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Original Article

Anti-Tyrosinase, Anti-Inflammatory, and Cytotoxic Activity of Si Boo Gan Tang Rice and Rice Bran Extracts

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ABSTRACT

Si Boo Gan Tang is a widely cultivated variety of rice in the Tak Bai district of Narathiwat province, located in the southern region of Thailand. This study aimed to evaluate the biological properties of Si Boo Gan Tang rice and rice bran extracts, focusing on their anti-tyrosinase activity, anti-inflammatory activity, and cytotoxicity. The anti-tyrosinase assay revealed that the aqueous extract of rice (RW) significantly inhibited tyrosinase activity by 41.47%. Similarly, the ethanolic extract of rice (RE) exhibited anti-tyrosinase activity at 35.34%. The aqueous (RBW) and ethanolic (RBE) extracts of rice bran showed anti-tyrosinase activity with percentages of 19.26% and 29.08%, respectively. The anti-inflammatory experiment, conducted using RAW 264.7 cells, demonstrated that all extracts (RW, RBW, RE, and RBE) from rice and rice bran exhibited anti-inflammatory properties by reducing the release of nitric oxide (NO) from lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. The extracts had IC_{50} values of over 400 µg/ml and CC_{50} values over 400 µg/ml. Cytotoxicity was assessed using the MTT assay on HaCaT cells. The findings indicated that the IC_{50} values for both aqueous and ethanolic extracts of rice and rice bran were greater than 800 µg/ml.

Keywords: Si Boo Gan Tang Rice, Anti-tyrosinase, Anti-inflammatory, Cytotoxicity, HaCaT cell.

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INTRODUCTION

Rice, scientifically known as *Oryza sativa* L., is a crucial staple crop in global food production, especially in Asian countries where it is a dietary mainstay. Thriving in hot and humid conditions, rice is ideally cultivated in Thailand, a country renowned for its high-quality rice cultivars. Thai rice varieties are popular worldwide among consumers for their quality and flavour. Rice grains consist of two primary components: the rice husk and the seed, known as the brown grain. The brown grain, which appears faintly red, is the part of the rice grain with the husk removed but unpolished. It contains various substances, including anthocyanin, protein, cellulose, hemicellulose, fat, amylose, and amylopectin.[1] Rice bran, another vital component, comprises 12.45% fat, 10.90% protein, 45.31% carbohydrates, and 13.51% fibre.[2,3] Additionally, rice bran is rich in bioactive compounds such as phenolic acids, flavonoids, vitamin E, and gamma oryzanol, surpassing vegetables, fruits, nuts, and other dried fruits in these nutrients.[4] Extracting these bioactive components from rice bran enhances its nutritional and medicinal value.[5] Rice varieties popular in specific regions are chosen for their adaptability to local climate and soil conditions, high yield potential, and resistance to diseases and pests.[6] In the southern border provinces of Thailand, common rice varieties include Si Boo Gan Tang and Hawm Gra Dang Ngah. Pattani Lep Nok and Sangyod rice are indigenous to this region.[7] Si Boo Gan Tang rice accounted for up to 90% of the cultivated rice, covering approximately 16,500 rai. Currently, there is significant research and development on coloured rice, particularly compounds from the outer layer of seeds or rice bran. These studies primarily focus on the antioxidant capacities of different extracts, emphasising monomeric anthocyanin, total phenolic compounds, and ferric reducing chemicals. It has been found that extracts with antioxidant properties can also inhibit collagenase activity, an enzyme in the matrix metalloproteinases (MMPs) category that degrades the extracellular matrix, especially collagen fibres in the skin's dermis layer.[8] Research on Sangyod rice, a type of red rice, indicates it has 3 to 5 times higher antioxidant activity than Jasmine rice 105 due to its high levels of phenolic compounds and flavonoids.[9] This antioxidant effect is linked to the prevention of chronic diseases such as diabetes, cardiovascular disease, and cancer.[10] Studies assessing the antioxidant properties of Sangyod rice from various southern provinces found that rice from Nakhon Si Thammarat had an IC_{50} value of 8.64 mg/ml. The highest antioxidant activity was observed in Krabi, Satun, Phatthalung, Songkhla, and Trang, respectively. Brown rice from Nakhon Si Thammarat also had the highest concentration of phenolic compounds at 11.91 mg GAE/g rice, followed by Krabi and Phatthalung, Satun, Songkhla, and Trang.[11]

Si Boo Gan Tang rice exhibits the typical attributes of a compact and erect growth habit, with stems that are quite firm in texture. The species has seeds that are a combination of white and scarlet. The nutritional composition of the food sample was determined to have a moisture content of 13.30%, an ash content of 1.29%, a protein content of 9.82%, a fat content of 8.07%, a carbohydrate content ranging from 67.18%, and a dietary fibre content of 0.54%.[11] The rice grains come in black and red varieties. The antioxidant

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efficacy of white rice grains is significantly lower in comparison to this. It is widely known that it possesses efficacy in combating free radicals. The level of independence is determined by the quantities of phenolic compounds and anthocyanin present in rice grains. In the Narathiwat province, farmers opt to cultivate Si Boo Gan Tang rice. Rice and rice bran extracts have not been extensively researched in terms of their biological qualities, despite being native species. Hence, the research team aims to investigate the biological properties of extracts derived from rice and rice bran of Si Boo Gan Tang. Specifically, they are interested in examining the antioxidant activity, anti-tyrosinase activity, antibacterial activity, cytotoxicity to HaCaT cells, and anti-inflammatory effects. The research team aims to utilise the acquired knowledge to advance the development of agricultural products into novel innovations. In response to the requirements of national policy and the policy of Princess of Naradhiwas University, which prioritises research on technology and innovation, our focus is on enhancing the quality of life for farmers by augmenting the value of agricultural goods. Additionally, it stimulates the economic growth of both the province and Thailand as a whole.

MATERIALS AND METHODS

Preparation of Si Boo Gan Tang Rice and Rice Bran Extracts

Rice Aqueous Extract (RW)

80 g of ground rice were mixed with 320 ml of water and left to stand 2 h. The mixture was then filtered through cheesecloth and No. 1 filter paper. After freezing at -20°C for 24 h, the resulting solution was air-dried until completely dry. The powdered crude extract was then stored at -20°C.

Rice Ethanol Extract (RE)

80 g of ground rice was mixed with 800 ml of 50% ethanol and stirred for 2 h. The mixture was then filtered using cheesecloth and No. 1 filter paper. The resulting mixture was evaporated, and ethanol was completely removed using a Rotary Evaporator. Subsequently, the mixture was freeze-dried until fully dried, yielding a powdered crude extract stored at -20°C.

Rice Bran Aqueous Extract (RBW)

320 ml of water was added to 80 g of rice bran and stirred, then left for 2 h. Subsequently, the mixture was filtered using cheesecloth and No. 1 filter paper. The resulting solution was then frozen at -20°C for 24 h and subsequently freeze-dried until completely dry. The resulting powdered crude 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. Next, the mixture was freeze-dried until fully dried. The powdered crude extract obtained was stored at -20°C.

Anti-tyrosinase activity assay of rice and rice bran extract

The determination of anti-tyrosinase activity was carried out using the method described by.[12] A solution of 10 µl of 0.1 M phosphate buffer at pH 6.8 was mixed with 50 µl of a 4 mmol solution of L-Dopa. This mixture was thoroughly combined and allowed to incubate for 10 mins at room temperature. Following this, 20 µl of the extract (RW, RE, RBW, and RBE) were added at concentrations of 18.50, 9.38, 4.69, 2.34, and 1.17 mg/ml. Subsequently, 20 µl of tyrosinase solution (0.5 mg/ml) were added, thoroughly mixed, and incubated for a further 10 mins. The optical density was then measured at a wavelength of 475 nm. The percentage of tyrosinase inhibition was calculated using the following formula:

%Tyrosinase inhibition = [A-(B-C)]/A] * 100

where A is the control absorbance, B is the sample absorbance, and C is the blank absorbance.

Anti-Inflammatory activity assay of rice and rice bran extract

The anti-inflammatory activity assay was conducted following the methodology described by.[13] The assay was performed on RAW 264.7 cells cultivated in DMEM medium, supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (10,000 U/mL penicillin and 10 mg/mL streptomycin), and incubated at 37°C with 5% CO_2.

RAW 264.7 cells, at a concentration of 5 x 10^5 cells/ml, were placed in a 6-well plate and incubated at 37°C for 24 h. Extracts (RW, RE, RBW, and RBE) at concentrations of 50, 100, 200, and 400 µg/ml were then added to the cells, which were incubated for an additional 6 h at 37°C. DMSO was used as a control substitute for the extracts. After this incubation period, the RAW 264.7 cells were stimulated with lipopolysaccharide (LPS) at a concentration of 1 µg/ml for 24 h. The samples were then centrifuged at 3000 rpm/min at 4°C for 10 mins. Following centrifugation, 100 µl of the supernatant was combined with 100 µl of Griess reagent and allowed to react for 15 mins in the dark at room temperature. The optical density was measured at a wavelength of 540 nm using a Bio-Rad microplate reader. Sodium nitrite, with concentrations ranging from 10 to 100 µmol, was used as the standard.

Cytotoxicity assay

A cytotoxicity experiment was conducted on HaCaT cells using the MTT technique, following the protocol outlined by.[14] The HaCaT cells were cultivated in DMEM medium at 37°C with 5% carbon dioxide and incubated for 72 h. The cells were then quantified to a density of 5 x 10^4 cells/ml in a 96-well plate and incubated at 37°C with 5% CO_2 for 24 h. Subsequently, 100 µl of extract (RW, RE, RBW, and RBE) at concentrations of 5, 10, 50, 100, 200, 400, and 800 µg/ml were added to each well and incubated at 37°C with 5% CO_2 for another 24 h. Subsequently, 100 µl of MTT [3-(4, 5-dimethyl thiazolyl-2)-2, 5-diphenyltetrazolium bromide] solution at a concentration of 5 mg/ml was introduced into each well and incubated at 37°C with 5% CO_2 for 2 h. Following this, the solution in the wells was removed and replaced with 100 µl of DMSO. The plate was left undisturbed for 1 h before the optical density was measured at a wavelength of 550 nm using a Bio-Rad microplate reader. The experiment was conducted in triplicate. The CC_{50} value was determined using the following formula:

% Viability of cell = (ODT/ODC) x 100

where ODC is the optical density of the control, and ODT is the optical density of the test sample.

Statistical analysis

All experimental results were presented as mean ± standard deviation (S.D.) using analytical software. Data analysis was conducted using paired t-tests, one-way ANOVA, and post hoc tests with Duncan's multiple range test (DMRT), with a significance level set at $p < 0.05$, to compare the control group and the test group. Statistical analysis was performed using IBM SPSS Statistics Standard Version 29.0.1.0.

RESULTS AND DISCUSSION

Anti-Tyrosinase activity

The inhibitory effects of aqueous and ethanolic extracts of rice (RW and RE) on the tyrosinase enzyme were found to be 41.47% and 35.34%, respectively. Similarly, aqueous and ethanolic extracts of rice bran showed inhibition rates of 19.26% and 29.08%, respectively. As shown in Figure 1, the standard compound, ascorbic acid, suppressed the activity of the tyrosinase enzyme by 78.40%.

A study by[12] found that Sangyod germinated brown rice extract, when soaked in pandanus leaf extract, effectively inhibited tyrosinase activity, with an IC_{50} value of 3.14 µg/ml. Additionally, extracts from the flowers, leaves, stems, and seeds of garden balsam demonstrated the ability to inhibit the tyrosinase enzyme. The seeds and flowers showed the most potent inhibitory effects at a volume of 50 µl, with inhibition rates of 61.11 ± 6.19% and 60.00 ± 5.01%, respectively.[14] The inhibitory action of garden balsam extract on the tyrosinase enzyme is attributed to its phenolic constituents, such as kojic acid, kaempferol, and quercetin, which are known to inhibit tyrosinase activity.[15,16] Additionally, five varieties of coloured rice available in the market, including red rice (Sang Yot rice and Mun Poo rice), purple rice (Riceberry), unpolished purple rice (Hom Nil rice), and black rice (Lum Pua rice), were extracted using a 50:50 mixture of water and ethanol. Although the IC_{50} values for these rice extracts could not be determined due to their limited effectiveness in inhibiting the tyrosinase enzyme, each type of rice extract showed a certain percentage of inhibition.

The concentrations recorded were: Sangyod rice at 15.45 ± 2.08 mg/ml, Mun Poo rice at 23.51 ± 10.88 mg/ml, Riceberry rice at 14.49 ± 3.61 mg/ml, Hom Nil rice at 14.27 ± 6.33 mg/ml, and Lum Pua rice at 19.56 ± 8.77 mg/ml.[17]

Anti-Inflammatory activity

Inhibiting nitric oxide (NO) production in RAW 264.7 cells stimulated with lipopolysaccharide (LPS), which causes inflammation, involves measuring the absorbance at 540 nm and comparing it with the standard curve of potassium nitrite (KNO_2). The experiment found that the percentage of nitric oxide (NO) secretion decreased by more than 80 % in the group receiving dexamethasone before stimulation with LPS, compared to the group stimulated with LPS alone. The IC_{50} of dexamethasone was determined to be 1.55 µg/ml. The Si Boo Gan Tang rice and rice bran extracts (RW, RE, RBW, and RBE) reduced nitric oxide (NO) secretion by 50 %, with IC_{50} values greater than 400 µg/ml and CC_{50} values also greater than 400 µg/ml, as shown in Table 1 and Figures 2 and 3.

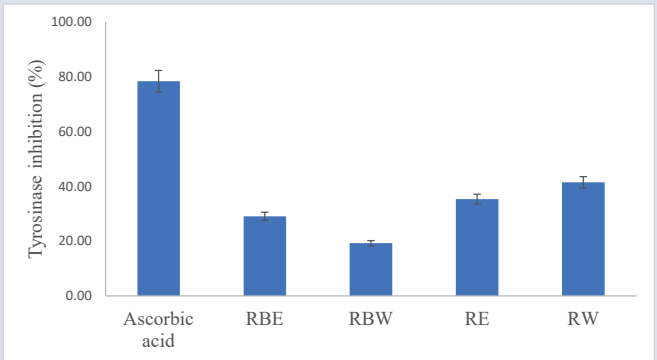

Figure 1: Tyrosinase inhibition activity of Si Boo Gan Tang Brown Rice and Rice Bran Extracts. Data are expressed as mean ± SEM (n = 3). (RBE = Rice Bran Ethanol Extract, RBW = Rice Bran Aqueous Extract, RE = Rice Ethanol Extract and RW = Rice Aqueous Extract).

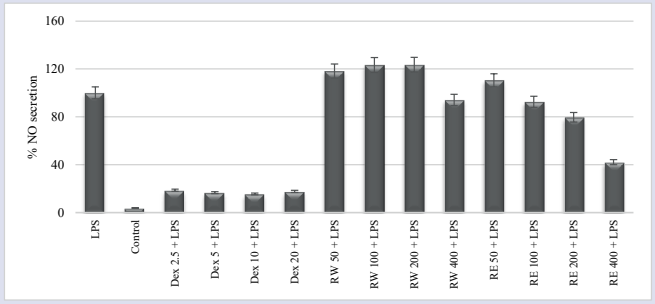

Figure 2: Nitric Oxide (NO) secretion (LPS = Lipopolysaccharide, Dex = Dexamethasone, RW = Rice Aqueous Extract, and RE = Rice Ethanol extract).

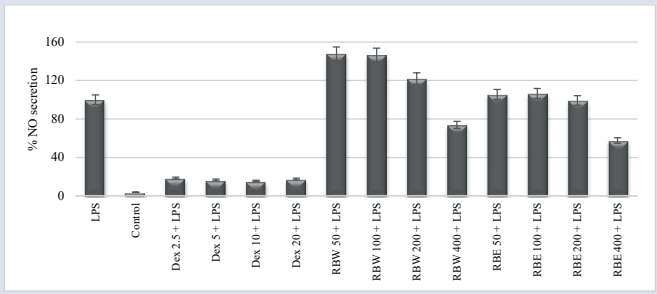

Figure 3: Nitric Oxide (NO) secretion (LPS = Lipopolysaccharide, Dex = Dexamethasone, RBW = Rice Bran Aqueous Extract, and RBE = Rice Bran Ethanol extract).

Table 1: Anti-Inflammatory activity of RW, RE, RBW and RBE extracts.

Sample	NO production (Mean±SE) (µmol/L)	%NO secretion	IC_{50} of anti-inflammatory activity (µg/ml)	CC_{50} of anti-cytotoxicity activity (µg/ml)
LPS	31.96±9.98	100.00		
Control	1.24±0.74	3.79		
Dex 2.5 + LPS	7.16±8.68	18.68		
Dex 5 + LPS	6.45±8.17	16.60	1.55	>400
Dex 10 + LPS	6.10±7.94	15.57		
Dex 20 + LPS	6.90±8.93	17.66		
RW 50 + LPS	38.07±14.30	118.29		
RW 100 + LPS	39.58±13.47	123.36	>400	>400
RW 200 + LPS	39.62±13.09	123.56		
RW 400 + LPS	30.05±10.12	94.13		
RE 50 + LPS	33.28±12.95	110.52		
RE 100 + LPS	28.64±13.08	92.57	>400	>400
RE 200 + LPS	24.95±12.18	79.60		
RE 400 + LPS	13.83±8.95	42.10		
RBW50 + LPS	47.31±18.29	147.55		
RBW100 + LPS	46.83±15.75	146.33	>400	>400
RBW200 + LPS	39.51±15.59	121.90		
RBW400 + LPS	23.88±8.96	73.94		
RBE 50 + LPS	33.74±11.09	105.42		
RBE100 + LPS	33.55±8.07	106.39	>400	>400
RBE200 + LPS	31.35±7.77	99.29		
RBE400 + LPS	18.486.37	57.57		

(RBE = Rice Bran Ethanol Extract, RBW = Rice Bran Aqueous Extract, RE = Rice Ethanol Extract and RW = Rice Aqueous Extract, DEX= Dexamethasone, LPS= lipopolysaccharide)

Moreover, gamma-oryzanol extract from Thai Kam rice bran has the ability to inhibit nitric oxide production in RAW 264.7 cells, with an IC_{50} value between 23.69 and 41.22 µg/ml.[18] Peptide hydrolysate from Dok Mali 105 rice bran, which had undergone oil extraction, at a concentration of 50 µg/ml showed anti-inflammatory effects by inhibiting nitric oxide production by 41.13% in RAW 264.7 cells stimulated with LPS.[19] The aqueous leaf extract of Thunbergia laurifolia was evaluated[20], and it was reported to effectively reduce nitric oxide production, enhance cell proliferation, and exhibit low-level cytotoxicity.

Additionally, turmeric extract exhibited anti-inflammatory properties by reducing nitric oxide production in RAW 264.7 cells stimulated with LPS, with an IC_{50} value of 35.20 ± 1.35 µg/ml.[21] Extract from Gam Luem Phua rice, a variety of purple rice grown in northern Thailand, also demonstrated the ability to inhibit nitric oxide (NO) production, with an IC_{50} value of 18.32 ± 0.82 µg/ml.[22]

Cytotoxicity assay

A cytotoxicity assay of RW, RE, RBW, and RBE extracts on HaCaT cells was performed using the MTT method. The results showed that the Si Boo Gan Tang extracts (RW, RE, RBW, and RBE) were non-toxic to HaCaT cells, with IC_{50} values exceeding 800 µg/ml, as shown in Figures 4-7. Additionally, the crude extract of Vernonia cinerea Less. exhibited moderate toxicity to myeloma cells (IC_{50} = 197.9 µg/ml) but was non-toxic to lymphocytes (IC_{50} = 305.9 µg/ml).[23] Furthermore, Sangyod germinated brown rice extract, when soaked in pandanus leaf extract, showed toxicity to HaCaT cells with an IC_{50} value >400 µg/ml, indicating its potential for development into medical cosmetics.[14]

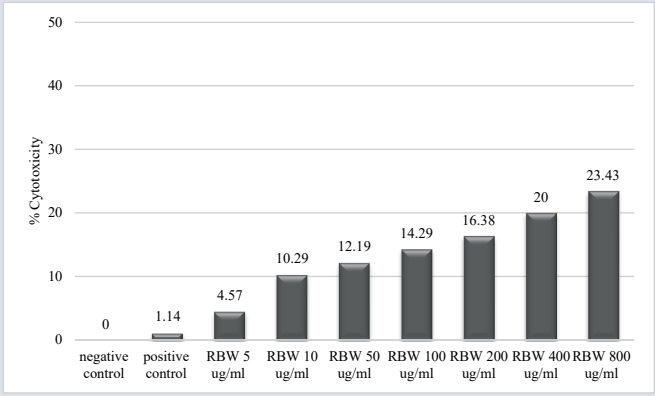

Figure 4: Cytotoxicity assay of Rice Bran Aqueous Extract (RBW) to HaCaT cell.

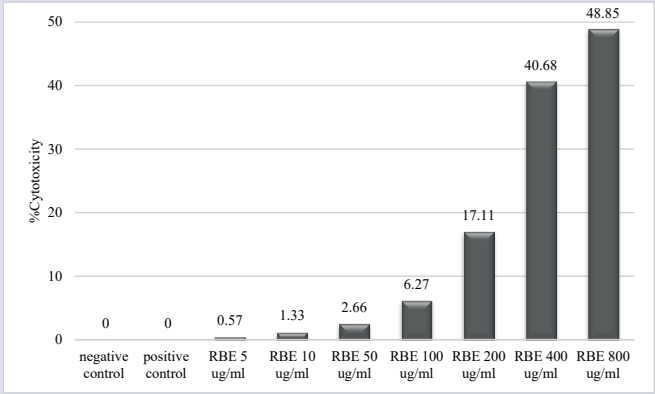

Figure 5: Cytotoxicity assay of Rice Bran Ethanol Extract (RBE) to HaCaT cell.

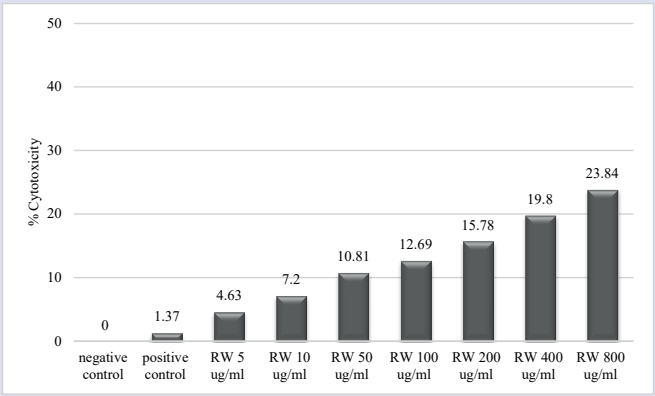

Figure 6: Cytotoxicity assay of Rice Aqueous Extract (RW) to HaCaT cell.

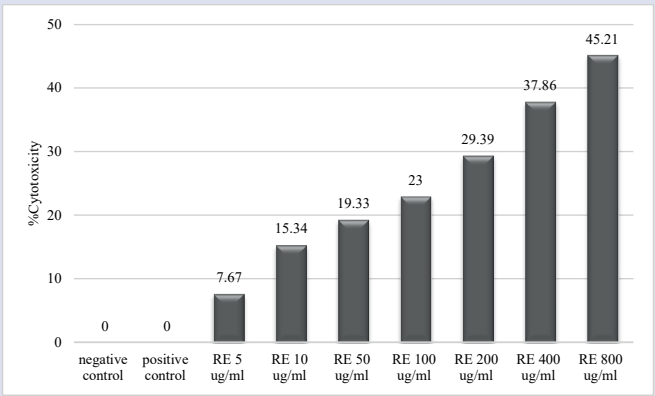

Figure 7: Cytotoxicity assay of Rice Ethanol Extract (RE) to HaCaT cell.

CONCLUSIONS

The findings of this study on the biological properties of Si Boo Gan Tang rice and rice bran extracts have revealed several promising attributes. All extracts exhibited significant anti-tyrosinase activity, suggesting their potential for development into cosmetic products aimed at reducing melasma and other forms of hyperpigmentation. Tyrosinase is an enzyme responsible for the formation of dark spots on the skin, and the inhibition of this enzyme by the extracts indicates their usefulness in skin-lightening applications.

Additionally, the extracts demonstrated notable anti-inflammatory effects at concentrations exceeding 400 µg/ml. This anti-inflammatory activity suggests that the extracts could be beneficial in soothing irritated skin and reducing inflammation, further enhancing their potential application in skincare products. Importantly, none of the extracts were toxic to HaCaT cells, which indicates their safety for human use. This non-toxicity supports the potential development of these extracts into medical body lotions and other topical applications, where skin safety is paramount.

Given these promising results, there is a strong case for further research and development. The combination of anti-tyrosinase and anti-inflammatory activities positions these extracts as valuable ingredients in formulations designed to lighten hyperpigmentation, improve skin tone, and soothe irritated skin. However, before any commercial product development can proceed, it is essential to conduct further testing in animal models. These studies will help to confirm the efficacy and safety of the extracts in a living system, providing more comprehensive data on their biological effects and potential side effects. This step is vital to ensure that the extracts can be safely and effectively used in humans.

In conclusion, Si Boo Gan Tang rice and rice bran extracts show great promise for use in cosmetic and medical products due to their anti-tyrosinase and anti-inflammatory properties, as well as their demonstrated safety in cell culture. Future research, including animal testing, will be crucial to fully realize their potential and bring these natural extracts to market.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study, in the data collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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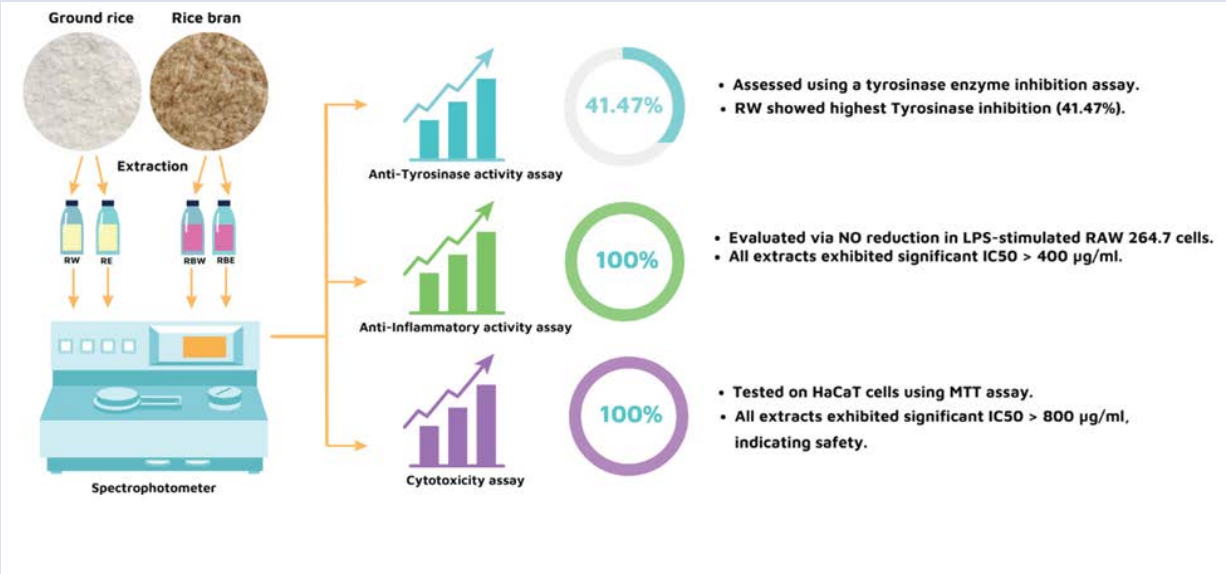
GRAPHICAL ABSTRACT



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