

# Phytochemical screening, quantitative analysis of cyanidin-3-O-glucoside content, and anticancer activity of novel rice bran (Tubtim Chumphae rice)

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## ABSTRACT

The objective of this research was to explore the phytochemicals in Tubtim Chumphae rice bran and determine their potential antioxidant and anticancer activities. The rice bran extract contained total phenolics, flavonoids, and anthocyanins as the major compounds. In addition, it exhibited the highest DPPH<sup>•</sup>, followed by ABTS<sup>•+</sup>, and FRAP radical scavenging activity. Moreover, the cytotoxic effect of the extract on Hep G2, SW620, KATO-III, BT474, and ChaGo-K-1 cancer cell lines was investigated; the obtained IC<sub>50</sub> values were 144.6 ± 0.39, 116.9 ± 0.68, 99.6 ± 0.10, 94.4 ± 0.33, and 25.6 ± 0.45 µg/mL, respectively. Overall, the abovementioned results suggest that rice bran extract has potent anticancer activity owing to the induction of cell death in cancer cell lines.

**Key words:** Anticancer, cyanidin-3-O-glucoside, rice bran extract (Tubtim Chumphae rice)

## INTRODUCTION

Pigmented rice, Tubtim Chumphae rice (TCR), is a Thai rice breed comprising Jasmine Rice (Hom Mali rice) and Sung Yod Phatthalung rice. It contains high amounts of polyphenols, phenolics, anthocyanins, and flavonoids in the aleurone layer, which appear red in rice grains.<sup>[1]</sup> In general, rice bran contains carbohydrates, minerals, proteins, fatty acids, vitamins, and dietary fiber.<sup>[2]</sup> Phytochemicals with biological activities, such as flavonoids and phenolic acids, have been found in rice bran.<sup>[3]</sup> Owing to this, it has received considerable attention owing to its potential pharmacological activities.

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Numerous phytochemicals in rice bran are responsible for preventing diseases, such as hypertension and diabetes. They also exhibit immunomodulatory activities.<sup>[4]</sup> Polyphenols in rice bran exhibit anti-inflammatory activity and activate the immune system against cancer cells.<sup>[5]</sup> The different fractions of rice bran extract consist of anthocyanins and phenolic acids, which have alpha-amylase and alpha-glucosidase inhibitory activities.<sup>[6]</sup> Anthocyanins in rice bran extract inhibit human cancer cell growth, including tongue, hepatocellular, and cervical carcinomas.<sup>[7]</sup> In addition, crude anthocyanin extract inhibits invasion by human lung cancer cells.<sup>[8]</sup> Black rice extracts containing high anthocyanin content prevent oxidative damage and improve lipid profiles *in vivo*.<sup>[9]</sup>

In this study, we screened the phytochemical constituents of the TCR bran (TCRB) extract and investigated their potential antioxidant and anticancer properties. Antioxidant

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activity was determined through DPPH, ABTS, and FRAP radical scavenging assays, whereas anticancer activity was evaluated *in vitro* through a cytotoxicity assay. The results provide data to assess the anticancer activity of TCRB extract, which could be applied in food or pharmaceutical products as a therapeutic ingredient in the future.

## MATERIALS AND METHODS

### Plant collection and extraction

TCRB was obtained from the Chumphae Rice Research Center in Khon Kaen Province, Thailand, and harvested on March 21. The TCRB was dried at 40°C in a hot air oven. Next, constituents of dried TCRB were extracted with 70% EtOH at a sample to solvent ratio of 1:5 (w/v) for 24 h on a rotary shaker (120 rpm). The mixture was evaporated using a rotary evaporator, and the final product was stored at -20°C until analysis.

### Qualitative phytochemical analysis

The presence of phytochemicals was confirmed by solution characteristics or color changes, depending on the reaction.<sup>[10]</sup> Changes in the color of the mixture were observed for terpenoids using the Salkowski test (reddish-brown), phenols/tannins (brownish green or blue-black color), anthocyanin (pinkish red to bluish violet coloration) and flavonoids (yellow).

### Total phenolic content

The total phenolic compound was determined according to Rungruang.<sup>[11]</sup> A volume sample of 20 µL (1 mg/mL) solution containing TCRB extract was prepared and added 100 µL of 10% Folin-Ciocalteu solution. Subsequently, 80 µL Na<sub>2</sub>CO<sub>3</sub> 7.5% (w/v) was reacted with sample, and the mixture was maintained for 60 min. The absorbance was detected at 760 nm (Biochrom EZ Read 2000, Cambridge, UK).

### Total flavonoid content

The aluminum chloride colorimetric method was used to quantify the total flavonoid content.<sup>[12]</sup> The extract (30 µL) was mixed with a 1:5 (w/v) mixture of NaNO<sub>2</sub> in 100 µL of MeOH and agitated for 6 min. Next, 15 µL of a 1:10 (w/v) solution of AlCl<sub>3</sub> was added to the mixture and incubated for 6 min. Finally, 1 M NaOH (55 µL) was added, and the reaction solution was incubated for 10 min at 25°C. Absorbance was detected at 510 nm. The results were expressed as quercetin equivalents (mg QE/g extract).

### Quantification of cyanidin-3-O-glucoside

Quantification of cyanidin-3-O-glucoside (Cy3G) was conducted as previously described.<sup>[12]</sup> Separation was achieved on an XDB-C18, 4.6 × 150 mm, 5 µm (Agilent Eclipse) analytical column using an HPLC equipment (Agilent Technologies Inc., California, USA) coupled to a detector (diode array detector, DAD). The injection volume

(1 mg/mL) was 100 µL, the flow rate was 0.4 mL/min, mobile phase A (0.1% trifluoroacetic acid in Milli-Q water) and mobile phase B (Acetonitrile). The peak areas were recorded at 530 nm and were quantified by comparison with pure Cy3G which was used as the standard.

### *In vitro* antioxidant assay using DPPH<sup>•</sup>

The DPPH assay was performed as previously described.<sup>[13]</sup> A total of 195 µL DPPH<sup>•</sup> solution was mixed with TCRB extract (5 µL) and incubated for 30 min in the dark. A reduction in absorbance was detected at 515 nm, and the results are expressed in mg TE/g extract.

### *In vitro* antioxidant assay using ABTS<sup>•+</sup>

The ABTS<sup>•+</sup> assay was conducted as previously described.<sup>[14]</sup> The mixing of 2.45 mM K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> and 7.0 mM ABTS was used to produce ABTS radical. The radical product was kept in the dark for 16 h. Before analysis, the ABTS solution was diluted using 95% EtOH until an absorbance of 0.70 ± 0.05 was obtained at 734 nm. The TCRB extract (40 µL) was mixed with 160 µL of diluted radical solution and allowed to stand for 6 min in the dark. The absorbance was recorded at 734 nm. All data are expressed as mg TE/g extract.

### *In vitro* antioxidant assay using FRAP

The chelating ability of TCRB extract was determined using the FRAP assay.<sup>[15]</sup> A freshly prepared FRAP reagent (150 µL) was pipetted into the TCRB extract (20 µL) and incubated at 37°C for 8 min. Absorbance was determined at 600 nm, and FRAP values are expressed as TE per 100 g dry weight sample based on a standard curve of Trolox (0–100 µM).

### Assay for cytotoxic activity

Cytotoxicity was determined using the MTT assay.<sup>[16]</sup> Human breast cancer (BT474), lung bronchus carcinoma (ChaGo-K-1) cells, and colorectal (SW620), liver (Hep G2), and gastric (KATO-III) cancer cells were cultured in TCRB extract (8, 20, 40, and 200 µg/mL), and the results were compared with those of 10 µg/mL doxorubicin (positive control) and 0.2% DMSO (negative control). The cells were incubated overnight in a 5% CO<sub>2</sub> atmosphere at 37°C for 24 h. The cultured cells were treated with TCRB extract and incubated further at 37°C for 72 h. The cells were then incubated with an MTT reagent for 4 h. The absorbance of the lysed cells was measured at 540 nm. The percentage of growth inhibition was calculated and IC<sub>50</sub> values were calculated from four concentrations of the extract and compared with those of doxorubicin. The fluorescence microscope (Olympus IX71) at a magnification of ×40 was used to observe cell morphology.

#### Cell viability percentage

$$= \frac{\text{Absorbance of sample} - \text{blank}}{\text{Absorbance of untreated} - \text{blank}} \times 100$$

### Statistical analysis

Statistical analysis was executed using analysis of variance

and SAS version 9 software (SAS Institute, NC, USA). All results are expressed as mean  $\pm$  standard deviation. The significant differences were set at  $P < 0.05$  by using duncan's multiple range test.

## RESULTS

### Yield and appearance of Tubtim Chumphae rice B extract

The yield of TCRB after extraction with 70% ethanol for 24 h was  $5.32 \pm 0.13\%$  w/w compared with the initial dry weight. The TCRB extract was dark brown and highly viscous.

### Phytochemical screening

Bioactive compounds, including phenols/tannins, flavonoids, anthocyanins, terpenoids, total phenolics, total flavonoids, and Cy3G, were screened using biochemical analysis techniques [Table 1].

The tabulated results show that the TCRB extract mostly contained total phenolic compounds, followed by total flavonoids. Total anthocyanins were detected as Cy3G [Table 1]. The Cy3G and standard solutions were eluted at 7.729 and 7.722 min, respectively [Figure 1].

### Antioxidant activity of Tubtim Chumphae rice bran extract

The scavenging activity against DPPH, ABTS, and FRAP radicals was compared with that of Trolox [Table 2].

### Potential anticancer activity of Tubtim Chumphae rice bran extract

Following treatment of investigated cells with TCRB extract, they shrank and lost their normal shape, and cell confluency diminished. Cells treated with doxorubicin became round and died, whereas negative control cancer cells exhibited normal shapes and no changes [Figure 2].

**Table 1: Phytochemical screening results of Tubtim Chumphae rice bran extract**

Phytochemical compounds	Result
Phenols/tannins	+
Flavonoids	+
Anthocyanins	+
Terpenoids	+
Total phenolic content (mg GAE/g extract)	$21.22 \pm 0.19$
Total flavonoid content (mg QE/g extract)	$17.31 \pm 0.26$
Cyanidin-3-O-glucoside content (mg Cy3G/g extract)	$0.188 \pm 0.02$

+: Detected

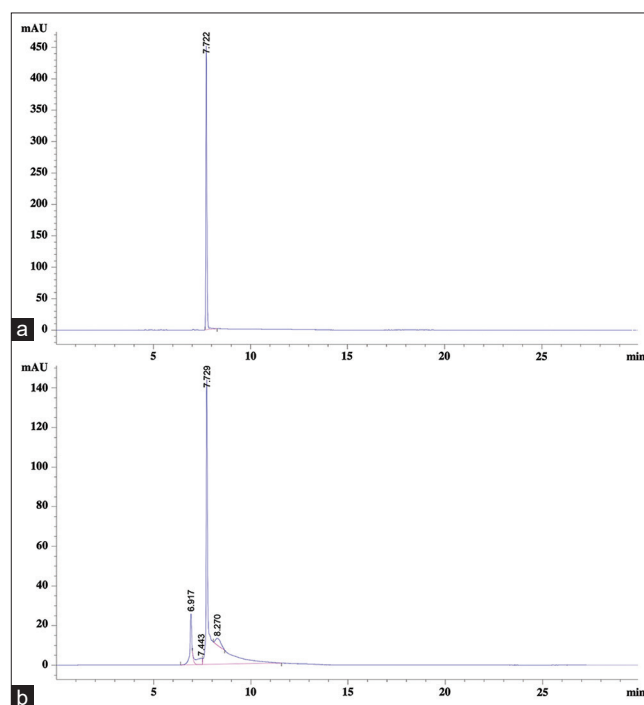
**Table 2: Antioxidant activity of Tubtim Chumphae rice bran extract**

DPPH (mg TE/g extract)	ABTS (mg TE/g extract)	FRAP (mM Trolox equivalents/g extract)
$35.48 \pm 1.50$	$9.74 \pm 1.77$	$5.03 \pm 1.32$

The  $IC_{50}$  values of the TCRB extract in ChaGo-K-1, BT474, SW620, Hep G2, and KATO-III cells are shown in Table 3. The viability of these cells was  $<90\%$  compared with that of the negative control, except for KATO-III cells [Figure 3]. However, doxorubicin caused the lowest cell viability at  $10 \mu\text{g/mL}$ .

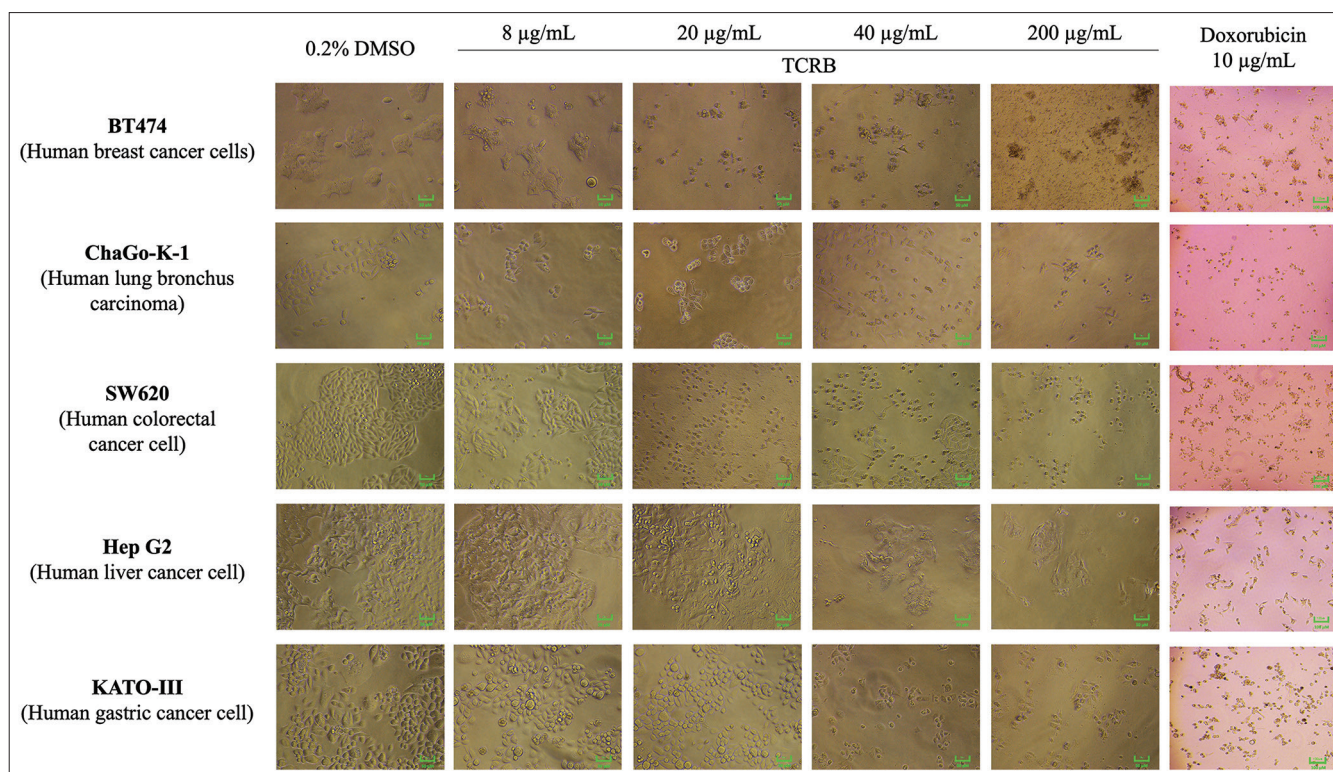
## DISCUSSION

Rice bran has received increasing attention because of its beneficial health effects. The highly effective extraction of thermally sensitive bioactive compounds from plant sources involves solvent maceration combined with shaking. This extraction process has been shown to increase mass transfer, diffusion, and cell destruction, leading to high yields.<sup>[17]</sup> Herein, following solvent maceration and shaking, the resultant TCRB extract appeared dark brown owing to the pigment in TCR, which contains high amounts of anthocyanins, polyphenols, phenolics, and flavonoids.<sup>[18]</sup> Therefore, phytochemical screening was performed using biochemical analysis techniques. Phenolic compounds and flavonoids, which are powerful chain-breaking antioxidants, are primarily found in plants.<sup>[19]</sup> The anthocyanin Cy3G has hypolipidemic effects,<sup>[20]</sup> reduces blood glucose levels, and suppresses oxidative stress.<sup>[21]</sup> Anthocyanins are the main pigments in black or colored rice and are the source of antioxidant activity. They prevent cardiovascular diseases, improve brain function, and reduce inflammation.<sup>[22]</sup> The TCRB extract showed antioxidant activity through hydrogen (DPPH $\cdot$  and

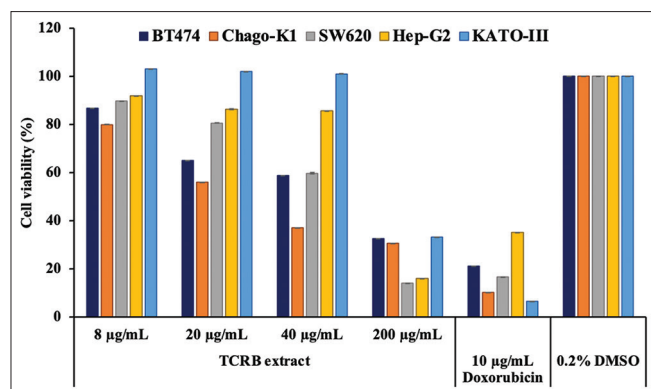


**Figure 1: Typical chromatograms of standard Cy3G (a) and TCRB extract (b)**





**Figure 2:** Morphology of human cancer cells (ChaGo-K-1, BT474, SW620, Hep G2, and KATO-III) treated with TCRB and doxorubicin



**Figure 3:** Cell viability of cancer cells (ChaGo-K-1, BT474, SW620, Hep G2, and KATO-III) treated with TCRB, doxorubicin, and 0.2% DMSO

ABTS<sup>•+</sup>) and electron (FRAP) donors in various *in vitro* assays.<sup>[23]</sup> Furthermore, the extract showed higher hydrogen than electron donor capacity. The main structure of the major compound in rice bran consists of multiple hydroxyl groups on different benzene rings. The absence of a hydroxyl group at the 3<sup>rd</sup> position and resonance at the 4<sup>th</sup>, 5<sup>th</sup>, or 7<sup>th</sup> positions through the pyrylium oxygen in the radical reaction system promotes hydrogen atom donation.<sup>[23]</sup> Moreover, the electron-donating ability might be a consequence of glycosylation and sugar units in the Cy3G structure. Compounds with more sugar groups are associated with higher electron-donating activity

**Table 3: Inhibition of proliferation of human cancer cells (ChaGo-K-1, BT474, SW620, Hep G2, and KATO-III) using Tubtim Chumphae rice bran and doxorubicin**

Human cancer cell line	IC <sub>50</sub> (µg/mL)	
	TCRB extract	Doxorubicin
BT474	94.4±0.33 <sup>b</sup>	0.23±0.04 <sup>d</sup>
ChaGo-K-1	25.6±0.45 <sup>a</sup>	0.12±0.06 <sup>c</sup>
SW620	116.9±0.68 <sup>d</sup>	0.04±0.03 <sup>a</sup>
Hep G2	144.6±0.39 <sup>e</sup>	0.04±0.01 <sup>a</sup>
KATO-III	99.6±0.10 <sup>c</sup>	0.07±0.02 <sup>b</sup>

Values with different superscript letters in the same column are significantly different ( $P < 0.05$ ). Values are expressed as mean±SD. SD: Standard deviation, TCRB: Tubtim Chumphae rice bran

than those with less.<sup>[24]</sup> In addition, the electron-donating activity could be attributed to the sugar group at the 3<sup>rd</sup> position.<sup>[25]</sup> In this study, the concentration-dependent inhibition of cancer cell viability decreased with increasing TCRB extract concentration. Cy3G affects carcinogenesis through multiple pathways, such as the induction of cancer cell apoptosis, cell cycle arrest, and DNA fragmentation.<sup>[26]</sup> The IC<sub>50</sub> of the TCRB extract was substantially different among the five types of cancer cell lines owing to their different biological properties. Moreover, the morphology of the cells was round and shrunken, and dead cells and cell debris were evident after treatment with 200 µg/mL TCRB. This result was used to predict the anticancer activity of the main bioactive compounds in the TCRB extract

## CONCLUSION

The findings of this study showed that the investigated TCRB extract exhibited radical scavenging activity following DPPH, ABTS, and FRAP assays. Moreover, the TCRB extract inhibited cancer cell growth. This was supported by changes in cell morphology of extract-treated ChaGo-K-1, BT474, SW620, Hep G2, and KATO-III cells. Overall, we conclude that TCRB extract has the potential to prevent the progression of various cancer types.

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## Conflicts of interest

There are no conflicts of interest.

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