International Food Research Journal 20(5): 2117-2124 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antioxidant properties and antiproliferative effect of brewers' rice extract (*temukut*) on selected cancer cell lines

[1]Tan, B. L., [1,2,*]Norhaizan, M. E., [1]Suhaniza, H. J., [1]Lai, C. C., [3]Norazalina, S. and [4]Roselina, K.

[1]Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, [2]Laboratory of Molecular Biomedicine and [3]Laboratory Cancer Research UPM-MAKNA, Institute of Bioscience, [4]Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Article history

Received: 12 February 2013
Received in revised form: 15 May 2013
Accepted: 18 May 2013

Keywords

Temukut
Brewers' rice
Colon cancer
Ovary cancer
Liver cancer cell lines

Abstract

Temukut, or brewers' rice, is a mixture of broken rice, rice bran, and rice germ. Extensive studies have been conducted on rice bran, which possesses various health benefits. *Temukut*, however has been less well studied. The present study aimed to investigate the antioxidant and growth inhibition properties of *temukut* extract using colon cancer (HT-29), ovary cancer (Caov-3), and liver cancer (HepG2) cell lines. The antioxidant activity was determined by the β-carotene bleaching assay, analysis of the DPPH radical scavenging capacity, and a FRAP assay. The total phenolic compounds, oryzanol, vitamin E, and phytic acid levels in *temukut* were also investigated. The antiproliferative activity was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. There was a significant difference in the cytotoxicity of two types of *temukut* extract (water and methanol) for HT-29 and Caov-3 cells ($p < 0.05$) but not for HepG2 cells. The HepG2 cell line is the least sensitive to *temukut*, (IC_{50} = 55.30 µg/mL), whereas the highest sensitivity was observed in Caov-3 cells (IC_{50} =36.67 µg/mL). No cytotoxic effect of *temukut* was observed on normal cells (BalBlc3T3). Although the content of the phytochemicals studied (total phenolic compounds, vitamin E, oryzanol, and phytic acid) in *temukut* was lower than that in rice bran, as has been previously reported, the present study demonstrated *temukut*'s potential to inhibit the proliferation of HT-29, Caov-3, and HepG2 cells.

Introduction

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries (World Health Organization, 2008). In Malaysia, cancer has become a major health problem and estimated to be 40,000 cancer cases were diagnosed in 2007 and registered in the National Cancer Registry (National Cancer Registry Malaysia, 2011). There are evidences showing that nutrition plays an important role in the etiology of cancer (Katyana *et al.*, 2002). These are further supported by epidemiological studies, pre-clinical and clinical interventions which highlighted that minor component of fiber, other nutrients, and phytochemicals present in foods may have protective role against various cancer (Ferguson and Harris, 1996).

Rice (*Oryza sativa* L.) is a food staple for nearly half of the world's population and is cultivated in over 100 countries (Ohtsubo *et al*., 2005). In addition to rice husk and rice bran, *temukut* or brewers' rice is also a byproduct of rice processing. *Temukut* is a mixture of broken rice, rice bran, and rice germ and is used almost exclusively in the production of beer and for animal feeds. There are extensive studies on the health benefits of rice bran but few on *temukut.* Although *temukut* is considered a lower quality product than rice bran due to its lower nutritional value, its phytochemical content might be significant due to the presence of rice germ and rice bran. Rice germ contains bioactive compounds such as vitamin E, oryzanol, and γ-aminobutyric acid (Yu *et al*., 2007), and rice bran contains vitamin E and oryzanol (Danielski *et al*., 2005). Research conducted in the last two decades has shown that rice bran contains a unique complex of antioxidant compounds (Moldenhauer *et al.*, 2003). These phytonutrients were found to be beneficial for the management of several metabolic disorders, such as diabetes, hypertension, cardiovascular disease, and cancer (Devi and Arumughan, 2007). The anticancer effects of rice bran-derived bioactive components are mediated through their ability to induce apoptosis, inhibit cell proliferation and alter cell cycle progression in malignant cells. Rice germ also has been reported to

*Corresponding author.
Email: *nhaizan@medic.upm.edu.my*
Tel.: +603-89472427; Fax: +603-89426769

play a key role in prevention of cancer (Katyama *et al*., 2002).

Cisplatin, doxorubicin, and 5-fluorouracil are commonly used to treat cancers (El-Sayyad *et al.*, 2009). However, these drugs produce unwanted side effects, such as low blood counts, poor appetite, and nausea (Alvarez-Cabellos *et al*., 2007; Ajani, 2008; Dank *et al.*, 2008). The present study will provide information on the bioactive compounds in *temukut* that can provide baseline data for future research. Therefore, the purpose of the present study was to determine the antioxidant properties and anti-proliferative potential of *temukut* extract on colon cancer (HT-29), ovary cancer (Caov-3), and liver cancer (HepG2) cells.

Materials and Methods

Chemicals and reagents

The high performance liquid chromatography (HPLC) grade acetonitrile; methanol; isopropanol; hexane; α-, β-, γ-, and δ-tocopherols; and α-, β-, γ-, and δ-tocotrienols were purchased from Merck (Darmstadt, Germany). Petroleum ether (40-60°C), boric acid, and sodium hydroxide were obtained from Fisher Scientific (Loughborough, Leicestershire, UK). The phytic acid standard, 5-fluorouracil, and fatty acid methyl ester standard mixtures were purchased from Sigma-Aldrich (St. Louis, USA), while the gamma-oryzanol standard was purchased from Santa Cruz (USA). All other chemicals and reagents used were analytical grade and purchased from Sigma-Aldrich.

Dulbecco's Modified Eagle Medium (DMEM), RPMI-1640 medium, fetal bovine serum, and penicillin–streptomycin were purchased from PAA (Austria). 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide were obtained from Sigma (USA).

Cell lines

The colon cancer (HT-29), ovary cancer (Caov-3), liver cancer (HepG2), and BALB/c 3T3 (mouse fibroblast) cell lines were obtained from the American Type Culture Collection (USA).

Sample preparation

Temukut (MR 219) was obtained from a local milling factory, BERNAS in Seri Tiram Jaya, Selangor, Malaysia. To stabilize *temukut*, it was heated for 4 min at 2450 MHz using a microwave oven (Model EMO-1706, ELBA). The stabilized sample was left to cool at room temperature before sealing the lid of the container and placing it in a black bag. The sample was stored at 4°C prior to analysis. *Temukut* was stabilized to prevent the development of oxidative rancidity during storage. The stabilized *temukut* was extracted with water or methanol following the modified method of Yu *et al.* (2007). Five g of finely ground *temukut* was extracted with 40 mL of methanol or distilled water in a shaker (Heidolph Inkubatorhaube, Germany) for 2 h at 40°C. The slurry was filtered through filter paper (Whatman no. 1). The filtrate from the methanol extract was dried using a rotary evaporator (Büchi Rotavapor R-200, Switzerland), while the filtrate from the water extract was freeze-dried to obtain a concentrated powder.

Growth inhibition assay

The colorectal cancer (HT-29) cell line was cultured in DMEM while the ovary cancer (Caov-3), the liver cancer (HepG2), and the normal fibroblast (3T3) cell lines were cultured in RPMI medium supplemented with 10% fetal bovine serum and 1% of penicillin-streptomycin. All cells were incubated in a humidified atmosphere, at 37°C under 5% CO_2. The tetrazolium salt assay was used to evaluate the proliferation of the cells following the modified methods of Shamsuddin *et al.* (1996) and Vucenik *et al.* (1998). After the cells reached 70-80% confluence, they were detached by trypsinization. The cells marked with trypan blue were counted and seeded at a density of 1×10^5 cells/well in 96-well microtiter plates overnight in an incubator. The medium was removed the next day, and 5 to 320 µg/mL of *temukut* extract (water or methanol) was added to each well. After 72 h, 20 µL of MTT solution was added and left for 4 h in the incubator. The formazan product was solubilized in 100 µL of dimethyl sulfoxide, and the absorbance was measured at 570 nm using an Elisa plate reader.

Determination of total phenolic compounds

The total phenolic compound content in the *temukut* extracts was determined using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Approximately 0.3 mL of *temukut* extract was added to 1.5 mL of Folin-Ciocalteu reagent (diluted 10 times with water) and 1.2 mL of sodium carbonate (7.5% w/v). The tubes were vortexed and allowed to stand for 30 min before the absorbance was measured at 765 nm. The total phenolic compound content was expressed as gallic acid equivalents (GAE).

Determination of antioxidant activity by the β-carotene bleaching test

The β-carotene bleaching test was performed using a modified method of Miller (1971). A solution

of 5 mg/10 mL of β-carotene was prepared in chloroform, and 3 mL of this solution was pipetted into a 100-mL round-bottom flask. The chloroform was removed by vacuum evaporation at 40°C. Following evaporation, 40 mg of linoleic acid, 400 mg of Tween 40 emulsifier, and 100 mL of distilled water were added to the flask, followed by vigorous shaking. An aliquot of 4.8 mL of this emulsion was transferred into a series of test tubes containing 200 µL of the extracts or methanol (control). Butylated hydroxytoluene (BHT) was used as the reference antioxidant. The zero time absorbance was measured at 470 nm immediately after the addition of the emulsion to each tube. The subsequent absorbance readings were recorded over a 2-h period at 20-min intervals by keeping the reaction tubes in a water bath set to 50°C. Blank samples devoid of β-carotene were prepared for background subtraction. The capacity of the extracts to protect against oxidation of β-carotene was determined as follows:

$$((A_{t\text{-}0\ Sample} - A_{t\text{-}0\ Blank}) - (A_{t\text{-}120\ min\ Sample} - A_{t\text{-}120\ min\ Blank})) / ((A_{t\text{-}0\ Control}) - (A_{t\text{-}120\ min\ Control})) = C$$

$$\beta\text{-carotene retention (\%)} = 100\% - (C) \times 100\%$$

where A is the absorbance at a particular time and C is the carotene depletion factor.

Determination of antioxidant activity by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging capacity

The DPPH radical scavenging capacity was determined using a UV-visible spectrophotometer (Shimadzu, Australia) following the method of Zhang and Hamauzu (2004). Approximately 1.5 mL of 0.1 nM DPPH in methanol was added to a 0.5-mL sample. The mixture was shaken for 15 s and allowed to stand for 1 h at room temperature. The absorbance was read at 517 nm. Ascorbic acid (standard) and solute controls were prepared using the same procedure. The results of the DPPH assay are reported as EC_{50}, which is the concentration of the sample required to scavenge 50% of the DPPH free radicals.

Determination of antioxidant activity from the ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power of *temukut* extracts was determined using the potassium ferricyanide–ferric chloride method (Oyaizu, 1986). A 1-mL of different dilutions of the extracts was added to 2.5 mL of 0.2 mol/L phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixtures were incubated at 50°C for 20 min after 2.5 mL of trichloroacetic acid (10%) was added. A 2.5-mL aliquot of the mixture was mixed with 2.5 mL of water and 0.5 mL of 1% ferric chloride. The absorbance was measured at 700 nm after allowing the solution to stand for 30 min. An aqueous solution of ferrous sulphate was used as the standard. The ferric reducing antioxidant power values were expressed as mg Fe/g, determined using a standard curve.

Determination of phytic acid content

The phytic acid content was determined using the rapid method described by Haug and Lantzsh (1983). Approximately 0.06 g of *temukut* was added to 10 mL of 0.2 mol/L hydrochloric acid for an overnight incubation at 4°C. A 0.5-mL aliquot of *temukut* extract was pipetted into a test tube, and 1 mL of ammonium iron (III) sulphate solution was added, followed by incubation in a boiling water bath for 30 min. After cooling to room temperature, 2 mL of (1% v/v) 2′,2-bipyridine solution was added, and the absorbance was measured at 519 nm, using distilled water as a blank control. Calibrations were performed with known concentrations of phytic acid.

Oil extraction

The oil contents of *temukut* were extracted using the Bligh and Dyer method following the protocol of Manirakiza *et al.* (2001). Five g of *temukut* was mixed with 20 mL of methanol and 10 mL of chloroform. The mixture was centrifuged (Zentrifugen, Germany) for 2 min at 2200 rpm. Then, 10 mL of chloroform was added, and the mixture was centrifuged again for 2 min. The mixture of *temukut* and solvents was filtered through Whatman No.1 filter paper. The *temukut* residue was extracted two more times using the same procedure. The mixture of solvents and oil were dried using a rotary evaporator (Büchi Rotavapor R-200, Switzerland). The oil was further dried in an oven at 40°C for 1 h.

Determination of oryzanol content

The oryzanol content was determined using the method of Rogers *et al.* (1993) by reverse-phase HPLC, which consisted of a Hewlett-Packard (Germany) Model G1311A HPLC connected to an ALS Autoinjector Series 1100 (Hewlett Packard, Germany). Acetonitrile/methanol/isopropanol (50:45:5 by volume) was used as the mobile phase with a flow rate of 0.8 mL/min. The injection volume was 10.0 µL. The column temperature was 40°C, and the ultraviolet detector (Hewlett-Packard Model 1100 Series Photodiode Array Detector) was set at 325 nm. The oryzanol from the sample was separated

on a Hewlett-Packard 250 x 4 mm column packed with 5 mm ODS (C18) Hypersil silica, and oryzanol was used as a standard for calibration.

Determination of vitamin E contents

The vitamin E content of *temukut* was analyzed using an HPLC protocol based on the modified method of Azrina (2005). The sample was extracted following the method of Panfili *et al*. (2003). A 4-g sample was added to 10 mL of ethanolic pyrogallol (60 g/L), 4 mL of ethanol (95%), 4 mL of sodium chloride (10 g/L), and 4 mL of potassium hydroxide (600 g/L), under nitrogen. The solution was incubated in a water bath (70°C) for 45 min and mixed every 5-10 min. The lipids were dried and dissolved with 1 mL of the mobile phase solution (hexane: propanol, 99:1, v/v) before being injected into a 2 mL vial. The vitamin E in *temukut* was analyzed a using YMC-Pack SIL column (150 × 6.0 mm ID, S-5 µm, 12 nm) with a fluorescence detector set to an excitation wavelength of 290 nm and an emission wavelength of 330 nm, at a flow rate 1.0 mL/min.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences version 17 software. Comparison between the *temukut* extracts' (water or methanol) content of total phenolic compounds and IC_{50} values was analyzed using the Independent-Samples T test. The antioxidant activities were analyzed using a one-way ANOVA. A value of $p < 0.05$ was considered significant.

Results and Discussion

MTT assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay is a simple and reliable assay that measures the viability of cells, and it can be used to screen antiproliferative agents (Manosroi *et al.*, 2006). Different doses of *temukut* extract (methanol or water), ranging from 5-320 µg/mL, were applied to colon cancer (HT-29), ovary cancer (Caov-3), liver cancer (HepG2) cell lines, whereas 0.2-200 µg/mL was used for mouse fibroblast (BalBlc3T3) cell lines. The cytotoxicity of the *temukut* extracts is expressed as IC_{50}, which is the concentration causing a 50% inhibition of cell proliferation. The results showed that the water and methanol *temukut* extracts had cytotoxicity activity against all cancer cell lines studied. The water extract of *temukut* exert cytotoxic effects against the HT-29, Caov-3, and HepG2 cells with IC_{50} values of 38.33 ± 6.51, 36.67 ± 5.77, and 55.30 ± 5.51 µg/mL, respectively, after 72 h of exposure (Table 1). As indicated in the National Cancer Institute Guidelines (USA), extracts with $IC_{50} < 100$ µg/mL are considered antiproliferative agents (Suffness and Pezzuto, 1990). The results demonstrated that there was a significant difference between the water and methanol extracts in terms of their cytotoxicity in the HT-29 and Caov-3 cells ($p < 0.05$), but not in the HepG2 cells. The Caov-3 and HT-29 cells were highly inhibited by the water extract of *temukut*. These results showed that the Caov-3 and HT-29 cells were more sensitive to the *temukut* extract than were the HepG2 cells. Identification of lowest cytotoxic dose of *temukut* extract for non-tumorigenic cells was performed using a normal cell lines (mouse fibroblast, BalBlc3T3) as comparison. BalBlc3T3 cell line is recommended by US National Institute of Environmental Health Sciences, Interagency Coordinating Committee in the Validation of Alternative Methods for determining basal cytotoxicity (National Institute of Environmental Health Sciences, 2001). No toxic effect on BalBlc3T3 cell was observed for either *temukut* extract (IC_{50} was not determined) (Figure 1). This result showed that the water and methanol extracts of *temukut* are selective anticancer agents because they caused cell death in the HT-29, Caov-3, and HepG2 cell lines but not the BalBlc3T3 cell line.

The commercially available drug 5-fluorouracil, which has been used in the treatment of several types of cancers, including breast cancer, head and neck, and gastrointestinal cancer (El-Sayyad *et al*., 2009), however, was toxic to the BalBlc3T3 cell line, as shown in Figure 1. Previous reports described histopathological and ultrastructural abnormalities

Table 1. Percentage of viable cells in the different cancer cell lines treated with *temukut* extracts

Cancer cell lines	IC_{50} (µg/mL)	
	Water extract	Methanol extract
HT-29	38.33±6.51[a]	54.00±5.29[b]
Caov-3	36.67±5.77[a]	62.33±2.52[b]
HepG2	55.30±5.51[a]	56.00±8.54[a]

Note: Values are reported as the mean ± SD from three replicate experiments (n = 3)
Values with different letter superscripts are significantly difference ($p < 0.05$)

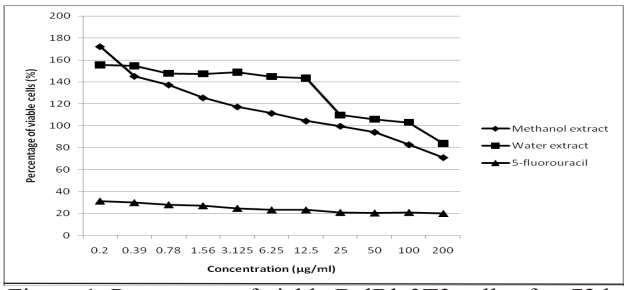

Figure 1. Percentage of viable BalBlc3T3 cells after 72 h exposure to *temukut* extracts or 5-fluorouracil

Table 2. Total phenolic content (TPC) and antioxidant activity of *temukut* determined using the β-carotene bleaching test, the DPPH assay, and the ferric reducing antioxidant power assay

TPC and antioxidant assays	Samples and standard			
	Water extract	Methanol extract	Ascorbic acid	BHT
Total phenolic content (mg GAE/g)	0.20±0.10[a]	0.32±0.05[a]		
β-carotene bleaching test (%)	71.97±3.44[ab]	61.40±6.93[a]		83.47±5.77[b]
DPPH EC_{50} (mg/mL)	3.97±0.86[a]	2.90±0.10[a]	0.14±0.006[b]	
Ferric reducing antioxidant power (mg Fe/g)	4.88±0.38[a]	4.77±0.17[a]		

Note: Values are reported in mean ± SD from three replicate assays (n = 3)
Values with different letter superscripts in a row are significantly difference ($p < 0.05$).
BHT, butylated hydroxytoluene.

in the liver after the intraperitoneal administration of 5-fluorouracil, such as apoptotic cell death, the presence of numerous inflammatory cells, and markedly affected cytoplasmic organelles with collagenous fibrils present in a number of the necrotic cells (El-Sayyad *et al*., 2009). We suspected that the antiproliferative effect of *temukut* could be due to the presence of various bioactive phytochemicals, such as polyphenols, oryzanol, vitamin E, and phytic acid. Therefore, the content of these phytochemicals in *temukut* was also studied.

Total phenolic compounds

Phenolic compounds, found mainly in plants, are powerful chain-breaking antioxidants with the ability to neutralize free radicals and play a crucial role in stabilizing lipid peroxidation (Sharma and Gupta, 2008). In this study, the total phenolic content was determined using the modified Folin–Ciocalteu method and expressed as mg of gallic acid equivalents (GAE) per g. The water and methanol extracts yielded 0.20 ± 0.10 and 0.32 ± 0.05 mg GAE/g of dry weight of total phenolic content, respectively (Table 2). The difference between the values was not significant ($p > 0.05$). The amount of total phenolic compounds in *temukut* was lower than that found in rice bran. Chotimarkorn *et al.* (2008) reported that the values for the total phenolic content of the bran from different cultivars of rice in Thailand ranged from 2.2-3.2 mg GAE/g. It is expected, as indicated by Zhou *et al.* (2004), that the phenolic acids of rice are mostly concentrated in the bran.

Antioxidant activity

In the β-carotene bleaching test, the oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of a hydrogen atom from the diallylic methylene groups (Kumaran and Karunakaran, 2006). The antioxidant activity of the water and methanol extracts of *temukut* and the BHT standard were determined by the β-carotene bleaching method to be 71.97 ± 3.44%, 61.40 ± 6.93%, and 83.47 ± 5.77%, respectively (Table 2). One-way ANOVA showed that there was no significant difference between the antioxidant activity of the water and methanol extracts ($p > 0.05$). BHT had a higher antioxidant activity than either extract, but it was not significantly different from that of the water extract. This result indicated that the ability of *temukut* to reduce the extent of β-carotene bleaching by neutralizing the linoleate-free radicals formed is not as strong as that of BHT. The water extract performed better than the methanol extract in reducing the oxidation of β-carotene.

The DPPH radical scavenging capacity assay was used to examine the antioxidant activity of *temukut*. The DPPH radical assay is based on the ability of a compound to transfer electrons from a donor molecule to the corresponding radical (Fugliano *et al*., 1999). It is the easiest method to measure the ability of antioxidants to intercept free radicals (Norhaizan *et al*., 2011). The EC_{50} values of the water and methanol extracts of *temukut* determined by the DPPH assay were significantly higher (3.97 ± 0.86 mg/mL and 2.90 ± 0.10 mg/mL, respectively) than that of ascorbic acid (0.14 ± 0.006 mg/mL) ($p < 0.05$) (Table 2). However, there was no significant difference in the DPPH radical scavenging capacity of the water and methanol extracts of *temukut* ($p > 0.05$). This result indicated that the ability of *temukut* to scavenge free radicals is lower than that of ascorbic acid. The EC_{50} value of *temukut* was higher than those of several types of rice bran from Thailand, which ranged from 0.38-0.74 mg/mL (Chotimarkorn *et al.*, 2008). This finding is supported by an earlier study by Butsat and Siriamornpun (2010), which showed that the bran and husk of rice had a higher antioxidant activity than did milled or brown rice, based on DPPH and ferric reducing antioxidant power assays.

The ferric reducing antioxidant power assay is a method to measure the reducing power of antioxidants.

Table 3. Bioactive compounds in *temukut*

Bioactive compounds		Amount
Phytic acid (mg/g)		0.38 ± 0.01
Oryzanol (mg/g)		14.20
Vitamin E (μg/g)		
	α-tocopherol	0.31
	γ-tocopherol	2.62
	δ-tocopherol	0.47
	α-tocotrienol	0.12
	γ-tocotrienol	3.10
	δ-tocotrienol	0.03

The ferric (Fe^{3+})-ferrous (Fe^{2+}) transformation in the presence of the extracts was investigated. The results demonstrated that the water extract had a higher ferric reducing antioxidant power capacity than the methanol extract although the difference was not significant ($p > 0.05$) (Table 2). The ferric reducing capacity of the *temukut* extracts may serve as an antioxidant activity, where the reducing power of the extracts increased as the concentration increased. An increase in the absorbance of the reaction mixture showed the increase of the reducing power of the sample. Antioxidant activity can be due to mechanisms such as the prevention of chain initiation, the binding of transition metal ion catalysts, the decomposition of peroxides, the prevention of continued hydrogen abstraction, reductive capacity, and free radical scavenging (Sharma and Gupta, 2008). The ferric reducing antioxidant power of different cultivars of rice bran in Thailand ranged from 0.10-0.53 mg/mL, which was comparatively lower than the values for both water and methanol extracts of *temukut*. Chotimarkorn *et al.* (2008) reported that the reducing power of rice bran extracts was associated with the total phenolic content. This finding is consistent with the total phenolic content of *temukut* being less than that of rice bran.

Phytic acid

Phytic acid has been shown to have antioxidant activity through several mechanisms (Norhaizan *et al.*, 2011). It may lower the incidence of colonic cancer and protect against inflammatory bowel disease (Nelson *et al*., 1989) through the chelation of iron, which is associated with the suppression of the iron-related initiation and promotion of carcinogenesis (Norhaizan *et al.*, 2011). Phytic acid binding to free iron will suppress a number of iron-driven oxidative reactions and serves as a potent antioxidant for the preservation of seeds (Norhaizan *et al*., 2011). The result of this study showed that the content of phytic acid in *temukut* was 0.38 mg/g (Table 3). The value is within the range reported by Nam *et al.* (2005) for rice bran, which was 0.22 to 2.22%.

Oryzanol and vitamin E contents of temukut

As reported by Imsanguan *et al.* (2008) and Nam *et al.* (2005), both tocotrienols and tocopherols have anti-oxidative as well as antimutagenic and anticancer activity. Several preliminary studies on animals have shown that γ-oryzanol may inhibit tumor cell growth (Asanamo *et al*., 1994). The data from our study showed that the concentrations of γ-oryzanol and vitamin E in *temukut* were 14.20 mg/g and 6.65 μg/g, respectively (Table 3). The content of oryzanol in *temukut* was lower than that of the rice bran of local mixed rice varieties, which ranged from 23.7-43.0 mg/g (Azrina *et al.*, 2008). The amount of vitamin E in *temukut* was much lower than that of rice bran and rice germ, which contained 612.19 and 106.01 μg/g of vitamin E, respectively (Yu *et al.*, 2007). Gamma-tocotrienol (3.10 μg/g) was the major type of vitamin E in *temukut*, followed by γ-tocopherol (2.62 μg/g), δ-tocopherol (0.47 μg/g), α-tocopherol (0.31 μg/g), α-tocotrienol (0.12 μg/g), and δ-tocotrienol (0.03 μg/g) (Table 3). This finding is in agreement with previous research done by Butsat and Siriamornpun (2010), who reported that the bran has the highest oryzanol and tocopherol content compared to other parts of rice.

The finding that the water extract of *temukut* demonstrated significantly better antiproliferative activity than the methanol extract on the cancer cells studied suggested that bioactive compounds other than polyphenol, vitamin E or oryzanol, which are soluble in water, might contribute to this anticancer effect. More research is necessary to identify the bioactive compounds in *temukut*; the one receiving the greatest attention currently is a bioactive peptide. As shown by Kannan *et al.* (2008), a bioactive peptide extracted from rice bran can inhibit the proliferation of human colon and liver cancer cells.

Conclusion

Even though the content of phytochemicals studied in *temukut* (total phenolic compounds, vitamin E, oryzanol, and phytic acid) was lower than that of rice bran, as previously reported, *temukut* has a potential use in inhibiting the proliferation of colon cancer (HT-29), ovary cancer (Caov-3), and liver cancer (HepG2) cells without cause any cytotoxicity on normal cells (BalBlc3T3). Moreover, because *temukut* has high carbohydrate content and is edible, it can be used in a variety of food products. Further *in vivo* and human studies need to be performed to evaluate the safety and clinical utility of our finding.

Acknowledgements

Special thanks to BERNAS, Seri Tiram Jaya, Selangor, Malaysia for supplying the *temukut* samples and to the laboratory staff of the Department of Nutrition and Dietetics and the Laboratory of Cancer Research (MAKNA) UPM for their technical assistance. We also acknowledge the financial assistance provided by Ministry of Higher Education, Malaysia (RUGS-2 project no. 05-02-12-1862RU).

References

Ajani, J. A. 2008. Optimizing docetaxel chemotherapy in patients with cancer of the gastric and gastroesophageal junction: evolution of the docetaxel, cisplatin, and 5-fluorouracil regimen. Cancer 113: 945-955.

Alvarez-Cabellos, R., Garcia-Carbonero, R., Garcia-Lacalle, C., Gomez, P., Tercero, A., Sanchez, D. and Paz-Ares, L. 2007. Fluorouracil-based chemotherapy in patients with gastrointestinal malignancies: influence of nutritional folate status on toxicity. Journal of Chemotherapy 19: 744-749.

Asanamo, M., Takahashi, K., Miyobi, M., Yamamoto, K., Yoshini, N., Mori, H. and Kawazoe, Y. 1994. Inhibitory effect of topical application of polymerized ferulic acid, a synthetic lignin on tumor promotion in mouse skin two-stage tumorigenesis. Carcinogenesis 15: 2069-2071.

Azrina, A. 2005. The effects of rice bran and blended rice bran oils on indices of coronary heart disease. Selangor, Malaysia: Universiti Putra Malaysia, Ph.D. Thesis.

Azrina, A., Maznah, I. and Azizah, A. H. 2008. Extraction and determination of oryzanol in rice bran of mixed herbarium UKMB; AZ 6807: MR 185, AZ 6808: MR 211, AZ6809: MR 29. ASEAN Food Journal 15: 89-96.

Butsat, S. and Siriamornpun, S. 2010. Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. Food Chemistry 119: 606-613.

Chotimarkorn, C., Benjakul, S. and Silalai, N. 2008. Antioxidant components and properties of five long-grained rice bran extracts from commercial available cultivars in Thailand. Food Chemistry 111: 636–641.

Corpet, D. E. and Taché, S. 2002. Most effective colon cancer chemopreventive agents in rats: a systematic review of aberrant crypt foci and tumor data, ranked by potency. Nutrition and Cancer 43: 1-21.

Danielski, L., Zetzl, C., Hense, H. and Brunner, G. 2005. A process line for the production of raffinated rice oil from rice bran. Journal of Supercritical Fluids 34: 133-141.

Dank, M., Zaluski, J., Barone, C., Valvere, V., Yalcin, S., Peschel, C., Wenczl, M., Goker, E., Cisar, L., Wang, K. and Bugat, R. 2008. Randomized phase III study comparing irinotecan combined with 5-fluorouracil and folinic acid to cisplatin combined with 5-fluorouracil in chemotherapy naive patients with advanced adenocarcinoma of the stomach or esophagogastric junction. Ann Oncology 19: 1450-1457.

Devi, R. R. and Arumughan, C. 2007. Antiradical efficacy of phytochemical extracts from defatted rice bran. Food and Chemistry Toxicology 45: 2014–2021.

El-Sayyad, H., Ismail, M. F., Shalaby, F. M., Abou-El-Magd, R. F., Gaur, R. L., Fernando, A., Raj, M. H. G. and Ouhtit, A. 2009. Histopathological effects of cisplatin, doxorubicin and 5-fluorouracil (5-FU) on the liver of male albino rats. International Journal Biology Science 5: 466-473.

Ferguson, L. R., and Harris, P. J. 1996. Studies on the role of specific dietary fibres in protection against colorectal cancer. Mutation Research 350: 173–184.

Fugliano, V., Verde, V., Randazzao, G. and Ritieni, A. 1999. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wine. Journal of Agricultural and Food Chemistry 47: 1035-1040.

Haug, W. and Lantzsch, H. J. 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. Journal of Science and Food Agriculture 34: 1423-1426.

Imsanguan, P., Roaysubtawee, A., Borirak, R., Pongamphai, S., Douglas, S. and Douglas, P. S. 2008. Extraction of α-tocopherol and γ-oryzanol from rice bran. LWT-Food Science and Technology 41: 1417-1424.

Kannan, A., Hettiarachchy, N. and Johnson, M. G. 2008. Human colon and liver cancer cell proliferation inhibition by peptide hydrolysates derived from heat-stabilized defatted rice bran. Journal of Agricultural and Food Chemistry 56: 11643-11647.

Katyama, M., Yoshimi, N., Yamada, Y., Sakata, K., Kuno, T., Yoshida, K., Qiao, Z., Vihn, P. Q., Iwasaki, T., Kobayashi, H. and Mori, H. 2002. Preventive effect of fermented brown rice and rice bran against colon carcinogenesis in male F344 rats. Oncology Report 9: 817-822.

Kumaran, A. and Karunakaran, R. J. 2006. Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromaticus. Food Chemistry 97: 109–114.

Lim, A. K. H. and Lim, G. C. C. 1993. The burden of advanced cancer in Malaysia. In Proceedings of the National Hospice Conference, p. 13-18. Penang: National Cancer Society of Malaysia, Penang Branch.

Manirakiza, P., Covaci, A. and Schepens, P. 2001. Comparative study on total lipid determination using Soxhlet, Roese-Gottlieb, Bligh and Dyer, and modified Bligh & Dyer extraction methods. Journal of Food Composition and Analysis 14: 93-100.

Manosroi, J., Dhumtanom, P. and Manosroi, A. 2006. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. Cancer Letters 235: 114-120.

Miller, H. E. 1971. A simplified method for the evaluation of antioxidants. Journal of American Oil Chemists' Society 48: 91.

Moldenhauer, K. A., Champagne, E. T., McCaskill, D.

R. and Guraya, H. 2003. Functional products from rice. In Mazza, G. (Eds). Functional Foods, p. 71-89. Switzerland: Technomic Publishing Co. Inc., Lancaster, Basel.

Nam, S. H., Choi, S. P., Kang, M. Y., Kozukue, N. and Friedman, M. 2005. Antioxidative, antimutagenic, and anticarcinogenic activities of rice bran extracts in chemical and cell assays. Journal of Agricultural and Food Chemistry 53: 816–822.

National Institute of Environmental Health Sciences (NIEHS). 2001. Report of the international workshop on *in vitro* methods for assessing acute systemic toxicity. NIEHS: Research Triangle Park, NC.

National Cancer Registry Malaysia. 2011. National Cancer Registry

Report: Malaysia Cancer Statistics- Data and Figure 2007. Ministry of Health Malaysia.

Nelson, R. L., Yoo, S. J., Tanure, J. C., Andrianopoulos, G. and Misumi, A. 1989. The effect of iron on experimental colorectal carcinogenesis. Anticancer Research 9: 1477-1482.

Norhaizan, M. E., Ng, S. K., Norashareena, M. S. and Abdah, M. A. 2011. Antioxidant and cytotoxicity effect of rice bran phytic acid as an anticancer agent on ovarian, breast and liver cancer cell lines. Malaysian Journal Nutrition 17: 367-375.

Ohtsubo, K., Suzuki, K., Yasui, Y. and Kasumi, T. 2005. Bio-functional components in the processed pre-germinated brown rice by a twin-screw extruder. Journal of Food Composition and Analysis 18: 303–316.

Oyaizu, M. 1986. Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Jpn. Journal of Nutrition 44: 307–315.

Panfili, G., Fratianni, A. and Irano, M. 2003. Normal phase high-performance liquid chromatography method for the determination of tocopherols and tocotrienols in cereals. Journal of Agricultural and Food Chemistry 51: 3940-3944.

Rogers, E. J., Rice, S. M., Nicolosi, R. J., Carpenter, D. R., McClelland, C. A. and Romanczyk, L. J., Jr. 1993. Identification and quantitation of γ–oryzanol components and simultaneous assessment of tocopherols in rice bran oil. Journal of American Oil Chemists' Society 70: 301-307.

Shamsuddin, A. M., Yang, G. Y. and Vucenik, I. 1996. Novel anti-cancer functions of IP6: growth inhibition and differentiation of human mammary cancer cell lines *in vitro*. Anticancer Research 16: 3287-3292.

Sharma, S. K. and Gupta, V. K. 2008. *In-vitro* antioxidant studies of *Ficus racemosa* linn. root. Pharmacognosy Magazine 4: 70-74.

Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144–158.

Suffness, M. and Pezzuto, J. M. 1990. Assays related to cancer drug discovery. In Hostettmann, K. (Ed). Methods in plant biochemistry: Assays for bioactivity, p. 71-133. London: Academic Press.

Vucenik, I., Kalebic, T., Tantivejkul, K. and Shamsuddin, A. M. 1998. Novel anticancer function of inositol hexaphosphate: inhibition of human rhabdomyosarcoma *in vitro* and *in vivo*. Anticancer Research 18: 1377-1384.

World Health Organization. 2008. The global burden of disease: 2004 Update. Geneva: World Health Organization.

Yu, S., Nehus, Z. T., Badger, T. M. and Fang, N. 2007. Quantification of vitamin E and gamma-oryzanol components in rice germ and bran. Journal of Agricultural and Food Chemistry 55: 7308-7313.

Zhang, D. and Hamauzu, Y. 2004. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chemistry 88: 503–509.

Zhou, Z., Robards, K., Helliwell, S. and Blanchard, C. 2004. The distribution of phenolic acids in rice. Food Chemistry 87: 401–406.