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

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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 inhibitory activity 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*, whereas the highest sensitivity was observed in Caov-3 cells (IC50 = 55.30 μg/mL). No cytotoxic effect of *temukut* was observed on normal cells (BalBc3T3). 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.

Keywords

Temukut, Brewers’ rice, Colon cancer, Ovary cancer, Liver cancer cell lines

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, preclinical 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 phytoneutrients 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...
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). All cell lines were purchased 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% CO₂. 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 1×10⁵ 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 (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 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:

\[
\frac{(A_{t=0 \text{ Sample}} - A_{t=0 \text{ Blank}}) - (A_{t=120 \text{ min Sample}} - A_{t=120 \text{ min Blank}})}{(A_{t=0 \text{ Control}}) - (A_{t=120 \text{ min Control}})} = C
\]

β-carotene retention (%) = 100% - (C) x 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 mM 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.

**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.
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 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 IC₅₀ 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 (BalBc3T3) cell lines. The cytotoxicity of the temukut extracts is expressed as IC₅₀, 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 IC₅₀ values of 38.33 ± 6.51, 36.67 ± 5.77, and 55.30 ± 5.51 μg/mL, respectively, after 72 h of exposure to temukut extracts or 5-fluorouracil.

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 BalBc3T3 cell line, as shown in Figure 1. Previous reports described histopathological and ultrastructural abnormalities

<table>
<thead>
<tr>
<th>Cancer cell lines</th>
<th>Water extract (μg/mL)</th>
<th>Methanol extract (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT-29</td>
<td>38.33 ± 6.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.00 ± 5.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Caov-3</td>
<td>36.67 ± 5.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.33 ± 2.52&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HepG2</td>
<td>55.30 ± 5.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.00 ± 8.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Values are reported as the mean ± SD from three replicate experiments (n = 3). Values with different letter superscripts are significantly different (p < 0.05).
in the liver after the intraperitoneal administration of 5-fluouracil, 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 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

<table>
<thead>
<tr>
<th>TPC and antioxidant assays</th>
<th>Water extract</th>
<th>Methanol extract</th>
<th>Ascorbic acid</th>
<th>BHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolic content</td>
<td>0.20±0.10</td>
<td>0.32±0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg GAE/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-carotene bleaching test</td>
<td>71.97±3.44</td>
<td>61.40±6.93</td>
<td>83.47±5.77</td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>DPPH EC$_{50}$ (mg/mL)</td>
<td>3.97±0.86</td>
<td>2.90±0.10</td>
<td>0.14±0.006</td>
<td></td>
</tr>
<tr>
<td>Ferric reducing antioxidant power (mg Fe/g)</td>
<td>4.88±0.38</td>
<td>4.77±0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>a</td>
<td>a</td>
<td></td>
</tr>
</tbody>
</table>

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.
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.

Table 3. Bioactive compounds in temukut

<table>
<thead>
<tr>
<th>Bioactive compounds</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytic acid (mg/g)</td>
<td>0.38 ± 0.01</td>
</tr>
<tr>
<td>Oryzanol (mg/g)</td>
<td>14.20</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>0.31</td>
</tr>
<tr>
<td>γ-tocopherol</td>
<td>2.62</td>
</tr>
<tr>
<td>δ-tocopherol</td>
<td>0.47</td>
</tr>
<tr>
<td>α-tocotrienol</td>
<td>0.12</td>
</tr>
<tr>
<td>γ-tocotrienol</td>
<td>3.10</td>
</tr>
<tr>
<td>δ-tocotrienol</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Asanamo et al. (2005), who reported that the bran has the highest oryzanol and tocopherol content compared to other parts of rice.

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

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