

Antibacterial and Antioxidant Activities of Red Rice/Brown Rice, Si Boo Gan Tang Rice and its Rice Bran Extracts

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Abstract

This study aimed to assess the biological properties of Si Boo Gan Tang Rice and bran extract. Antibacterial activity was evaluated using the agar well diffusion method, revealing inhibitory effects exclusively on gram-positive bacteria by both ethanolic Si Boo Gan Tang Rice (RE) and bran (RBE) extract. The inhibition zone of RE to methicillin-resistant *Staphylococcus aureus* (MRSA142), *Staphylococcus aureus* TISTR517, *Bacillus cereus* ATCC 11778, and *Micrococcus luteus* TISTR 884 was 2.07 ± 0.06 , 1.94 ± 0.07 , 1.95 ± 0.03 , and 2.04 ± 0.03 cm, respectively. For RBE, the inhibition zones to methicillin-resistant *S. aureus* (MRSA142), *Staphylococcus aureus* TISTR517, *B. cereus* ATCC 11778, and *M. luteus* TISTR 884 were 2.07 ± 0.06 , 2.00 ± 0.04 , 1.67 ± 0.04 , and 2.42 ± 0.56 cm, respectively. Scanning electron microscope (SEM) analysis indicated changes in the morphology of *Staphylococcus aureus* TISTR517 and Methicillin-resistant *Staphylococcus aureus* (MRSA142) cells treated with the RE and RBE, characterized by cell swelling and the formation of a fibrous network around them, contrasting with the control group. Antioxidant activity was assessed via DPPH, ABTS, and FRAP assay. The RBE displayed significant antioxidant potential, with IC_{50} values of 0.78 and 0.69 mg/mL in the DPPH and ABTS assays, respectively. Conversely, the aqueous rice bran (RBW) extract had IC_{50} values of 3.83 and 0.90 mg/mL in the same assays. For the RE, IC_{50} values were 1.11 mg/mL (DPPH) and 0.78 mg/mL (ABTS), while the aqueous rice (RW) extract had IC_{50} values of 4.45 mg/mL (DPPH) and 1.75 mg/mL (ABTS). In the FRAP assay, all the extracts RW, RBW, RE and RBE demonstrated ferric level reductions of 37.82, 36.85, 56.24, and 81.45 $\mu\text{mol/g}$ extract, respectively. Phenolic content in the extracts ranged from 34.82 to 154.31 mg GAE/g extract, while flavonoid concentrations varied between 2.17 and 8.78 mg QE/g extract.

Keywords: Biological properties, Si Boo Gan Tang Rice Extract, Si Boo Gan Tang Rice bran Extract, Antibacterial activity, Scanning electron microscope, Antioxidant activity, Phenolic compounds, Flavonoid compound

Introduction

Nowadays, most people are turning to their health and popular to consume nutritious food. Therefore, foods with biological properties are widely popular, such as vegetables, fruits, and unpolished rice. Colored rice, including purple rice, red rice, or brown rice, is popular because it contains many nutrients. In Thailand, rice (*Oryza sativa* L.) stands as a crucial pillar of both sustenance and economic prosperity. Rice grains are

comprised of 2 key parts: The husk, encompassing a sizable husk, a small husk, a tail, a seed pole, and a seed petal; and the seed, often referred to as brown rice, retaining its outer husk yet lacking the glossy appearance of polished rice. The edible portion, known as the pericarp, imparts brown coloration and frequently contains anthocyanins that its mechanism is to interact with the bacterial membrane, resulting in the inhibition

of the growth of bacterial cells. Rich in protein, cellulose, and hemicellulose, the cell wall forms a fibrous structure. Surrounding the endosperm, the seed coat, primarily lipid-based, encases the aleurone layer, abundant in proteins, fats, cellulose, and hemicellulose. The endosperm, primarily starch (amylose and amylopectin), is nestled within, while the embryo, or germ, harbors juvenile plants, immature roots, and nascent plant membranes rich in protein and fat [1].

Rice bran, the outer layer of rice grains removed during polishing, serves various purposes, including animal feed production and extraction of rice bran oil, adding economic value. Comprising 12.45 % fat, 10.90 % protein, 45.31 % carbohydrates, and 13.51 % fiber, Rice bran is a nutrient-dense food, abundant in essential nutrients [2,3]. The high concentration of bioactive chemicals in this substance, such as phenolic acids, flavonoids, vitamin E, and gamma oryzanol, is especially remarkable. The phenolic and flavonoid compounds are the main parameters that exhibit antioxidant activity. These levels exceed those reported in numerous fruits, vegetables, nuts, and dried fruits [4]. Therefore, extracting bioactive components from rice bran is essential for nutritional supplementation and medical applications, enhancing its overall value. Si Boo Gan Tang rice, commonly cultivated in Thailand's southern border regions, particularly in Pattani, Yala, and Narathiwat provinces, is known for its nutritional richness. Classified as a type of red rice or brown rice, it is consumed either as brown rice or coarse rice due to its abundant nutritional content, making it a valuable natural food for promoting health and well-being. Si Boo Gan Tang rice exhibits notable chemical characteristics, boasting a high amylose content of approximately 28.51 %. Moreover, it offers significant nutritional value, providing 349.30 kilocalories of energy, 7.31 g of protein, 2.14 g of fat, 75.20 g of carbohydrates, 3.45 g of dietary fiber, and 22.85 milligrams of GABA (gamma amino butyric acid) per 100 g [5].

Despite its nutritional significance, there is limited research on the biological properties of Si Boo Gan Tang rice extract compared to Hawm Gra Dang Ngah rice, another brown rice variety cultivated in Narathiwat province. Further investigation into the biological characteristics of Si Boo Gan Tang rice extract is warranted, especially considering its similarities with

Hawm Gra Dang Ngah rice. Therefore, the objectives of this study are to assess the antibacterial activity of Si Boo Gan Tang rice and rice bran extracts against various bacterial strains, evaluate the antioxidant activity of the extracts using DPPH, ABTS, and FRAP assays, and analyze the phenolic and flavonoid content of the extracts to determine their potential health benefits.

Materials and methods

Preparation of si boo gan tang rice and rice bran extracts

Rice ethanol extract (RE)

A total of 80 g of ground rice was combined with 800 mL of ethanol that had a concentration of 50 %. The mixture was then stirred for a duration of 2 h. The solution was subjected to filtration using cheesecloth and No. 1 filter paper, followed by ethanol removal using rotary evaporation and leave it at room temperature until the ethanol has completely evaporated. The brown crude extract was kept at a temperature of -20°C .

Rice aqueous extract (RW)

80 g of ground rice was mixed with 320 mL of water and left to stand for 2 h. The mixture was filtered using cheesecloth and No. 1 filter paper and frozen at -20°C for 24 h, finally freeze-drying. The resulting light brown powdered extract was stored at -20°C .

Rice bran ethanol extract (RBE)

800 mL of 50 % ethanol was added to 80 g of rice bran and stirred. The mixture was then allowed to sit for 2 h. Subsequently, the mixture was filtered using cheesecloth and No. 1 filter paper. After filtering, the resulting mixture was evaporated, ensuring complete evaporation of ethanol using a Rotary Evaporator. The white to light yellow crude extract obtained was stored at -20°C .

Rice bran aqueous extract (RBW)

80 g of rice bran was mixed with 320 mL of water and left for 2 h. The solution was subjected to filtration using cheesecloth and No. 1 filter paper, followed by freezing at a temperature of -20°C for a duration of 24 h. Subsequently, the frozen solution was subjected to

freeze-drying. The resulting white to light yellow powdered extract was stored at -20 °C.

Antibacterial properties of rice and rice bran extracts

Seven different bacterial strains were used in the study, including: 1) *Pseudomonas aeruginosa* TISTR1467, 2) *Escherichia coli* ESBL182, 3) *Staphylococcus aureus* TISTR517, 4) *Micrococcus luteus* TISTR884, 5) *Salmonella typhimurium* TISTR292, 6) Methicillin-resistant *Staphylococcus aureus* (MRSA142), and 7) *Bacillus cereus* ATCC 11778. These strains were cultured in Mueller Hinton Agar (MHA) for 18 h at 37 °C, then transferred to Mueller Hinton Broth (MHB) for 4 h. Their concentration was adjusted to 0.5 McFarland (1.5×10^8 CFU/mL) using 0.85 % NaCl. The antibacterial activity was assessed using the agar well diffusion method as outlined by On-Anong Somsap in 2019. Every bacterial strain was applied onto MHA plates, and wells with a diameter of 0.6 cm were created using a sterile tip. Concentrations of 9.375, 18.75, 37.5, 75, 150, and 300 mg/mL of Rice Aqueous Extract (RW), Rice Ethanol Extract (RE), Rice Bran Aqueous Extract (RBW), and Rice Bran Ethanol Extract (RBE) were applied to the wells. The plates were kept at room temperature for 5 h and then at 37 °C for 18 h. The widths of the inhibitory zones were measured in cm using a vernier caliper. The positive control of the experiment was conducted in triplicate using Gentamicin at a concentration of 10 µg/mL.

Scanning electron microscope (SEM) analysis

Following the methodology of Samsap *et al.* [6], treated cells of indicator strains (RW, RE, RBW, and RBE) were prepared. The cells were scraped and washed twice with 0.15 M phosphate buffer (pH 7.2), then fixed with 2.5 % glutaraldehyde for 1 - 2 h. After 2 additional washes with distilled water and phosphate buffer, the cells were dehydrated using acetone concentrations from 5 to 100 %. Critical Point Drying was used to dry the cells, which were then mounted on stubs with carbon tape and paint, coated with gold using a sputter coater, and observed under SEM.

Analysis of antioxidant activity in rice and rice bran extracts

1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity

The researchers utilized a revised DPPH assay Samsap *et al.* [7] employing 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich) as the oxidizing agent. The rice and rice bran extracts (RW, RE, RBW, and RBE) were diluted to concentrations of 18.50, 9.38, 4.69, 2.34, and 1.17 mg/mL. Each 20 µl extract was combined with 180 µl of DPPH solution and allowed to incubate at room temperature for 30 min in the absence of light. The Bio-Rad microplate reader was used to measure the absorbance at a wavelength of 517 nm. Methanol was substituted for the extract in the control group, while solely methanol was utilized in the blank group. Ascorbic acid was used as a reference substance at concentrations ranging from 10 to 100 mg/mL. The quantification of antioxidant activity was expressed in mg/mL of ascorbic acid equivalent, using the following formula:

$$\% \text{ Radical scavenging} = [(A_0 - A_{30}) / A_0] \times 100 \quad (1)$$

The IC₅₀ was determined by plotting the percentage inhibition against the concentration of Si Boo Gan Tang Rice and Rice Bran extracts (RW, RE, RBW, and RBE) on a graph.

2,2'-azino-bis -3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay

A minor modification was made to the ABTS assay method described previously [7]. The ABTS solution, with a concentration of 7 mM, and the potassium persulfate solution, with a concentration of 2.45 mM, were mixed in a ratio of 1:2. The mixture was then left to incubate for a duration of 12 h. The concentrations of RW, RE, RBW, and RBE extracts were diluted to 9.38, 4.69, 2.34, and 1.17 mg/mL, respectively. Each 20 µl extract was combined with 180 µl of the working solution and left to incubate in darkness for a duration of 30 min. The measurement of absorbance was conducted at a wavelength of 734 nm using a Bio-Rad microplate reader. Ascorbic acid was used as a reference, and the antioxidant activity was measured in mg of ascorbic acid per g of fresh weight.

FRAP (ferric reducing antioxidant power) assay

From the study by Samsap *et al.* [7], the FRAP method was modified marginally. A FRAP solution was made by mixing 25 mL of 300 mM acetate buffer, 2.5 mL of 10 mM TPTZ solution, and 2.5 mL of 20 mM FeCl₃ solution in a ratio of 10:1:1. The concentrations of RW, RE, RBW, and RBE extracts were diluted to 18.50, 9.38, 4.69, 2.34, and 1.17 mg/mL, respectively. Each 20 µl extract was combined with 180 µl of the FRAP solution and then kept in darkness at room temperature for 15 min. The absorbance was quantified at a wavelength of 595 nm using a Bio-Rad microplate reader. Ascorbic acid served as the reference substance, while distilled water functioned as the control. The antioxidant activity was quantified and reported as micromoles per g of fresh weight.

Assessment of phytochemical content in rice and rice bran extracts

Total phenolic content

The quantification of the overall phenolic content was conducted by employing a modified Folin-Ciocalteu method, as described by [7]. A solution was prepared by dissolving 1 g of rice or rice bran in 5 mL of ethanol, followed by filtration. A total of 20 µl of RW, RE, RBW, and RBE extracts were combined with 100 µl of a 20 % Na₂CO₃ solution and 50 µl of Folin-Ciocalteu's phenol reagent. The mixture was then left to incubate for 1 h at room temperature in the absence of light. The optical density was quantified at a wavelength of 760 nm using a Bio-Rad microplate reader. Calibration was performed using Gallic acid standards ranging from 0.02 to 0.4 mg/mL. The total phenolic content was quantified as terms of gallic acid equivalent (mg of GAW/g extract).

Total flavonoid content

The total flavonoid concentration was determined using the Folin-Ciocalteu technique, with some modification based on a prior study conducted by [7]. A solution was made by combining 50 µl of a 5 % NaNO₂ solution with 20 µl of RW, RE, RBW, and RBE extracts. After a comprehensive mixing process, 50 µl of a 10 % solution of AlCl₃ was introduced into the reaction mixture, along with an additional 50 µl of a 1M solution of NaOH. Subsequently, the mixture was vigorously

agitated once again and allowed to incubate for a further 10 min at ambient temperature. Following optical density measurements were conducted at a precise wavelength of 510 nm using a Bio-Rad microplate reader. Calibration was performed using Quercetin standards with concentrations ranging from 1 to 10 mg/mL. The extract's total flavonoid concentration was measured as terms of quercetin equivalent (mg of QE/g extract).

Statistical analysis

All experimental results were presented as mean ± standard deviation (S.D.) using an analytical software program. Data analysis was conducted utilizing one-way ANOVA, with a significance level set at $p < 0.05$, to compare between the control group and the test group. The statistical analysis was performed using IBM SPSS Statistics Standard Version 29.0.1.0.

Results and discussion

Antibacterial efficacy of rice and rice bran extracts (RW, RE, RBW, and RBE)

The study examined the bactericidal activity of RW, RE, RBW, and RBE extracts. Results showed resistance of gram-negative bacteria to all extracts, as presented in **Table 1**, with only gram-positive bacteria being inhibited by the ethanolic extract of rice (RE) and rice bran (RBE). Similarly, an ethanolic extract from purple rice cultivated in northern Thailand, specifically Khao Gam Luem Phua, inhibited gastrointestinal pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella enteritidis*, *Escherichia coli*, and *Vibrio parahaemolyticus* [8]. Moreover, Sangyod rice bran extract, with 95 % ethanol at a dosage of 0.25 mg/mL, promoted the growth of *Lactocaseibacillus paracasei* by encouraging surface adherence [9]. Polysaccharides isolated from Gam Doi Saket rice bran using hexane inhibited *S. aureus* and *E. coli* [10]. Bains *et al.* [11], while rice husk extract inhibited the growth of *S. aureus* [12]. In addition, some research stated that colored rice contains substances, anthocyanins, that are extracted from various plants that exhibit antimicrobial properties. Anthocyanin was grouped in polyphenol, which interacts with bacterial membranes, resulting in bactericidal or bacteriostatic to bacterial cells [13-16]. From the experiment, it was found that the aqueous extract (RW and RBW) did not show antibacterial

activity because water is a lower polarity solvent than ethanol. Therefore, the effective extract will be in small quantities, and this extraction method does not use heat. As a result, all aqueous extracts may not contain enough active ingredients to inhibit bacteria. In this study we used rice extract (RW, RE) and rice bran extract (RBW, RBE) and found that extraction with ethanol showed high activity against gram-positive bacteria because it has a high value of flavonoids and phenolic compounds. Yuan *et al.* [17] reported that the mechanism of action of flavonoids is to gram-positive bacteria by acting at the main site (cell membrane) and damaging phospholipid bilayers or resulting in the respiratory chain or fuel synthesis of cells.

The effect of ethanolic extract of rice and rice bran on Gram-positive bacterial strains was investigated using scanning electron microscopy (SEM) to observe any changes in the morphology of the bacterial cells, such as cell swelling, cell rupture, cell wilting, or cell elongation. The study's findings, depicted in **Figure 1**, reveal the untreated *S. aureus* TISTR517's normal cell shape in panel A (1). When subjected to RBE, it was observed that the cell structure appeared normal in comparison to untreated cells. However, it was

discovered that a network of fiber was present around the cell clusters, as depicted in **Figure 1(A)** (2). Similarly, when *S. aureus* TISTR517 was treated with RE extract, a network of fiber resembling a mesh was observed surrounding the clusters of cells, as depicted in **Figure 1(A)** (3). However, the size of the *S. aureus* TISTR517 cells was found to be lower in comparison to the control group. In panel B, the untreated MRSA142 cell exhibited normal cells, as depicted in **Figure 1(B)** (1). Subsequently, the cell was treated with RBE, as illustrated in **Figure 1(B)** (2), and subsequently treated with RE, as shown in **Figure 1(B)** (3). The cells manifested signs of enlargement and swelling. In contrast to the control, which bore a uniform surface, the cell surface exhibited a coarse texture. This result revealed that *S. aureus* TISTR517 and MRSA142 cells were damaged bacterial cell walls by RE and RBE extracts. Similarly, Zheng *et al.* [18] reported that the extract of *Polygonum chinense* L. aqueous showed an antimicrobial effect by causing strong damage of the bacterial cell walls and extracellular solutes existed. Moreover, the autolysis of bacterial cells was demonstrated in treated cells.

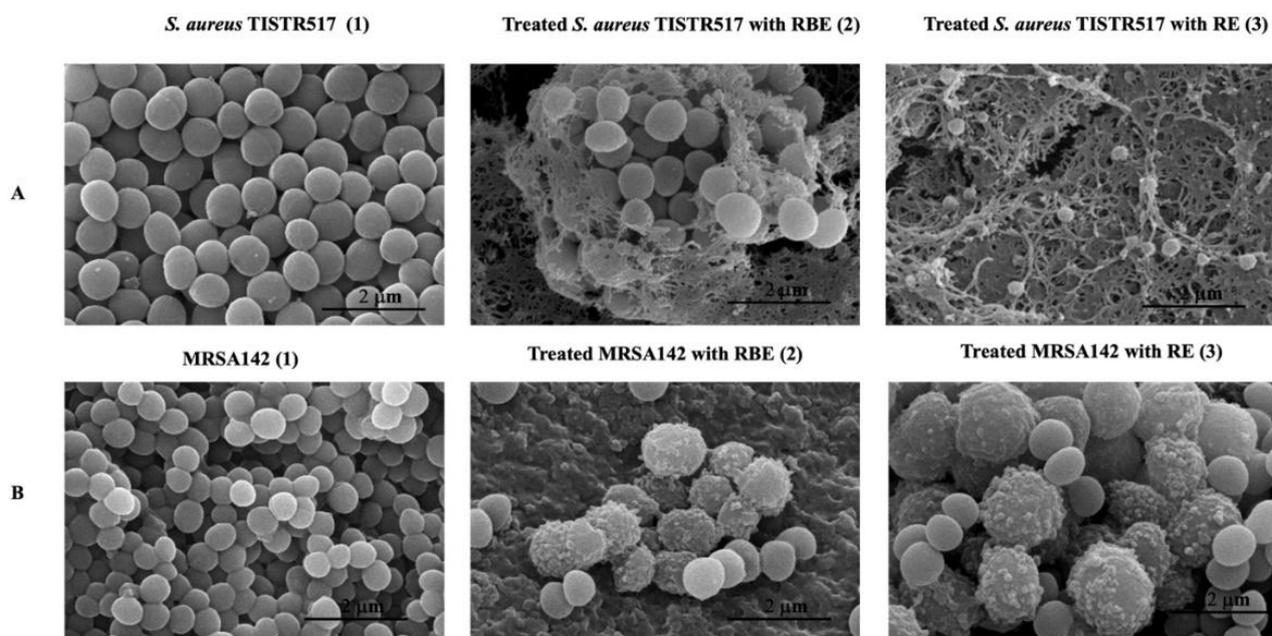


Figure 1 Scanning electron micrographs of untreated *S. aureus* TISTR517 and MRSA142 (A) and (B) and treated cells with RBE (2) and RE (3). Magnification of x 30000. (Upper panel, *S. aureus* TISTR517; Lower panel, MRSA142).

Table 1 Antibacterial activity and inhibition zones of RW, RE, RBW, and RBE extracts.

Extract (100 µl)	Inhibition zone (cm) (mean ± SE)						
	<i>Methicilin-resistant S. aureus (MRSA142)</i>	<i>S. aureus TISTR 517</i>	<i>B. cereus ATCC 11778</i>	<i>M. luteus TISTR 884</i>	<i>S. typhimurium TISTR292</i>	<i>E. coli ESBL 182</i>	<i>P. aeruginosa TISTR1467</i>
Gentamicin (10µg/mL)	-	1.65 ± 0.11	1.47 ± 0.03	1.53 ± 0.04	-	-	-
DMSO	-	-	-	-	-	-	-
Distilled water	-	-	-	-	-	-	-
RE (300 mg/mL)	2.07 ± 0.06	1.94 ± 0.07	1.95 ± 0.03	2.04 ± 0.03	-	-	-
RBE (300 mg/mL)	2.07 ± 0.06	2.00 ± 0.04	1.67 ± 0.04	2.42 ± 0.56	-	-	-
RW (300 mg/mL)	-	-	-	-	-	-	-
RBW (300 mg/mL)	-	-	-	-	-	-	-

Antioxidant activity of RW, RE, RBW and RBE by DPPH assay

The DPPH assay revealed IC₅₀ values for rice and rice bran extracts as follows: 1.11 mg/mL (RE), 4.54 mg/mL (RW), 0.78 mg/mL (RBE), and 3.83 mg/mL (RBW) (Figure 2). Furthermore, the peptide hydrolysate derived from Khao Dok Mali 105 rice bran, subjected to oil extraction, demonstrated the ability to scavenge free radicals (DPPH) with an IC₅₀ value of 1.15 mg/mL. This study illustrates that the peptide hydrolysate from Khao Dok Mali 105 rice bran could donate hydrogen atoms (H⁺) to the DPPH free radical [19]. Sangyod germinated brown rice extract soaked in pandanus leaf showed an EC₅₀ of 53.42 ± 3.56 µg/mL, consistent with the total phenolic content study, highlighting phenolic compounds radical scavenging properties [20]. The IC₅₀ values of leaf and flower extracts of garden balsam were determined to be 1.34 ± 0.15 and 0.99 ± 0.07 µg/mL, respectively [21]. The antioxidant activity of the aqueous extract derived from Luem Pua black glutinous rice bran is comparable to that of the aqueous extract obtained from Jasmine rice bran, with an equivalent of 73 ± 0.69 % [22]. Dok Mali 105 rice bran extract exhibits antioxidant activities, with the lowest EC₅₀ value recorded at 0.53 mg/mL [23]. In Iraq, a study conducted by Talib *et al.* [24] found that methanolic rice bran extract possesses antioxidant activities, with an IC₅₀ value of 114 µg/mL. The market

offers 5 varieties of colored rice: Red rice (Sang Yot rice and Mun poo rice), purple rice (Rice berry), purple rice (Hom Nil Rice), and black rice (Lum Pua Rice). It was discovered that water and ethanol (in a 50:50 ratio) were used to extract all 5 types of rice. Lum Pua rice extract exhibited the highest antioxidant activity when assessed using the DPPH method, with an IC₅₀ value measured at 0.034 ± 0.002 mg/mL [25]. Polysaccharides derived from Kam doi rice bran were extracted using hexane and ethanol. The hexane extraction resulted in the production of polysaccharides that exhibited antioxidant activity, as evidenced by an IC₅₀ value of 1.1 mg/mL. Similarly, the ethanol extraction method also produced polysaccharides that exhibited antioxidant activity, although the IC₅₀ value was slightly higher at 5.2 mg/mL [10]. The antioxidant activity of red rice extracts decreased by 54.23 - 49.78 % after being stored for 10, 15, and 30 days. However, red rice extract prepared using an emulsion formula maintained consistent antioxidant activity of 56.32 - 55.89 % throughout the storage period [11]. Chen *et al.* [26] reported that antioxidant activity of the polysaccharide extract depended on molecular weight and type of monosaccharides. The main parameter that showed antioxidant activity is phenolic compound [27]. The mechanism of polysaccharide to antioxidant activity is by scavenging free radicals. Monosaccharides donated an electron of the hydrogen group to a free radical that

converted into a more stable product [28]. Anthocyanin was classified as a group of phenol that act as antioxidant activity is by donated the hydrogen atom [29].

Antioxidant activity of RW, RE, RBW and RBE by ABTS assay

The ABTS radical scavenging experiment revealed that the extracts RW, RE, RBW, and RBE displayed IC_{50} values of 1.75, 0.78, 0.90, and 0.69 mg/mL, respectively, as detailed in **Figure 3**. Kaewpiboon *et al.* [20] reported that the germinated brown rice extract of Sangyod, soaked in pandanus leaf extract, achieved an EC_{50} value of $9.92 \pm 2.12 \mu\text{g/mL}$. This result aligns with the study's overall findings on phenolic content, highlighting the radical scavenging abilities of phenolic compounds. Rice bran extract obtained from a rice bran oil factory exhibited an antioxidant activity of 232.4 mg Trolox/g, determined by the ABTS technique [30]. Pattanandecha *et al.* [8] reported that ethanolic compounds derived from purple

rice grown in northern Thailand, specifically Gam Luem Phua rice (Khao Gam Luem Phua), exhibited free radical inhibitory activity at a value of 524.26 ± 4.63 vitamin C mg/100 g equivalent (VCEAC). The market offers 5 varieties of colored rice: Red rice (Sang Yot rice and Mun poo rice), purple rice (Riceberry), unpolished purple rice (Hom Nil Rice), and black rice (Lum Pua Rice). Water and ethanol (in a 50:50 ratio) were employed for extracting all 5 varieties of rice. The extract from Lum Pua rice exhibited the highest antioxidant activity when assessed using the ABTS method. The IC_{50} value for Lum Pua rice extract was reported to be 0.016 ± 0.001 mg/mL [25]. According to Surin *et al.* [10], polysaccharides obtained from Kam doi rice bran using hexane exhibited antioxidant activity with an IC_{50} value of 0.6 mg/mL, whereas ethanol extraction yielded an IC_{50} value of 1.8 mg/mL. Therefore, the antioxidant activity of all these extracts is expected to be due to anthocyanin in polysaccharide. Anthocyanin is a natural polyphenol compound that is soluble in high polar solvents [31].

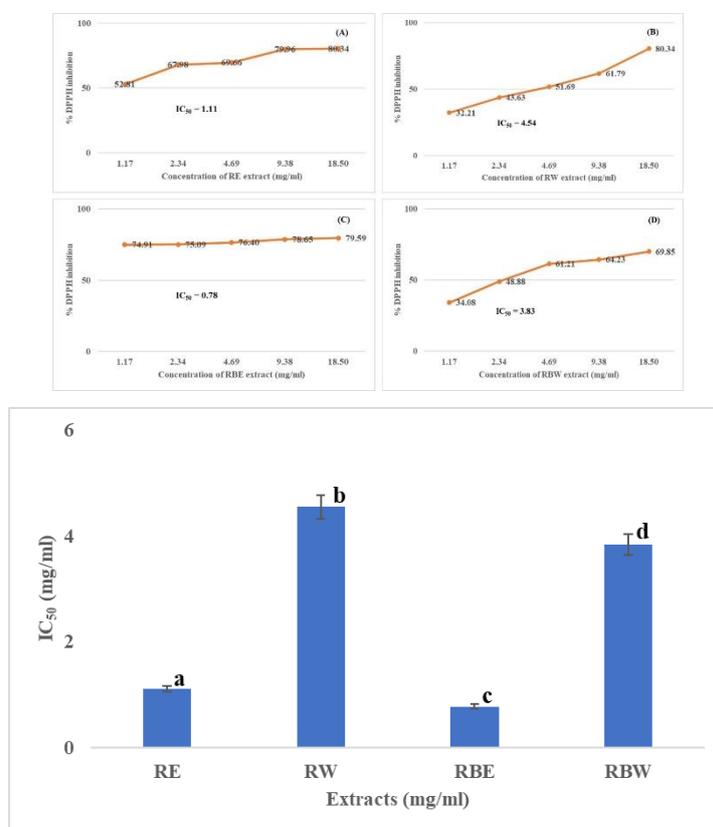


Figure 2 DPPH radical scavenging activity of Si Boo Gan Tang rice and rice bran extracts (RE, RW, RBE and RBW). The IC_{50} value are expressed as mg/mL. (RBE = Rice Bran Ethanol Extract, RBW = Rice Bran Aqueous Extract, RE = Rice Ethanol Extract and RW = Rice Aqueous Extract). Results are presented as mean \pm standard deviation ($n = 3$). Indicates significant difference at $p < 0.05$.

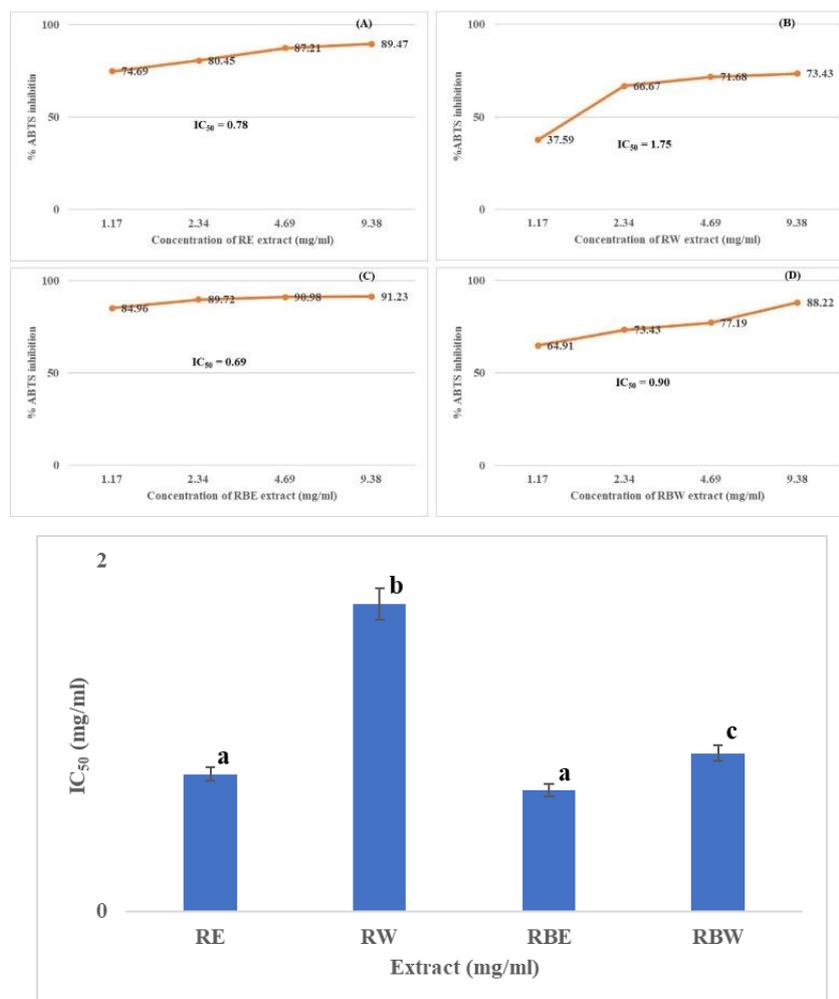


Figure 3 ABTS radical scavenging activity of Si Boo Gan Tang rice and rice bran extracts (RE, RW, RBE and RBW). The IC₅₀ value are expressed as mg/mL. (RBE = Rice Bran Ethanol Extract, RBW = Rice Bran Aqueous Extract, RE = Rice Ethanol Extract and RW = Rice Aqueous Extract). Results are presented as mean \pm standard deviation ($n = 3$). Indicates significant difference at $p < 0.05$.

Antioxidant activity of RW, RE, RBW and RBE by FRAP assay

The FRAP assay reveals that the polysaccharide from RW extract possesses a reducing power of $37.82 \pm 0.01 \mu\text{mol Fe}^{2+}/\text{g}$ extract, while the polysaccharide from RE extract exhibits a reducing power of $56.24 \pm 0.02 \mu\text{mol Fe}^{2+}/\text{g}$ extract. Additionally, the rice bran extracts RBW and RBE display reducing powers of $36.85 \pm 0.01 \mu\text{mol Fe}^{2+}/\text{g}$ extract and $81.45 \pm 0.01 \mu\text{mol Fe}^{2+}/\text{g}$ extract, respectively as shown in **Figure 4**. Moreover, the peptide hydrolysate derived from Dok Mali 105 rice bran, subjected to oil extraction, showcases the ability to reduce ferric acid (FRAP) to a level of $157.12 \pm 1.72 \mu\text{mol Fe}^{2+}/\text{g}$ extract. This suggests that the peptide hydrolysate from Dok Mali 105 rice bran possesses a structure capable of transferring electrons (e^-) to the

ferric ion (Fe^{3+} -TPTZ) [19]. The rice bran extract from Tubtim Chumphae demonstrates antioxidant activity, determined by the FRAP technique, with an equivalent value of 50.12 mg Trolox per gram [30]. Polysaccharides extracted from Kam doi rice bran using hexane exhibit an antioxidant impact of $28.8 \mu\text{mol Fe}^{2+}/\text{g}$ extract, while extraction with ethanol results in an antioxidant effect of $194.4 \mu\text{mol Fe}^{2+}/\text{g}$ extract [10]. The FRAP mechanism is based on electron transfer rather than hydrogen atom transfer by using the ability of pH to reduce Fe^{3+} to Fe^{2+} . Colored rice contents of phenolic, flavonoid, and anthocyanin that are effective to antioxidant activity. This substance contains in polysaccharide which soluble in more stable and high polar solvent such as ethanol exhibited antioxidant activity.

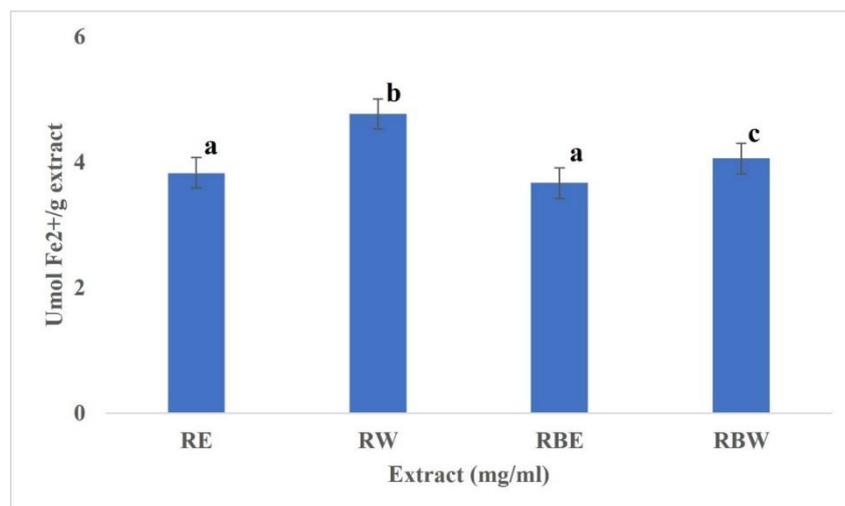


Figure 4 FRAP radical scavenging activity of Si Boo Gan Tang rice and rice bran extracts (RE, RW, RBE and RBW). The reducing power value are expressed as $\mu\text{mol Fe}^{2+}/\text{g}$. (RBE = Rice Bran Ethanol Extract, RBW = Rice Bran Aqueous Extract, RE = Rice Ethanol Extract and RW = Rice Aqueous Extract). Results are presented as mean \pm standard deviation ($n = 3$). Indicates significant difference at $p < 0.05$.

Total phenolic compound assay

The garden balsam leaves and flowers were reported by Koodkaew *et al.* [21] to contain the highest phenolic levels, with 122.39 ± 8.50 and 111.53 ± 1.42 mg GAE/g, respectively. Kaewpiboon *et al.* [20] reported that Sangyod germinated brown rice extract, soaked in pandanus leaf extract, had a phenolic content of 134.52 ± 5.21 mg GAE/g. In comparison, the phenolic compound test for Si Boo Gan Tang rice extract revealed total phenolic contents of 34.82 mg GAE/g for RW and 41.21 mg GAE/g for RE. Rice bran extracts RBW and RBE showed phenolic contents of 34.82 and 154.31 mg GAE/g, respectively (**Table 2**). Jasmine rice bran aqueous extract had a phenolic concentration of 65.24 ± 1.74 mg GAE/g, differing significantly from black glutinous rice bran, Leum Pua, and Sangyod rice bran extracts [22]. Dok Mali 105 rice

extract exhibited a phenolic concentration of 3.21 mg GAE/g [23]. Tubtim Chumphae rice bran had a total phenolic content of 87.14 mg GAE/g [30]. Purple rice extract, specifically Gam Luem Phua rice from northern Thailand, showed a phenolic concentration of 595.53 ± 7.36 mg GAE/g [8]. Market varieties of colored rice-red (Sang Yot and Mun Poo), purple (Riceberry), unpolished purple (Hom Nil), and black (Lum Pua) were extracted using a 50:50 water-ethanol mixture, revealing phenolic contents between 128 - 322 mg GAE/g [25]. Seechamnaturakit *et al.* [32] stated that many compounds are contained in rice such as phenolic acids, flavonoids and anthocyanins. Moreover, phenolic acid contains only found in non-pigmented rice, but polyphenol group contain in pigmented rice. In addition, the most common phenolic compounds are found in red and black rice grains [33].

Table 2 Total phenolic content of RW, RE, RBW and RBE.

Extract	mg Gallic acid/g extract
RW	34.82 ± 0.01^a
RE	41.21 ± 0.01^b
RBW	34.82 ± 0.01^a
RBE	154.31 ± 0.02^c

(RBE = Rice Bran Ethanol Extract, RBW = Rice Bran Aqueous Extract, RE = Rice Ethanol Extract and RW = Rice Aqueous Extract). Results are presented as mean \pm standard deviation ($n = 3$). Indicates significant difference at $p < 0.05$.

Total flavonoid compound assay

Following experimentation, the quantities of flavonoids in rice (RW, RE) and rice bran (RBW, RBE) extracts were determined. Extracts from RW and RE contained 2.17 and 2.50 mg QE/g, respectively, while RBW and RBE extracts had 4.21 mg and 8.78 mg/g, respectively (**Table 3**). Koodkeaw *et al.* [21] reported that garden balsam flower and leaf extracts had higher flavonoid concentrations than seed and stem extracts, with the flower extract containing 44.74 ± 0.82 mg QE/g and the leaf extract containing 25.48 ± 0.65 mg QE/g. Jaroennon *et al.* [22] found that flavonoid concentrations in jasmine rice bran, Leum Pua black sticky rice bran, and Sangyod rice bran aqueous extracts

ranged from 0.02 to 0.05 mg QE/g. Gam Luem Phua rice, from northern Thailand, contained 379.35 ± 4.26 mg QE/g extract [8]. Five colored rice varieties (red, purple, unpolished purple, and black) showed flavonoid concentrations between 86 - 341 mg CE/g extract when extracted with a 50:50 water-ethanol solution [25]. Seechamnaturakit *et al.* [32] stated that many compounds contain in rice such as phenolic acids, flavonoids and anthocyanins while Shao *et al.* [33] stated that most flavonoid in pigmented rice is anthocyanin. Moreover, the kaempferol and quercetin were classified as flavonols found in pigmented rice [34].

Table 3 Total flavonoid content of RW, RE, RBW and RBE.

Extract	mg Quercetin/g extract
RBE	8.78 ± 0.01^a
RBW	4.21 ± 0.01^b
RE	2.5 ± 0.01^c
RW	2.17 ± 0.01^c

(RBE = Rice Bran Ethanol Extract, RBW = Rice Bran Aqueous Extract, RE = Rice Ethanol Extract and RW = Rice Aqueous Extract). Results are presented as mean \pm standard deviation (n = 3). Indicates significant difference at $p < 0.05$.

Conclusions

The findings revealed that Si Boo Gan Tang rice and rice bran extracts exclusively inhibit the growth of gram-positive bacteria. Observations using a Scanning Electron Microscope (SEM) showed changes in the morphology of treated cells, including cell swelling and the formation of a fiber network around the cells. All extracts (RW, RE, RBW, and RBE) demonstrated significant antioxidant activity, as determined by the DPPH, ABTS, and FRAP assays. These extracts contained valuable phytochemical compounds, such as phenolic and flavonoid compounds. Additionally, the extracts exhibited favorable biological properties, showing no harmful effects on skin cells, and aiding in the reduction of inflammation. Future research will focus on comprehensive genetic-level examinations, animal testing, and further refinement for medical applications.

Future work

In future work may be test about growth curve of indicator strains after adding the extract in culture media and test release of intracellular UV-absorbing material to confirm the mechanism of extract to interrupt cell membrane resulting leak of intracellular material out of the cell. In this research we do not study anthocyanin, but in the future, we will test it. Because some research reported anthocyanin has an activity against microbes. And the future work we will anticancer tests and wound healing tests.

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