvests per year. In order to support these efforts and enhance national food security, it is necessary to develop early-age and high-yielding rice varieties.

The food needs of the population tend to increase each year in line with population growth, the development of the food industry, the anticipation of crisis situations, and reduce import. Thus, it is strategically important to increase and stabilize our nation's food security. One way to achieve the goal of food self-sufficiency in the context of national food security is to increase food production through superior seed management. It is believed that the consistent use of high-quality seed varieties by farmers will contribute significantly to increasing the productivity of food crops. Consequently, we must work to ensure that high-quality seeds of superior crop varieties are available at the right time, in the right locations, in appropriate quantities, and at affordable prices to achieve competitive and sustainable production of high-quality agricultural products.

Whether rice attains high economic value is determined by the variety of rice cultivated and the cultivation techniques used (Srujana et al. 2017). People of the Toraja ethnic group in Indonesia often favor the taste of glutinous rice, including popular varieties such as Pare Kombong, Pare Bau', Pulu' Mandoti, Pare Barri, and others (Limbongan et al. 2019). Rice (*Oryza sativa* L.) is a widely consumed staple food that provides energy for people worldwide, especially in Southeast Asia. Recently, pigmented rice varieties have become popular for their high phytochemical content, which endows them with antioxidant and anti-inflammatory qualities as well as other beneficial health effects (Alves et al. 2016). Rice varieties are categorized as pigmented rice (red rice and black rice) or non-pigmented rice (brown rice) based on the bran color. Anthocyanins are the major phytochemicals in black rice, and they contribute to its color, whereas pro-

anthocyanidins are the major phytochemicals in red rice, and they contribute to its color (Gong et al. 2020; Pang et al. 2018).

The trend of using quality seeds has increased as the number of superior varieties available has increased, thus ensuring a stable market. Efforts to improve the quality and quantity of yields through crossbreeding should be encouraged to increase the diversity of existing varieties; it is also hoped that such crossbreeding can produce seeds or varieties with better quality (Gour et al. 2017).

MATERIALS AND METHODS

Plant crossbreeding, collection and identification

Five local Toraja rice were selected based on a preliminary study (Limbongan and Djufry 2015). These rice varieties were Pare Bau' (aromatic and white rice), Pare Kombong (aromatic, white, and sticky rice), Pare Lea (red rice), Pare Ambo' (aromatic and black rice), and Pare Lallodo (aromatic, black, and sticky rice). These local rice were single-crossed with Inpari 4 (National rice) to obtain F1 seed rice. The F1 seed rice were planted to obtain F2 seed rice. The F2 seed rice were planted and selected based on the harvest time, quality of the rice (color and aroma), and production/ha as presented. Thirty lines of F2 seed rice were selected as shown in Table 1.

The 30 line samples (aromatic rice/red rice/black rice) were collected in Toraja. After plant identification, plant voucher specimens were deposited in the Laboratory of Forest Product Chemistry, Faculty of Forestry, Mulawarman University.

Sample extraction

The 30 line samples (aromatic rice/red rice/black rice) were dried and powdered with an electric grinder. Next, an extraction process was conducted by soaking them in methanol (MeOH) for 3 x 24 hours. The extracts obtained from the samples (aromatic rice, red rice, and black rice) were filtered and evaporated under pressure to obtain dry extracts. The dry extracts were stored at 4°C in a refrigerator for further analysis.

Phytochemical analysis

The phytochemical test was used to screen for flavonoids, alkaloids, terpenoids, tannins, steroids, saponins, phenolics, carotenoids, coumarin, and carbohydrates. The screening tests for these major phytochemicals were carried out using standard qualitative procedures as described by Harborne (1984), Kokate et al. (2017), and Viji et al. (2013) as follows.

Detection of alkaloids

Five mL of extract was mixed carefully with 2 mL of HCl in a test tube, and 1 mL Dragendorff reagent was added. The formation of a yellow-colored precipitate showed a positive result for alkaloids in the extracts (Kokate et al. 2000).

Detection of flavonoids

An extract (1 mL) was treated with 5 drops of 1% sodium hydroxide solution. The formation of an intense yellow color, which developed into a colorless solution upon the addition of a dilute acid (HCl 1%), indicated the presence of flavonoids in the extracts (Harborne 1984).

Detection of saponins

Extracts (60 mg) were mixed with 2 mL of acetone and 3 mL of hot water was then added. The mixture was cooled and then shaken vigorously for 10 seconds. The formation of bubbles or persistent foam with a height of 1-10 cm for 10 min following the addition of one drop of HCl 2N, and the froth did not disappear indicated the presence of saponins in the extracts (Harborne 1984).

Detection of triterpenoid and steroid

A mixture of 10 drops of acetic anhydride and two drops of concentrated sulfuric acid with 1 mL of extracts was diluted in acetone. The resultant mixture was shaken vigorously. The production of red or purple indicated the presence of triterpenoids, and the production of a blue-green color showed that steroids were present (Kokate et al. 2000).

Detection of carotenoids

An extract sample of 1 mL was diluted with 5 mL chloroform in a test tube and shaken vigorously, and four drops of 85% sulphuric acid were added. The production of blue color on the surface of the mixture indicated that carotenoids were present (Viji et al. 2013).

Detection of coumarins

A 1 mL sample of each extract was treated with four drops of sodium hydroxide and alcohol. The solution turned yellow if coumarins were present (Viji et al. 2013).

Detection of tannins

An extract sample (10 mL) was mixed with three drops of freshly prepared 1% lead acetate. The formation of yellow precipitates was considered to show the presence of tannins (Kokate et al. 2000).

Detection of carbohydrates

One mL of extract was dissolved in 1 mL of acetone in a test tube and was treated with one drop of *Molisch* reagent. The resultant mixture was shaken vigorously and treated with 1 mL of sulphuric acid. The production of purple rings between two layers of the mixture indicated that carbohydrates were present (Kokate et al. 2000).

Radical scavenging (DPPH) assay

This assay was performed as described by Arung et al. In this assay, 500 μL of 60 μM DPPH and 467 μL of ethanol were used as working solutions. The positive control was Vitamin C. The percentage of free radical scavenging activity was calculated using the following equations (note that "Abs" indicates absorbance): % scavenging activity = (Abs control-Abs sample)/ Abs control x 100. The lower the absorbance was, the higher the

free radical scavenging activity. The 50% inhibitory concentration (IC_{50}) represents the concentration of extract required to inhibit 50% of free radical DPPH. According to Miryanti et al. (2011), antioxidant activity is considered to be extremely high if the value of IC_{50} is less than 50 µg/mL, high if the value of IC_{50} is between 50-100 µg/mL, moderate if the value is between 100-150 µg/mL and low if the value is between 151-200 µg/mL.

Cytotoxicity assay

The cell lines were obtained from the RIKEN Cell Bank (Japan). The cells were maintained in Eagle's Minimum Essential Medium (EMEM) or *Dulbecco's Modified Eagle Medium* (DMEM). Cell viability was determined using the microculture tetrazolium technique (MTT). The MTT assay provides a quantitative measure of the number of viable cells by determining the amount of formazan crystals produced by metabolic activity in treated versus controlled cells. In brief, confluent cells in a 96-well plate or 24-well plate were treated with either vehicle or samples of different concentrations for 72 h and the cell viability was then checked using an MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] solution. After a 4-h incubation period, the MTT solution was removed and HCl-isopropanol solution was added to each well. The plate was incubated in the dark for 4 more hours, and the absorbance of the resulting solution was measured at 570 nm with a microplate reader (EL9800; Biotech; Winooski, Vermont, USA) (Arung et al. 2010). Cell viability was calculated using the ratio of the absorbance of the sample-treated wells to that of the vehicle-treated wells. The IC_{50} was inferred from the viability-dose-dependent curve.

RESULTS AND DISCUSSION

Thirty selected lines of rice

Based on Limbongan and Djufry (2015), the selected local rice such as Pare Bau', Pare Kombong, Pare Lea, Pare Ambo', and Pare Lallodo are varieties that taste good but require long harvesting time (approximately 5-7 months) and low productivity. The selection process of the F2 was conducted, and 30 lines were selected. The 30 selected lines (Table 1) were had a short growth period until harvesting (approximately 3 months), and had high productivity (data not shown). These 30 lines were analyzed for their health benefits and their phytochemical content, e.g., through antioxidant and anticancer assays.

Phytochemical analysis

In this experiment, we tested hybrid rice products (Table 1) that were found in Toraja, South Sulawesi. Phytochemical screening of aromatic rice, red rice, and black rice extracts was performed. The results revealed that the 30 rice lines tested contained various secondary metabolites such as tannins, alkaloids, flavonoids, triterpenoids, steroids, carotenoids, carbohydrates, coumarins, and saponins (Table 2). Some rice extracts lines were yellow in color when in basic conditions and became colorless solutions in acidic conditions, indicating the possible presence of flavonoids. The lead acetate test confirmed the presence of tannins in the extracts through the formation of yellow precipitates after the presence of coumarins was confirmed by their appearing yellow in color when in a basic condition. Furthermore, other phytochemicals such as alkaloids, triterpenoids, steroids, carotenoids, carbohydrates, and saponins were found to be randomly present in all rice varieties. Secondary metabolites in these rice varieties may influence their biological functions in individual or synergistic manners. For example, secondary metabolites such as phenolics, flavonoids, tannins, and coumarins are widely distributed in various plants that exhibit bioactivities such as antioxidant, anti-inflammatory, and enzyme inhibition activities (Alves et al. 2016; Ghasemzadeh et al. 2018; Sequeda-Castañeda et al. 2019; Bubols et al. 2013). In addition, alkaloids have been reported to have antimalarial (Dua et al. 2013), cytotoxic (Thite et al. 2013), and antimicrobial effects (Raji et al. 2019). Triterpenoids are known to have α-glucosidase inhibition (Ramadhan et al. 2020) and anticancer activities (Yan et al. 2013). Recently, other studies reported the presence of phytochemical components in methanolic extracts of red rice and non-colored rice from Minahasa, North Sulawesi (Moko et al. 2014). Shao et al. (2018) also reported a quantitative analysis of secondary metabolites such as phenols, flavonoids, and proanthocyanidins contained in non-pigmented, red, and black rice. These phytochemical components are known to have medicinal effects. As far as we know, there have been no reports involving the phytochemical screening of 30 lines of aromatic and pigmented rice hybrid products that were studied in the present research. Therefore, the present phytochemical investigation provides preliminary findings on rice hybrid products that appear to have benefits for human health.

Radical scavenging (DPPH) assay

The antioxidant activity of aromatic rice, black rice, and red rice is associated with their medicinal potency. In this study, the antioxidant potential of the 30 lines of aromatic rice, black rice, and red rice was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The results were expressed in terms of the concentration of the rice line to scavenge 50% of the free radical DPPH (IC_{50}). The inhibitory effects of the 30 lines of aromatic rice, black rice, and red rice were compared with those of a positive standard ascorbic acid (vitamin C, IC_{50}, 32.93 µg/mL) (Table 2). Among the 30 lines that were studied, thirteen lines of rice, including red rice, black rice, and aromatic rice, had good potential to inhibit free radical DPPH. Lower IC_{50} values against DPPH radicals indicate higher antioxidant activity. The antioxidant activity test showed that the F2A93, F2A205, and F2BA90 (IC_{50} 12.5 µg/mL) categorized as black rice and aromatic rice, respectively that exhibited the strongest activity against free radical DPPH. According to Miryanti et al. (2011) IC_{50} values < 50 µg/mL for antioxidant was very strong. The lower IC_{50} values of 30 lines of aromatic rice, black rice, and red rice against DPPH radical indicate the higher antioxidant activity. The findings of this study in line with Shao et al.

2018 reported that red and black rice had significant antioxidant activity. Furthermore, Saikia et al. 2012 also reported that aromatic pigmented and non-pigmented rice varieties had good scavenging activity against free radical DPPH.

Cytotoxicity assay

In this study, we investigated the cytotoxicity effects of methanol extracts from 30 lines of aromatic, black, and red rice by subjecting them to a cytotoxicity assay, which could demonstrate antioxidant activity and the presence of phytochemical content.

The cytotoxicity activity of the 30 lines aromatic rice, black rice, and red rice was evaluated against a breast cancer cell line (MCF-7), a colon cancer cell line (Caco-2), and an ovarian cancer cell line (OVK-18). The results of the cytotoxicity assay are presented in Table 4. Almost 30 rice lines tested exhibited lower cytotoxicity activity against MCF-7, Caco-2, and OVK-18 compared with the positive control (5-FU), with cell viability percentages of 88.4%, 91.9%, and 78.8%, respectively. Furthermore, the lines were tested against other cancer cells such as other colon cancer cell lines. These results showed good cytotoxicity activity that was quite similar to that of the positive control (5-FU). Moreover, among the 30 lines tested, the F2LD20 line (aromatic/black rice) showed the lowest of viability percentage against the ovarian cancer cell line (OVK-18), which indicated that this line had the strongest activity against these cancer cells. In general, our results revealed that the 30 lines of aromatic rice, black rice, and red rice had potency as anticancer agents. These results are consistent with the findings of Ghasemzadeh et al. (2018), who reported that black rice extracts exhibited potent antiproliferative activity, whereas red rice showed moderate activity against a breast cancer cell line (MCF-7). In food supplements, one component may deliver the desired therapeutic benefits while others may have toxic effects. The American National Cancer Institute recommends that crude herbal extracts that do not reduce the viability of normal cells below 76% are safe for human consumption (WHO, 2013). In the present study, the 30 lines investigated not only contained a variety of phytochemicals and showed antioxidant activity, but they also showed cytotoxicity activity.

Table 1. Characterization of 30 rice lines

Samples	Parental		Characterization	Samples	Parental		Characterization
	Male	Female			Male	Female	
F2 L 154	Padi Lea	Inpari 4	Red rice	F2 A 274	Padi Ambo'	Inpari 4	Aromatic, black rice
F2 L 239	Padi Lea	Inpari 4	Red rice	F2 A 93	Padi Ambo'	Inpari 4	Black rice
F2 L 93	Padi Lea	Inpari 4	Red rice	F2 A 205	Padi Ambo'	Inpari 4	Black rice
F2 L 70	Padi Lea	Inpari 4	Red rice	F2 A 49	Padi Ambo'	Inpari 4	Aromatic, black rice
F2 L 27	Padi Lea	Inpari 4	Red rice	F2 A 295	Padi Ambo'	Inpari 4	Black rice
F2 BA 12	Padi Bau'	Inpari 4	Aromatic, white rice	F2 RB 55	Inpari 4	Padi Bau'	Aromatic, white rice
F2 BA 90	Padi Bau'	Inpari 4	Aromatic, white rice	F2 RB 4	Inpari 4	Padi Bau'	Aromatic, white rice
F2 BA 35	Padi Bau'	Inpari 4	Aromatic, white rice	F2 RB 80	Inpari 4	Padi Bau'	Aromatic, white rice
F2 BA201	Padi Bau'	Inpari 4	Aromati, white rice	F2 RB 70	Inpari 4	Padi Bau'	Aromatic, white rice
F2 BA134	Padi Bau'	Inpari 4	Aromatic, white rice	F2 RB 90	Inpari 4	Padi Bau'	Aromatic, white rice
F2 LD 70	Padi Lallodo	Inpari 4	Aromatic, black, sticky rice	F2 K 12	Padi Kombong	Inpari 4	Aromatic, white, sticky rice
F2 LD223	Padi Lallodo	Inpari 4	Aromatic, black, sticky rice	F2 K 13	Padi Kombong	Inpari 4	Aromatic, white, sticky rice
F2 LD 69	Padi Lallodo	Inpari 4	Aromatic, black, sticky rice	F2 K 11	Padi Kombong	Inpari 4	Aromatic, white, sticky rice
F2 LD 20	Padi Lallodo	Inpari 4	Aromatic, black, sticky rice	F2 K 10	Padi Kombong	Inpari 4	Aromatic, white, sticky rice
F2 LD298	Padi Lallodo	Inpari 4	Aromatic, black, sticky rice	F2 K 18	Padi Kombong	Inpari 4	Aromatic, white, sticky rice

Table 3. DPPH radical-scavenging activity of methanol extracts of aromatic rice, black rice, and red rice

Samples	IC_{50} (µg/mL)	Samples	IC_{50} (µg/mL)	Samples	IC_{50} (µg/mL)
F2LD20	50.74	F2RB4	36.63	F2L239	42.32
F2LD69	51.32	F2RB55	49.94	F2L27	100.0
F2LD70	50.74	F2RB70	166.78	F2L70	100.0
F2LD223	49.35	F2RB80	50.83	F2L93	36.74
F2LD298	121.25	F2RB90	50.50	F2L154	87.13
F2A49	16.34	F2K11	41.54	F2BA12	13.38
F2A93	12.5	F2K12	140.15	F2BA35	201.75
F2A205	12.5	F2K13	25.0	F2BA90	12.5
F2A274	49.69	F2K18	87.79	F2BA134	90.37
F2A295	25.0	F2K10	61.24	F2BA201	50.0
Vitamin C (Ascorbic acid)					32.93

Table 2. Phytochemicals screening of aromatic rice, black rice, and red rice extract

Samples	Secondary metabolites								
	Tannins	Alkaloids	Flavonoids	Triterpenoids	Steroids	Carotenoids	Carbohydrates	Coumarins	Saponins
F2 L 154	-	+	+	-	-	+	+	+	-
F2 L 239	+	-	+	+	-	-	+	-	-
F2 L 93	-	+	-	-	+	+	+	+	+
F2 L 70	+	+	+	-	-	+	+	-	+
F2 L 27	+	-	+	-	+	-	+	+	+
F2 BA 12	-	+	-	+	-	-	+	-	+
BA F2 90	+	-	+	-	-	+	+	+	-
F2 BA 35	+	-	+	-	+	-	+	-	+
FA BA 201	-	+	-	-	-	+	+	+	-
F2 BA 134	-	-	+	-	+	-	+	-	+
F2 LD 70	-	+	+	-	-	+	+	-	-
F2 223	+	-	+	+	-	+	+	+	-
F2 LD 69	-	-	+	-	+	-	+	-	+
F2 LD 20	-	-	-	-	+	+	+	-	+
F2 LD 298	+	+	+	+	+	+	+	+	+
F2 A 274	+	-	+	-	+	-	+	-	-
F2 A 93	-	+	+	-	+	+	+	-	+
F2 A 205	+	-	+	+	-	-	+	+	-
F2 A 49	-	+	-	+	+	-	+	+	-
F2 A 295	-	-	+	-	-	+	+	+	+
F2 RB 55	-	+	-	-	+	-	+	-	+
F2 RB 4	+	-	+	+	-	+	+	+	-
F2 RB 80	+	+	+	-	+	-	+	+	-
F2 RB 70	-	-	+	-	-	+	+	-	+
F2 RB 90	-	+	-	+	-	+	+	-	+
F2 K 12	+	-	+	-	-	+	+	-	+
F2 K 13	-	-	+	-	-	+	+	-	+
F2 K 11	-	+	+	+	-	-	+	-	-
F2 K 10	+	+	-	+	+	+	+	-	-
F2 K 18	-	-	-	-	+	+	+	+	+

Note: +, Presence;-, Absence

Table 4. Viable cells of ethanol extracts of aromatic rice, black rice, and red rice

Samples	Extracts (g)	Cell viability (%)		
		MCF-7	Caco-2	OVK-18
F2LD20	0.0774	84.7±0.02	92.4±0.05	59.8±0.03
F2LD69	0.0909	106.5±0.01	99.4±0.09	67.6±0.01
F2LD70	0.0767	102.4±0.07	96.7±0.08	92.5±0.06
F2LD223	0.0703	109.2±0.04	98.4±0.03	96.6±0.06
F2LD298	0.0776	107.8±0.03	92.7±0.07	82.2±0.05
F2A49	0.0683	104.3±0.05	95.5±0.17	89.7±0.01
F2A93	0.0699	92.9±0.03	98.6±0.04	92.9±0.04
F2A205	0.0995	112.9±0.01	105.1± 0.14	102.1±0.08
F2A274	0.1132	117.0±0.02	114.4±0.06	97.0±0.03
F2A295	0.0740	117.9±0.04	110.0±0.18	94.0±0.04
F2L239	0.1609	99.9±0.02	95.6±0.05	95.6±0.02
F2L27	0.0977	113.9±0.05	113.8±0.08	113.8±0.01
F2L70	0.1487	109.8±0.03	84.3±0.06	84.3±0.02
F2L93	0.0865	112.1±0.05	106.4± 0.12	106.4±0.04
F2L154	0.1496	118.1±0.07	101.3±0.03	101.3±0.01
F2RB4	0.1171	113.7±0.05	105.9±0.05	105.9±0.06
F2RB55	0.0890	108.9±0.03	99.2±0.09	99.2±0.06
F2RB70	0.0777	118.8±0.09	98.7±0.12	98.7±0.03
F2RB80	0.0818	104.5±0.03	102.9±0.11	102.9±0.05
F2RB90	0.1206	105.8±0.02	108.3±0.04	108.3±0.02
F2K11	0.2167	104.2±0.01	112.7±0.12	94.0±0.02
F2K12	0.2794	109.7±0.03	113.2±0.18	94.3±0.01
F2K13	0.3825	109.3±0.03	136.7±0.13	114.0±0.04
F2K18	0.1710	112.1±0.02	118.3±0.17	98.8±0.03
F2K10	0.2468	104.3±0.02	122.9±0.04	110.9±0.07
F2BA12	0.1671	105.8±0.03	110.5±0.06	114.2± 0.04
F2BA35	0.1976	98.6±0.05	119.1±0.06	102.4±0.04
F2BA90	0.1135	106.7±0.01	124.7±0.16	110.6±0.02
F2BA134	0.2281	109.5±0.03	129.4±0.02	105.5±0.03
F2BA201	0.1804	98.7±0.02	110.2±0.13	98.9±0.05
5-FU (Positive Control)		88.4± 0.02	91.9± 0.06	78.8± 0.04
Control		100.0± 0.05	100.0± 0.22	100.0± 0.31

Note: Data are given as the means ± SDs (n=3)

In summary, to the best of our knowledge, this is the first report to show that hybrid rice products derived from local rice in the Toraja region have antioxidant activity as well as cytotoxicity activity. Furthermore, extracts containing high levels of phytochemicals and good bioactivity need to be studied further to isolate secondary metabolites that may be responsible for their anticancer and antioxidant activities. Moreover, this study provides scientific evidence for the use of local rice in the traditional healthcare system.

ACKNOWLEDGEMENTS

Our sincere gratitude goes to DRPM, Directorate General of Higher Education, Ministry of Education and Culture (Kemendikbud DIKTI), Indonesia for the research grant (14/LPPM-UKIT.01/KP/2018), as well as the Indonesian Christian University of Toraja, for their financial support throughout the course of this study.

REFERENCES

Alves GH, Ferreira CD, Vivian PG, Monks JLF, Elias MC, Vanier NL, Oliveira M. 2016. The revisited level of free and bound phenolics in rice: Effects the extraction procedure. Food Chem 208: 116-123. DOI: 10.1016/j.foodchem.2016.03.107.

Bubols GB, Vianna DR, Medina-Remón A, Poser G, Lamuela-Raventos RM, Eifler-Lima VL, Garcia SC. 2013. The antioxidant activity of coumarins and flavonoids. Mini-Rev Med Chem 13 (3): 318-334. DOI: 10.2174/138955713804999775.

Dua VK, Verma G, Singh B, Rajan A, Bagai U, Agarwal DD, Gupta NC, Kumar S, Rastogi A. 2013. Anti-malarial property of steroid alkaloid

conessine isolated from the bark of *Hollarhena antidysenterica*. Malaria J 12 (1): 1-6. DOI: 10.1186/1475-2875-12-194.

Ghasemzadeh A, Karbalaii MT, Jaafar HZE, Rahmat A. 2018. Phytochemical constituents, antioxidant activity, and antiproliferative properties of black, red, and brown rice bran. Chem Central J 12: 1-13. DOI: 10.1186/s13065-018-0382-9.

Gong ES, Liu C, Li B, Zhou W, Chen H, Li T, Wu J, Zeng Z, Wang Y, Si X, Lang Y, Zhang Y, Zhang W, Zhang G, Luo S, Liu RH. 2020. Phytochemical profiles of rice and their cellular antioxidant activity against ABAP induced oxidative stress in human hepatocellular carcinoma HepG2 cells. Food Chem 318: 126484. DOI: 10.1016/j.foodchem.2020.126484 .

Gour L, Koutu GK, Singh SK, Patel DD, Shrivastava A, Singh Y. 2017. Genetic variability, correlation and path analyses for selection in elite breeding materials of rice (*Oryza sativa* L.) genotypes in Madya Pradesh. Pharm Innov J 6 (11): 693-696.

Harborne JB. 1984. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Springer, Berlin.

Limbongan Y, Djufry F. 2015. Characterization and observation of five local rice accessions of Toraja Plateau, South Sulawesi. Buletin Plasma Nutfah 21 (2): 61-70. DOI: 10.21082/blpn.v21n2.2015.p61-70. [Indonesian]

Limbongan YL, Malamassam D, Sjahril R. 2019. Identification and characterization of Toraja local rice germplasm. The 1st Biennial Conference on Tropical Biodiversity. IOP Conf Ser Earth Environ Sci 27 (1): 012025. DOI: 10.1088/1755-1315/270/1/012025.

Kokate CK, Purohit AP, Gokhale SB. 2017. Pharmacognosy-1. Third Edition. Nirali Prakashan, Pune, IndiaMiryanti A, Sapei L, Budiono K, and Indra S. 2011. Extraction of Antioxidants from The Skin of Mangosteen Fruit (*Garcinia mangostana* L.). University of Catholic Parahyangan, Bandung. [Indonesian]

Moko EM, Purnomo H, Kusnadi J, Ijong FG. 2014. Phytochemical content and antioxidant properties of colored and non-colored varieties of rice bran from Minahasa, North Sulawesi, Indonesia. Int Food Res J 21 (3): 1053-1059.

Organization WHO. 2013. WHO traditional medicine strategy: 2014-2023. World Health Organization, Geneva.

Pang Y, Ahmed S, Xu Y, Beta T, Zhu Z, Shao Y, Bao J. 2018. Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice. Food Chem 240: 212-221. DOI: 10.1016/j.foodchem.2017.07.095

Raji P, Samrot AV, Keerthana D, Kharisma S. 2019. Antibacterial activity of alkaloids, flavonoids, saponins and tannins mediated green synthesized silver nanoparticles against *Pseudomonas aeruginosa* and *Bacillus subtilis*. J Cluster Sci 30 (4): 881-895. DOI: 10.1007/s10876-019-01547-2.

Ramadhan R, Phuwapraisirisan P, Kusuma IW, Amirta R. 2020. Ethnopharmacological evaluation of selected east Kalimantan flora for diabetes therapy: The isolation of lupane triterpenoids as α-glucosidase inhibitors from *Ceriops tagal* (Perr) C.B. Robb. Rasayan J Chem 13 (3): 1727-1734. DOI: 10.31788/RJC.2020.1335559.

Saikia S, Dutta H, Saikia D, Mahanta CL. 2012. Quality characterization and estimation of phytochemicals content and antioxidant capacity of aromatic pigmented and non-pigmented rice varieties. Food Res Int 46 (1): 334-340. DOI: 10.1016/j.foodres.2011.12.021.

Sequeda-Castañeda LG, Muñoz-Realpe CC, Celis-Zambrano CA, Guttiérrez-Prieto SJ, Luengas-Caicedo PE, Gamboa F. 2019. Preliminary phytochemical analysis of *Berberis goudotti* Triana & Planch. ex Wedd. (Berberidaceae) with anticariogenic and antiperiodontal activities. Sci Pharm 87 (1): 1-19. DOI: 10.3390/scipharm87010002.

Shao Y, Hu Z, Yu Y, Mou R, Zhu Z, Beta T. 2018. Phenolic acids, anthocyanins, proanthocyanidins, antioxidant activity, minerals and their correlations in non-pigmented, red and black rice. Food Chem 239: 733-741. DOI: 10.1016/j.foodchem.2017.07.009.

Srujana G, Suresh BG, Lavanya GR, Ram BJ, Sumanth V. 2017. Studies on genetic variability, heritability and genetic advance for yield and quality components in rice (*Oryza sativa* L.). J Pharmacog Phytochem 6 (4): 564-566.

Thite SV, Chayan YR, Aparadh VT, Kore BA. 2013. Preliminary phytochemical screening of some medicinal plants. Intl J Pharm Chem Biol Sci 3 (1): 87-90.

Viji GS, Vasanthe B, Suresh K. 2013. Screening and antibacterial activity analysis of some important medicinal plants. Intl J Innov Appl Stud 2 (2): 146-152.

Yan XJ, Gong LH, Zheng FY, Cheng KJ, Chen ZS, Zhi Z. 2013. Triterpenoids as reversal agents for anticancer drug resistance treatment. Drug Discov Today 19 (4): 482-488. DOI: 10.1016/j.drudis.2013.07.018.

Agronomic characteristics of 30 promising lines of aromatic, red, and black rice and their antioxidant and cytotoxic effects in some cancer cells

ORIGINALITY REPORT

6%	2%	4%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Enos Tangke Arung, Rudianto Amirta, Qinchang Zhu, Yhiya Amen, Kuniyoshi Shimizu. "Effect of wood, bark and leaf extracts of Macaranga trees on cytotoxic activity in some cancer and normal cell lines", Journal of the Indian Academy of Wood Science, 2018 Publication	4%
2	bmcchem.biomedcentral.com Internet Source	2%
3	Black Rice, 2016. Publication	1%

Exclude quotes	On	Exclude matches	< 10 words
Exclude bibliography	On		