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Phytochemical content, antioxidant capacity, and *in vitro* antibacterial activity of bran extracts of Philippine rice *(Oryza sativa)* cultivars

Rosaly V. Manaois*[1], John Edward I. Zapater[2], Amelia V. Morales[1], and Jay Carl A. Cacerez[1]

[1]Rice Chemistry and Food Science Division, Philippine Rice Research Institute, Maligaya, Science City of Muñoz, Nueva Ecija
[2]Microbial Insecticides Laboratory, University of the Philippines – Los Baños – National Institute of Molecular Biology and Biotechnology, Los Baños, Laguna

ABSTRACT

Rice bran is a by-product of the milling process that contains compounds with possible therapeutic properties. However, it is underutilized. This study assessed the phytochemical content, namely free, bound, and total phenolic (TPC), flavonoid (TFC), and anthocyanin (TAC) contents; antioxidant capacity using the 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging assay; and *in vitro* antibacterial activity of crude extracts from the bran of six (one white, two red, three black) selected Philippine rice varieties. Cyanidin-3-glucoside (C3G) and trans-ferulic acid (TFA) were quantified through High-Performance Liquid Chromatography. The *in vitro* antibacterial activities of extracts (0.1, 0.5, and 0.7 g/mL) against the gram-positive food pathogens *Staphylococcus aureus* and *Clostridium perfringens* were evaluated by agar well diffusion assay. Of the five samples, the red rice Kamanga recorded the highest free phenolics (121.01 mg gallic acid equivalents (GAE)/g), TFC (90.14 mg rutin hydrate equivalents/g), and DPPH antioxidant capacity (368.23 µmol Trolox equivalents/g). Generally, black brans had higher TAC, especially Ominio (17.81 mg C3G equivalents/g), which also had the highest bound phenolic content (125.6 mg GAE/g). C3G was only detected on black brans, with at least six-fold more in Ominio than in the other tested varieties. Only the extracts from pigmented varieties, except Ominio, displayed inhibitory potential against the food pathogens, implying that C3G is not the major component imparting the inhibitory activity in the black rice varieties. Therefore, pigmented rice brans can be explored as cheap sources of phytochemicals with antioxidant capacities and natural antibacterial agents for the food and pharmaceutical industries.

*Corresponding author
Email Address: rvmanaois@philrice.gov.ph
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INTRODUCTION

Rice bran is the portion removed during the polishing of rice. It includes the pericarp and aleurone layer (Hernandez et al. 2000) and accounts for roughly 10-12% of the rice grain (Kahlon 2009). Approximately 789 x 10^6 tons of rice are produced worldwide in 2021 (FAO 2023), which translates to at least 78.9 x 10^6 tons of bran being produced as by-product of the rice-milling process. In the Philippines, bran is mainly used in animal feeds (Doliente

KEYWORDS

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and Samsatli 2018). However, recent studies show that phytochemicals like oryzanols, tocopherols, tocotrienols, and ferulic acid are abundant in rice bran. These phytochemicals exert beneficial effects important for human nutrition (Piironen et al. 2000; Hu et al. 2003; Jiang and Wang 2005; Friedman 2013; Sapwarobol et al. 2021). They function as free radical scavengers and antioxidants that inhibit the formation and reduce the concentration of reactive cell-damaging agents, which causes lifestyle diseases such as cancer, diabetes, and cardiovascular diseases (Nam 2006; Lee et al. 2012; Zhang et al. 2012). In addition to these potential health benefits, phytochemical-rich plant samples have been shown to exert antimicrobial effects, which could find applications in the food or pharmaceutical industries (Nithiyanantham et al. 2012; Agourram et al. 2013). Due to the many side effects linked with synthetic antimicrobials (Al-Bakri and Afifi 2007), the commonly limited access of a large part of the population to these substances, and their high costs (Cowan 1999), there is an emerging interest in the use of plants as natural antimicrobial agents.

Staphylococcal enterotoxin-associated foodborne poisoning was among the most common in the Philippines in the period 1995-2018 (Azanza 2006; Azanza et al. 2019). Rice bran extracts, particularly those from pigmented varieties, were previously reported to inhibit diarrhea-causing bacterial pathogens such as *Staphylococcus aureus, Vibrio cholerae, Vibrio vulnificus, Salmonella spp., Shigella spp.* and *Escherichia coli* with minimum inhibitory concentrations as high as 0.976 mg/mL for the *V. cholerae* strain (Kondo et al. 2011). In another study by Ghazi et al. (2016), *Salmonella enterica* subsp. serovar *Typhimurium* is dose-dependently inhibited by red rice bran extracts in the small intestine epithelial (MSIE) and intestinal porcine epithelial cells (IPEC-J2) in mice. Luteolin, a type of polyphenol that is found in bran extracts, is shown to inhibit the growth of a wide range of gram-positive bacteria and yeasts (Singh et al. 2016). The Philippines has a rich collection of rice varieties, either pigmented or non-pigmented. However, limited information on the potential antimicrobial activity of bran from pigmented local rice varieties is currently available. Profiling the antioxidant and antibacterial properties of brans of local rice varieties is a step to identify potential sources of natural antimicrobial agents for the food and/or pharmaceutical industries. Hence, this study was conducted to assess the bran of selected Philippine rice varieties for their phytochemical content, antioxidant capacity, and *in vitro* antibacterial potential against two common foodborne gram-positive bacterial pathogens, namely *S. aureus* and *Clostridium perfringens*.

MATERIALS AND METHODS

A. Samples and Chemicals

Six rice samples composed of five pigmented and one non-pigmented rice varieties were collected in Banaue, Ifugao (4), Los Baños, Laguna (1), and PhilRice, Maligaya, Science City of Muñoz, Nueva Ecija (1) from the 2015 Wet Season and 2017 Dry Season harvests. The characteristics of the samples are listed in Table 1.

Table 1: Rice samples used in the study.

Rice Variety	Classification	Pericarp Color	Place of Collection
NSIC Rc 222	Modern	Brown	Muñoz, Nueva Ecija
Minaangan	Traditional	Red	Banaue, Ifugao
Kamanga	Traditional	Red	Banaue, Ifugao
Inipot Ibon	Traditional	Black	Los Baños, Laguna
Ingopon	Traditional	Black	Banaue, Ifugao
Ominio	Traditional	Black	Banaue, Ifugao

The following chemicals were sourced from Sigma Aldrich (St. Louis, MO, USA): Folin-Ciocalteu phenol reagent and gallic acid (GA) for phenolic analysis; 2,2-diphenylpicrylhydrazyl (DPPH) and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) for antioxidant assay; and cyanidin-3-O-glucoside (C3G), rutin hydrate (RH), chlorogenic acid (CGA), gallic acid (GA), quercetin, (+)-catechin hydrate, and trans-ferulic acid (TFA) standards for chromatographic measurements. Hexane was obtained from JT Baker (Center Valley, PA, USA) and methanol from Labscan (Bangkok, Thailand). All other reagents were of analytical grade.

B. Preparation and Collection of Bran Samples

Around 5 kg of paddy forms of each variety were dehulled using a THU-35A Satake machine and polished using a McGill No. 3 machine (Dayton Industrial Motor, TX, USA). The collected bran was passed through a 0.634-mm sieve to remove large particle impurities.

C. Stabilization of Rice Bran

To inactivate the lipase in the bran and prevent rancidity from setting in, the bran samples were stabilized according to the procedure of Damayanthi (2001), with modifications. Briefly, the rice bran samples were placed in cheesecloth and steam-heated at 95-100°C for 15 min, cooled, and then oven dried at 70°C for 1 hr. The samples were cooled to room temperature and kept in resealable polyethylene bags at -20°C until further analysis.

D. Sample Extraction for Phytochemical Analysis

The extraction of antioxidants used for total flavonoid content (TFC) and DPPH radical scavenging activity measurements was conducted by adopting the methods of Khairunnuur et al. (2009) and Singh et al. (2012), with modifications. Briefly, 0.3 g bran was placed into 15-mL centrifuge tubes and then added with 10 mL of 85% methanol. The contents of the tube were thoroughly mixed using a vortex mixer and a digital shaker at 300 rpm for 12 hr and then centrifuged (3,000 rpm, 15 min, 25°C). The extracts were transferred into another set of 15-mL centrifuge tubes, covered with aluminum foil, and then stored at 4°C until analyzed.

E. Preparation of Extracts for Free and Bound Phenolic Content

The extraction of free and bound phenolics was based on the procedure of Zhang et al. (2010). A rice bran sample (1 g) was added with 4 mL of 80% ethanol in a centrifuge tube. The tube was shaken at 3,000 rpm for 10 min at 4°C and then centrifuged (2,500 *x g*, 10 min, 4°C). The residues were set aside for the bound phenolic extraction, while the supernatants were transferred into a clean 50-mL centrifuge tube. The extraction was repeated once with the supernatants pooled and then evaporated to dryness at 30°C under a fume hood. The residues were re-dispersed in 2.5 mL of distilled water and then stored at 4°C until analyzed.

For the bound phenolics, the residue was defatted twice using hexane (Zhang et al. 2010). The samples were then hydrolyzed

with 25 mL of 2 N NaOH by shaking at 300 rpm for 1 hr. The pH of the solution was adjusted to 1.0 N using 6N HCl. The mixture was then extracted twice with 30 mL of ethyl acetate in a separatory funnel and evaporated at 30ºC. The residue was re-dissolved in 2.5 mL distilled water and stored at 4ºC until analyzed.

F. Phytochemical Analysis

1. Total Phenolic Content (TPC)

The phenolic content of the free and bound extracts was analyzed using the modified procedure of Singleton et al. (1999). Briefly, 2.5 mL of Folin-Ciocalteu reagent (1:10 dilution) was added to 0.5 mL of the sample extract, mixed vigorously, and then allowed to stand for 15 min. Two milliliters of 7.5% sodium carbonate were added into the mixture, which was incubated for 1 hr at room temperature before reading at 765 nm against gallic acid standards. The TPC was determined from the sum of the free and bound phenolic measurements and expressed as mg gallic acid equivalents (GAE) per gram of sample.

2. Total Anthocyanin Content (TAC)

The method of Abdel-Aal and Hucl (1999), with few modifications, was followed in the analysis of the TAC of the bran extracts. Briefly, 1 g of bran was added with 10 mL of 85% ethanol acidified with 1N HCl (pH 1.0) and the mixture was shaken for 1 hr. The tube was centrifuged at 3,000 rpm for 15 min and the supernatant was transferred into another centrifuge tube. The procedure was repeated twice, with the supernatants pooled in the same tube. Acidified ethanol (5 mL) was added to the residue. The tubes were shaken for 30 min and then centrifuged and the supernatants were pooled and measured at 535 nm against acidified ethanol as blank. The results of the analysis were expressed as mg cyanidin-3-glucoside equivalents per g (C3G Eq/g) of the sample.

3. Total Flavonoid Content (TFC)

The TFC was determined using the method of Bao et al. (2005), with some modifications. To a tube containing 2 mL of distilled water, 0.5 mL of diluted bran extract and 0.15 mL of 5% $NaNO_2$ were added, mixed, and incubated for 5 min. The mixture was added with 0.15 mL of 10% $AlCl_3 \cdot 6H_2O$, and then incubated for another 5 min. Finally, 1 mL of 1 N NaOH was added. The absorbance was read at 510 nm against rutin hydrate standards after 15 min. Results were expressed as rutin hydrate equivalents per gram (RHE/g) of the sample.

G. 2'2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity Analysis

The method of Brand-Williams et al. (1995), with modifications, was adopted in the determination of the DPPH radical scavenging activities of the extracts. Briefly, 0.5 mL of diluted extract was transferred in a 15-mL test tube, added with DPPH solution (0.01 mM, 5 mL), and mixed. The mixture was left to stand for 1 hr at room temperature and read at 517 nm, together with 85% methanol as reagent blank and different concentrations (0, 20, 40, 60, 100, 160, 200, 240, 280, and 320 µmol/L) of Trolox standards. The analysis was done in a dim place at ambient temperature. Results were expressed as µmol Trolox equivalents per gram (TE/g) of the sample.

H. Sample Preparation for Chromatographic Separation

The stabilized rice bran samples were extracted with HPLC-grade reagents, according to the previously modified extraction procedure of Khairunnuur et al. (2009) and Singh et al. (2012). The extracts were passed through a 0.45-micron cellulose acetate syringe filter (Waters, Milford, MA 01757, USA) into 2-mL amber vials for the HPLC analysis. HPLC standards (catechin hydrate, CGA, C3G, GA, quercetin, RH, and TFA) were individually prepared in 1 mg/mL concentrations by dispersing in HPLC-grade methanol. The standards were filtered and stored as described above.

I. Chromatographic Separation and Quantification of Selected Phenolics

Chromatographic analysis was conducted as reported by Manaois et al. (2020) using a Waters Alliance e2695 HPLC module (Milford, MA, USA) equipped with a Waters 2998 photodiode array detector (Milford, MA, USA) and a BRISA LC^2 C18 column (250 mm x 4.6 mm x 0.46 µm) (Teknokroma, Barcelona, Spain) and EMPOWER 3 PDA Software (Waters, Milford, MA 01757, USA) for data acquisition and processing. Quantification was based on the wavelength of maximum absorbance of each compound: CGA and GA, quercetin, RH, and TFA, 320 nm; (+)-catechin hydrate, 280 nm; and C3G, 530 nm.

J. *In vitro* Antibacterial Activity Determination

1. Preparation of Crude Extract for Antibacterial Assay

Stabilized rice bran (100g) was soaked in 500 mL of each of two solvents to evaluate the efficiency of extracting the potential antibacterial components in the bran: 80% ethanol and 80% methanol overnight. The mixtures were passed through a sterile qualitative filter paper and the solvents were removed using a rotary vacuum evaporator (Eyela N-1, Tokyo Rikakikai) set at 60ºC. The crude extracts were placed in sterile centrifuge tubes and then frozen overnight. The frozen extracts were subsequently lyophilized (Scanvac Coolsafe, Biobase), powdered, weighed, and then stored at -20ºC until use. Test solutions (0.1, 0.5 and 0.7 g/mL) were prepared by dispersing the powdered lyophilized crude extracts in an appropriate amount of distilled water.

2. Agar Well Diffusion Assay

The agar well diffusion assay of Widsten et al. (2014) was adopted. A loopful of each of *S. aureus* and *C. perfringens* was inoculated into 10 mL of sterile Mueller Hinton Broth and incubated overnight at 37ºC. The mixture was centrifuged (5 min, 4,000 rpm) to obtain bacterial pellets, which were then washed twice with phosphate-buffered saline solution and re-suspended in 1% salt peptone water (10 mL). Bacterial dilutions with a final concentration between 1 x 10^5 to 1 x 10^6 CFU/mL were pour-plated onto Mueller-Hinton agar. Three wells were made into the agar using a sterile 6-mm cork borer and 35 µL of rice bran extracts (0.1, 0.5, and 0.7 g/mL) were added into the wells. The inoculated medium was incubated in 24 hr at 37ºC and the length of zones of inhibition was measured. The antibacterial index of rice bran extract was calculated according to Villaseñor et al. (2004) as:

$$Antibacterial\ index = \frac{diameter\ of\ clear\ zone - diameter\ of\ well}{diameter\ of\ well}$$

For the negative control, 6-mm Whatman filter paper discs were soaked in each solvent (80% ethanol and 80% methanol) and sterile distilled water; air-dried for about 15 min; and oven-dried at 40ºC for 30 min. A disc without any extract was also included in each plate as the negative control. Commercial antimicrobial discs (10 µg each of Ampicillin, Amoxicillin, and Gentamycin) from Oxoid Limited (Hampshire, United Kingdom) were used as positive controls.

K. Statistical Analysis

Values are expressed as means ± standard deviation of triplicate measurements unless otherwise stated. Analysis of variance of the data on DPPH antioxidant activity, phenolic content, TFC, TAC, and subsequent multiple comparisons by Tukey's studentized range test were carried out using IBM SPSS version 20. The level of significance used in all tests was $p<0.05$.

RESULTS AND DISCUSSION

A. Total Phenolic Content (TPC)

The primary phytochemicals in plants, phenolic compounds, are also mainly responsible for their antioxidant capacities (Takebayashi et al. 2013; Kameya et al. 2014). They exist in either free form as soluble conjugates or in the insoluble bound form within cell wall polysaccharides and lignins (Zhou et al. 2004; Aguilar-Garcia et al. 2007; Vitaglione et al. 2008). As reported by Goufo and Trindade (2014), bran is the richest source of phenolic compounds in rice. Table 2 summarizes the results of the analysis of the phenolic content of the six rice bran samples.

Table 2: Free, bound, and total phenolic content of bran samples.

Rice Variety	Pericarp Color	Phenolic Content (mg GAE/g sample)[1]		
		Free	Bound	Total
NSIC Rc 222	Brown	15.12 ± 0.70^{e}	1.42 ± 0.20^{d}	16.54 ± 0.9^{e}
Minaangan	Red	56.83 ± 0.82^{c}	96.70 ± 9.40^{b}	153.53 ± 9.7^{b}
Kamanga	Red	121.01 ± 5.84^{a}	34.43 ± 1.60^{c}	155.44 ± 6.9^{b}
Inipot Ibon	Black	60.73 ± 0.51^{c}	42.90 ± 3.00^{c}	103.64 ± 2.5^{c}
Ingopon	Black	46.27 ± 3.38^{d}	42.79 ± 3.00^{c}	89.07 ± 2.2^{d}
Ominio	Black	67.75 ± 1.70^{b}	125.60 ± 7.70^{a}	193.35 ± 9.1^{a}

Means ± SD (n=3). Mean values with the same letter within the same column are not significantly different (p≤0.05).

[1]GAE = Gallic Acid Equivalents

The free phenolic content varied from 15.12 to 121.01 mg GAE/g, contributing 35.0-91.4% to the TPC of the samples (Table 2). The crude extracts from the bran of the red rice variety Kamanga recorded the highest free phenolic content (121.01 mg GAE/g), followed by the black rice varieties Ominio and Inipot Ibon. The bound phenolic fraction ranged from 1.42 to 125.60 mg GAE/g, which contributes 8.6 to 65.0% to the TPC. Meanwhile, Ominio bran crude extracts had the highest bound phenolic content (125.60 mg GAE/g). According to Laokuldilok et al. (2011), black rices tend to have higher levels of gallic, hydroxybenzoic, and protocatechuic acids in bound form. The TPC of the rice bran extracts ranged from 16.54 to 193.35 mg GAE/g. The free, bound, and TPC in the bran of pigmented rice varieties were significantly higher than that of the non-pigmented NSIC Rc 222, which agrees with the report of Goufo and Trindade (2014) and Friedman (2013).

B. Total Anthocyanin Content (TAC)

Anthocyanins are compounds that contribute to the red and purple color of pigmented rices (Hu et al. 2003). The anthocyanin content of the bran samples ranged 0.08-17.81 mg C3G Eq/g (Table 3). Black-colored bran samples had at least 77% more anthocyanin than red-colored brans and 96% more than the non-pigmented NSIC Rc222 bran. The characteristic dark purple shade of the black rice bran could be mainly due to two anthocyanins, namely C3G and peonidin-3-*O*-glucoside (P3G) (Semmarath et al. 2022). In red rice, proanthocyanidins account for most of its antioxidant activity instead of anthocyanin (Surarit et al. 2015). Unlike anthocyanins, proanthocyanidins are colorless but give off red color when oxidized (Goufo and Trindade 2014).

Table 3: Total anthocyanin content (TAC) of the bran samples.

Rice Variety	TAC (mg C3G Eq/g)[1]
NSIC Rc 222	0.08 ± 0.05^{e}
Minaangan	0.38 ± 0.23^{d}
Kamanga	0.50 ± 0.09^{d}
Inipot Ibon	5.60 ± 1.27^{b}
Ingopon	2.20 ± 0.89^{c}
Ominio	17.81 ± 2.71^{a}

Means ± SD (n=3). Mean values with the same letter are not significantly different (p≤0.05).

[1]C3G = Cyanidin-3-glucoside

C. Total Flavonoid Content (TFC)

Pigmented rice samples were observed to have higher levels of flavonoids than NSIC Rc 222 bran (Table 4). The TFC of bran from pigmented rice varieties varied from 42.75 to 90.14 mg RHE/g, which were significantly higher than that of NSIC Rc 222 bran. Crude extracts from the bran of Kamanga exhibited the highest TFC (90.14 ± 2.10mg RHE/g), followed by those of Ingopon and Inipot Ibon. Flavonoids have been shown to possess anti-inflammatory, antimicrobial, antiallergenic, and antioxidant activity. Some of the flavonoids that were observed to possess antibacterial activity include apigenin, luteolin, quercetin, and kaempferol and its derivatives (Cushnie and Lamb 2005). Goufo and Trindade (2014) reported that rice bran contains high amounts of the said compounds, along with tricin, isorhamnetin, and myricetin, suggesting that rice bran can be a potential resource of natural antibacterial agents.

Table 4: Total flavonoid content (TFC) of the bran samples.

Rice Variety	TFC (mg RHE/g)[1]
NSIC Rc 222	7.61 ± 0.58^{f}
Minaangan	49.28 ± 0.88^{d}
Kamanga	90.14 ± 2.10^{a}
Inipot Ibon	68.83 ± 1.35^{c}
Ingopon	73.76 ± 2.35^{b}
Ominio	42.75 ± 2.87^{e}

Means ± SD (n=3). Mean values with the same letter are not significantly different (p≤0.05).

[1]RHE = Rutin Hydrate Equivalent

D. DPPH Radical Scavenging Activity

Phenolic compounds have been reported as major contributors to the high radical scavenging activities in plants (Takebayashi et al. 2013; Kameya et al. 2014). The DPPH assay is one of the most widely used methods to measure the antioxidant capacity of various samples. It relies on the loss of the purple color of the solution via the reduction of the radical DPPH with the antioxidant as the hydrogen atom donor (Baang et al. 2015). Table 5 shows the DPPH antioxidant capacities of the rice bran extracts.

The DPPH values ranged from 13.34 to 368.23 µmol TE/g sample (Table 5). Similar to the results of the TPC, TAC, and TFC, crude extracts of brans of pigmented rice samples had higher DPPH radical scavenging activities than that of the non-pigmented NSIC Rc 222. This can be attributed to the high levels of phytochemicals found in pigmented rice. Kamanga crude extracts showed the highest antioxidant capacity (368.23 ± 9.2 µmol TE/g sample), followed by Ingopon, Inipot Ibon, and Minaangan.

Table 5: 2,2-diphenylpicrydrazyl (DPPH) radical scavenging activity of the rice bran extracts.

Rice Variety	DPPH (μmol TE/g sample)[1]
NSIC Rc 222	13.34 ± 0.12^{e}
Minaangan	187.35 ± 4.78^{c}
Kamanga	368.23 ± 9.20^{a}
Inipot Ibon	204.78 ± 3.03^{c}
Ingopon	250.38 ± 15.41^{b}
Ominio	150.86 ± 3.07^{d}

Means ± SD (n=3). Mean values with the same letter within the same column are not significantly different (p≤0.05)

[1]TE = Trolox Equivalent

E. Quantification of Selected Phenolic Compounds

The levels of individual phenolic compounds as measured using the HPLC are presented in Table 6. Most of the phenolic compounds were not detected, except for C3G and TFA. In the study of Faid (2015), CGA, quercetin, and GA were detected in brown rice bran. In this study, however, none of these compounds were present in the samples, which may be due to the effect of heat during the stabilization process (Tang et al. 2015).

Table 6: Levels of secondary metabolites in the rice bran extracts.

Rice Variety	Content (mg/g sample)[1,2]	
	C3G	TFA
NSIC Rc 222	ND	ND
Minaangan	ND	0.03 ± 0^{b}
Kamanga	ND	0.03 ± 0^{b}
Inipot Ibon	0.61 ± 0.03^{b}	0.03 ± 0^{b}
Ingopon	0.36 ± 0.01^{c}	0.04 ± 0^{a}
Ominio	4.07 ± 0.24^{a}	0.03 ± 0^{b}

[1]C3G = cyanidin-3-glucoside; TFA = trans-ferulic acid; catechin hydrate, chlorogenic acid, gallic acid, quercetin, and rutin hydrate were not detected in all samples.

[2]Means ± SD (n=3). Mean values with the same letter within the same column are not significantly different at p≤0.05; ND = Not Detected

Only black bran samples had high detectable values of C3G (Table 6). Jang and Xu (2009) reported that C3G was the dominant anthocyanin of black rice, comprising 90% of total anthocyanins. This was followed by cyanidin-3-galactoside and peonidin-3-glucoside. However, they reported higher levels, which were approximately 26,400 mg/kg (26.4mg/g) from the inner bran fraction and 3,200 mg/kg (3.2 mg/g) from the outer bran fraction (Jang and Xu, 2009). Another study by Min et al. (2014) puts the C3G content of black rice at 13.12 mg/g. These values were far above the levels measured in this study. Similar to the findings on the other phytochemical compounds, anthocyanins in rice are heat-labile (Bhawamai et al. 2016; Yamuangmorn et al. 2018) and the stabilization process, which involved steam heating, could have caused the transformation or breakdown of anthocyanin. Stabilization was performed to prolong the shelf-life of the bran by inactivating the enzyme lipase (Yilmaz Tuncel and Yilmaz Korkmaz 2021). Lipase can cause hydrolytic rancidity and other chemical changes in the bran, which might also affect the concentrations of bioactive compounds and the antioxidant capacity of the bran samples. TFA was not detected in NSIC Rc 222 (Table 6). It was present at 0.03 mg/g in the rest of the sample crude extracts, except in Ingopon, which recorded the highest value at 40 mg/100 g.

F. Antibacterial Activity of Rice Bran Extracts

S. aureus and *C. perfringens* are two of the most common pathogens that cause foodborne disease outbreaks in different parts of the world, such as the United States (Bennett et al. 2013) and Latin America (CSPI 2005, as cited by IAASTD 2009). In the Philippines, *Staphylococcal* enterotoxin-associated foodborne poisoning was among the most common during the period 1995-2018 (Azanza 2006; Azanza et al. 2019). The antibacterial properties of the crude rice bran extracts against *S. aureus* and *C. perfringens* are shown in Tables 7 and 8, respectively. Ethanolic extracts generally had higher inhibitory properties than methanolic extracts, suggesting the higher efficiency of ethanol as the solvent for antibacterial analysis. Ethanolic extract of Inipot Ibon at its highest concentration (0.7 g/mL) had the highest zones of inhibition among all samples for both *S. aureus* (20.5 mm) (Table 7) and *C. perfringens* (17.0 mm) (Table 8). Their antibacterial indices were 2.4 (Table 7) and 1.8 (Table 8), respectively. Kamanga also generally exhibited higher values than the other samples, specifically its methanolic extracts. These strong inhibitory properties were similarly displayed as those of the positive controls Ampicillin, Amoxicillin, and Gentamycin. It must be noted that the crude bran extracts were used in determining the antibacterial properties. Comparison of activities of plant crude extracts with positive controls is widely used in scientific literature (Arullappan et al. 2009; Njateng et al. 2017; Bereksi et al. 2018; Mohammed et al. 2020).

Table 7: Zone of inhibition and antimicrobial index of stabilized rice bran crude extracts against *S. aureus* using agar well diffusion assay (*n*=2).

Sample	Solvent	Concentration	Zone of inhibition (mm)	Antibacterial index
Rice bran extract		g/mL		
NSIC Rc222	Ethanol	0.1	6.0 ± 0.0	0.0
		0.5	6.0 ± 0.0	0.0
		0.7	6.0 ± 0.0	0.0
	Methanol	0.1	6.0 ± 0.0	0.0
		0.5	6.0 ± 0.0	0.0
		0.7	6.0 ± 0.0	0.0
Minaangan	Ethanol	0.1	6.0 ± 0.0	0.0
		0.5	10.0 ± 2.8	0.7
		0.7	12.5 ± 0.7	1.1
	Methanol	0.1	6.5 ± 0.7	0.1
		0.5	8.0 ± 0.0	0.3
		0.7	10.5 ± 0.7	0.8
Kamanga	Ethanol	0.1	10.0 ± 2.8	0.7
		0.5	10.5 ± 2.1	0.8

		0.7	16.0 ± 2.8	1.7
	Methanol	0.1	9.0 ± 2.8	0.5
		0.5	12.5 ± 0.7	1.1
		0.7	13.5 ± 0.7	1.3
Inipot Ibon	Ethanol	0.1	9.5 ± 0.7	0.6
		0.5	15.0 ± 0.0	1.5
		0.7	20.5 ± 2.1	2.4
	Methanol	0.1	8.5 ± 0.7	0.4
		0.5	14.0 ± 0.0	1.3
		0.7	15.0 ± 1.4	1.5
Ingopon	Ethanol	0.1	7.0 ± 1.4	0.2
		0.5	8.0 ± 1.4	0.3
		0.7	8.0 ± 1.4	0.3
	Methanol	0.1	8.0 ± 0.0	0.3
		0.5	10.5 ± 0.7	0.8
		0.7	11.5 ± 2.1	0.9
Ominio	Ethanol	0.1	6.0 ± 0.0	0.0
		0.5	6.0 ± 0.0	0.0
		0.7	6.0 ± 0.0	0.0
	Methanol	0.1	6.0 ± 0.0	0.0
		0.5	6.0 ± 0.0	0.0
		0.7	6.0 ± 0.0	0.0
Antibiotic disc, (+) Control		µg		
Ampicillin		10	14.5 ± 0.7	1.4
Amoxicillin		10	14.5 ± 0.7	1.4
Gentamycin		10	10.0 ± 1.4	0.7
Solvent disc, (-) Control		%		
	Ethanol	80	6.0 ± 0.0	0.0
	Methanol	80	6.0 ± 0.0	0.0
	Distilled water	n/a	6.0 ± 0.0	0.0
Blank disc, (-) Control		n/a	6.0 ± 0.0	0.0

n/a = not applicable

Table 8: Zone of inhibition and antimicrobial index of stabilized rice bran crude extracts against *C. perfringens* using agar well diffusion assay (*n*=2).

Sample	Solvent	Concentration	Zone of inhibition (mm)	Antibacterial index
Rice bran extract		g/mL		
NSIC Rc 222	Ethanol	0.1	6.0 ± 0.0	0.0
		0.5	6.0 ± 0.0	0.0
		0.7	6.0 ± 0.0	0.0
	Methanol	0.1	6.0 ± 0.0	0.0
		0.5	6.0 ± 0.0	0.0
		0.7	6.0 ± 0.0	0.0
Minaangan	Ethanol	0.1	6.0 ± 0.0	0.0
		0.5	7.0 ± 0.0	0.2
		0.7	9.0 ± 1.4	0.5
	Methanol	0.1	6.0 ± 0.0	0.0
		0.5	6.0 ± 0.0	0.0
		0.7	9.5 ± 0.7	0.6

Kamanga	Ethanol	0.1	9.0 ± 1.4	0.5
		0.5	12.5 ± 0.7	1.1
		0.7	13.0 ± 1.4	1.2
	Methanol	0.1	6.0 ± 0.0	0.0
		0.5	13.0 ± 1.4	1.2
		0.7	17.0 ± 1.4	1.8
Inipot Ibon	Ethanol	0.1	8.5 ± 2.1	0.4
		0.5	16.5 ± 0.7	1.8
		0.7	17.0 ± 0.0	1.8
	Methanol	0.1	6.0 ± 0.0	0.0
		0.5	12.0 ± 2.8	1.0
		0.7	13.5 ± 0.7	1.3
Ingopon	Ethanol	0.1	6.0 ± 0.0	0.0
		0.5	6.0 ± 0.0	0.0
		0.7	7.0 ± 1.4	0.2
	Methanol	0.1	6.0 ± 0.0	0.0
		0.5	6.0 ± 0.0	0.0
		0.7	6.0 ± 0.0	0.0
Ominio	Ethanol	0.1	6.0 ± 0.0	0.0
		0.5	6.0 ± 0.0	0.0
		0.7	6.0 ± 0.0	0.0
	Methanol	0.1	6.0 ± 0.0	0.0
		0.5	6.0 ± 0.0	0.0
		0.7	6.0 ± 0.0	0.0
Antibiotic disc, (+) Control		µg		
Ampicillin		10	7.0 ± 0.0	0.2
Amoxicillin		10	9.0 ± 0.0	0.5
Gentamycin		10	10.5 ± 0.7	0.8
Solvent disc, (-) Control		%		
	Ethanol	80	6.0 ± 0.0	0.0
	Methanol	80	6.0 ± 0.0	0.0
	Distilled water	n/a	6.0 ± 0.0	0.0
Blank disc, (-) Control		n/a	6.0 ± 0.0	0.0

n/a = not applicable

Ominio did not have an inhibitory action for both gram-positive bacteria despite its high anthocyanin content (Tables 7 and 8). In the previous report of Puupponen-Pimia et al. (2001), C3G, an anthocyanin dominant in rice, has been shown to only inhibit the growth of *E. coli*, a gram-negative bacterium. However, it was unable to inhibit bacteria from gram-positive species, especially *Enterococcus faecalis* and those belonging to the genus *Lactobacillus* (Puupponen-Pimia et al. 2001). Georgiev et al. (2012) reported that ferulic acid in 96% ethanol inhibited *S. aureus* bacteria with a minimum inhibitory concentration of 125 µg/mL. In the crude extracts used in this study, however, the concentration of ferulic acid was only about 28 µg/mL even for the variety with the highest TFA level, Ingopon. A rice bran extract with a concentration higher than 28 µg/mL could not be quantitatively used because of the syrupy nature of the extract. It is also important to note that Inipot Ibon did not have the highest levels for all phytochemical assays performed: TAC, TFC, TPC, and DPPH. Other bioactive compounds present in the crude extract of the bran of this variety that were not measured may have accounted for the observed antibacterial activities.

The study has other potential limitations. Variations on the levels of bioactive compounds based on environmental factors and cultural management were not evaluated. All varieties were sourced from different ecosystems, i.e. locations where they are conventionally grown to ensure the expression of maximum levels of bioactive compounds. Also, traditional varieties may be grown organically. Nevertheless, this study provided a general information about the bran extracts of local pigmented varieties: that they generally exhibited inhibitory action against the two gram-positive foodborne pathogens and that these varieties could be further explored for use as natural antimicrobial agents to prevent food spoilage or contamination or even in pharmacological applications. A comprehensive analysis of the antibacterial properties of the bioactive components of these specific varieties and the influence of environmental and cultural management factors on their levels is worth investigating.

CONCLUSION

Pigmented rice bran contained significantly higher levels of bioactive compounds, such as free, bound, and total phenolics, flavonoids, and anthocyanins, than the bran of non-pigmented variety. Kamanga had the highest levels of free phenolics, TFC, and DPPH antioxidant capacity, while Ominio had the highest bound phenolic content and TAC. The anthocyanin C3G was detected in black-colored brans only, with the highest levels in Ominio. TFA was detected in all pigmented samples, with Ingopon containing the highest amount (0.04 mg/g). Except for Ominio, extracts of pigmented rice bran showed inhibitory properties against *S. aureus* and *C. perfringens,* with ethanolic extracts of Inipot Ibon (0.7 g/mL) displaying the highest potency against both *S. aureus* and *C. perfringens.* In conclusion, the brans of pigmented rice can be potential sources of phytochemicals with antioxidant capacities and serve as natural antibacterial agents for the food and/or pharmaceutical industries.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

RVM conceived and designed the experiments, performed the experiments on *in vitro* antibacterial activity and phenolic analysis, interpreted the data, and wrote the manuscript. JEIZ performed all the experiments, analyzed and interpreted the data, and wrote the manuscript. AVM designed and performed the experiments on *in vitro* antibacterial activity, analyzed and interpreted the data, and wrote the manuscript. JCAC performed the experiments, analyzed the data, and wrote the manuscript. All authors approved the final version of the manuscript.

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