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Cytotoxic activity and Antioxidant Potentials of Cold Pressed Rice Bran Oil

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Abstract: Rice bran is a byproduct of rice milling process which contains significant amount natural phytochemicals. A diverse panel of four rice bran oil verities was analyzed for growth inhibition of human colon cancer using the Methyl Thiazol Tetrazolium (MTT) assay. The antioxidant property was evaluated by free radical diphenyl-picryhydrazyl scavenging assay (DPPH) and ferric reducing antioxidant power (FRAP) assay. The result of MTT assay revealed that inhibit proliferation of colon cancer at 0.19-0.68 mg/ml, inhibition varied from 5.16-99.42%. In term of antioxidant activities demonstrated 51.23-100%, 0.67-98.5% against DPPH method and FRAP assay, respectively, depending on the verities of bran oil and concentration used. In this study found that Hom-Mali Gorkho has high potential for both anti cancer and antioxidant activities. It was concluded that varieties of rice bran oil is a potential source of bioactive compound against colon cancer cell line and antioxidant activity.
Keywords: cytotoxicity, antioxidant property, cold press, rice bran oil.

Introduction

Rice is the most important agricultural product in Thailand, since it is a staple food for most of the population in this region. Rice milling yields 70% of rice (endosperm) as the major product and byproducts consist of 20% rice husk, 8% rice bran and 2% rice germ. Rice bran can be used as feed or as a source of rice bran oil. Earlier studies have reported that rice bran oil is an edible oil of unsaturated fatty acid (monounsaturated fatty acid 45% and polyunsaturated fatty acid 37%)[1]. In Rice bran oil (RBO) comprises the rich source of many nutraceutical like, oryzanol, tocopherols, vitamin E, ferulic acid, phytic acid, lecithin, inositol and wax[2]. These bioactive rice bran components were found to display range of antioxidant activities that could be directly related to their anticancer activity[3]. Colon cancer is rapidly rising in Asia. The incidence in many Asian countries are in fact on par with the west. The occurrence of colon cancer is strongly related to age, with 90% of the case arising in

people who are 50 years or older[4]. It is now the third most common malignant diseases in both men and women in Asia[5]. Conventional treatment such as surgical resection, radiation therapy and chemotherapy are not still satisfactory and prevention of this disease or at least stopping it at its inception is important[6]. To relieve certain symptoms of cancer and to alleviate the side effects, which come with the use of conventional treatments, the medical world has now turned to complementary therapies for assistance. Ethno-traditional use of plant-derived natural products such as oils has been a major source for discovery of potential medicinal agents. Therefore, this study compares the IC_{50} (concentration needed for 50% cell growth inhibition) of rice bran oil across four rice varieties in order to explore their effects on the inhibition of cell growth and antioxidant activities from *Sativa oryza* L. (Hom-Pathum rice, Hom-Mali rice, Hom-Mali Gorkha rice, and Khaw-Khaw).

Materials and Methods

Chemical

RPMI-1640 medium, trypsin EDTA, penicillin, streptomycin, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), phosphate buffer saline (PBS), dimethyl sulfoxide (DMSO), fetal bovine serum (FBS), 1,1- diphenyl-2-picryl-hydrazyl (DPPH), **2,4,6-Tri(2-pyridyl)-*s*-triazine, TPTZ, gallic acid and trolox were purchased from Sigma Chemical Co., USA.**

Rice varieties

Four rice varieties were obtained from a local milling company in Thailand. The rice bran samples were used from different Thai rice varieties (Hom-Pathum, Hom-Mali, Hom-Mali Gorkha, and Khaw-Khaw). The samples were passed through sieve number 20 and immediately extracted under cold press conditions. The bran is extracted with screw press machine. The rice bran oil is keep in sterile bottle and store at room temperature for future use.

Cell culture and treatment conditions

COL205 human colon cancer cell lines were cultured in RPMI-1640 medium, supplemented with 10% FBS, 100 units/ml penicillin and 100 µg/ml streptomycin (complete medium). Cells were grown to confluence at 37 ^{o}C in humidified chamber with 5% CO_2 as monolayer adherent cultures in 75 cm^2 tissue culture flasks. Rice bran oil extracts were resuspended in cell culture medium at concentrations of 0.25, 0.5, 1, 2, 3 and 5 mg/ml.

Cytotoxic activity assay

The cytotoxic activity was determined by cell proliferation analysis using MTT assay as described from Watanapokasin *et al*.[7]. Cells were cultured in 96-well plates at a density of 1×10^4 cells/well in complete medium supplemented with 10% heat-inactivated new born calf serum, 50 IU/mL penicillin G sodium, 50 µg/mL streptomycin sulphate. Then the cells were treated with varying concentrations of sample and incubated at 37 ^{o}C for 24 h. The final DMSO concentration in each well is 0.05%, at which concentration no appreciable effect on cell proliferation is seen. Then, 50 µL of MTT solution is added to each well and then the plate is further incubated for 4 h. All remaining supernatant are removed and 200 µl of DMSO is added to dissolve the formed crystal formazan. MTT assay reading is performed using microplate reader.

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging system

The determination of antioxidant activity through DPPH scavenging system was carried out according to the method of Brand and Williams[8]. DPPH assay is a common antioxidant assay. The hydrogen atoms, or electron donation ability, of the corresponding extract were measured from the bleaching of purple color of DPPH solution. Each 100 µL of various concentrations of the extracts/gallic acid was added to 100 µL of a 200 µM ethanol solution of DPPH. After a 30 minutes incubation period at room temperature, the absorbance was read compared to a blank at the wavelength of 517 nm. Gallic acid was taken as the standard reference. The percentage of scavenging activity was calculated using the following equation:

$$\% \text{ Inhibition of DPPH radical} = [(A_0 - A_1 / A_0) \times 100]$$

where A_0 is the absorbance of the control

A_1 is the absorbance of the sample extracts

Determination of ferric reducing antioxidant power (FRAP) assay

The determination of antioxidant activity through FRAP was carried out according to the method of Benzie and Strain[9]. The FRAP assay measures the reducing ability of plant extracts. The FRAP reagent was freshly prepared by mixing of 10 mM of TPTZ in 40 mM hydrochloric acid, 20 mM $FeCl_3 \cdot 6H_2O$ in distilled water and 300 mM acetate buffer pH 3.6. 30 µL of sample extracted/trolox was added to a 96 well plate followed by 270 µL of FRAP reagent. The absorbance was measured at 593 nm after 30 minutes incubation at room temperature. Trolox was taken as the standard reference. The percentage of scavenging activity was calculated using the following equation The percentage of ferric reducing antioxidant was calculated using the following equation:

% Inhibition of FRAP reducing $= [(A_0 - A_1 / A_0) \times 100]$

where A_0 is the absorbance of the control

A_1 is the absorbance of the sample extracts

Statistics

To verify the statistical significance of all parameters, the values of mean ± standard deviation (SD) were calculated. Analysis of variance (ANOVA) was performed to determine the efficiency of the solvents for extraction as well as establish the differences in the content of antioxidant activity. A *p*-value of less than 0.05 was considered to be statistically significant.

Results and Discussions

The IC_{50} value of the colonic cancer cell lines, after 24 h incubation with serial dilutions of verities rice bran oil showed in Table 1. The Hom-Mali Gorkho showed the highest inhibition of colon cancer cell line. These results were then used to rank the relative anticancer activity of each variety of rice bran extract. Rice bran oil cell treatment extracts showed a range difference in cell growth inhibition (Figure 1). A illustrates COLO 205 cells treated with rice bran oil at concentration of 0.3 mg/ml, Hom-Mali Gorkho had the greatest inhibitory effect with cell viability at 80.56% followed by Khaw-Khaw (18.93%), Hom-Pathum (6.04%) and Hom-Mali (5.16%). These results demonstrate that the *in vitro* cancer inhibitory properties of rice bran extracts differ based on rice variety. In rice bran oil has ɣ-oryzanol, which had been reported for the treatment of hyperlipaemia in F344 rat and B6C3F1 mice[10]. Inhibitory effect of cycloartenol ferulate, a component of rice, on tumor promotion in two stage carcinogenesis in mouse skin was studied[11]. According to their study the active components of rice bran, sitosterol ferulate, 24-methylcholesterol ferulate, cycloartenol ferulate and 24-methylenecycloartanol ferulate inhibited markedly the TPA-induced inflammation in mice. Cycloartenol ferulate, a component of ɣ-oryzanol in rice bran oil shows marked inhibition on tumor promoting effect of TPA in 7, 12-dimethylbenz [a] anthracene-initiated mice[12]. Hirose studied the modifying effects of phytic acid and ɣ-oryzanol on the promotion stage of rat carcinogenesis[13]. Sugano *et al.* [14] have studied the health benefit of rice bran oil and its anticancer property.

Table: 1. Rice bran oil varieties differences for inhibition of cancer cell viability, DPPH method and FRAP assay

Rice varieties	IC_{50} (mg/ml)		
	Cancer cell	DPPH method	FRAP assay
Hom-Pathum	0.21	0.137	7.675
Hom-Mali	0.69	0.197	8.195
Hom-Mali Gorkho	0.19	0.136	2.273
Khaw-Khaw	0.20	3.533	6.327

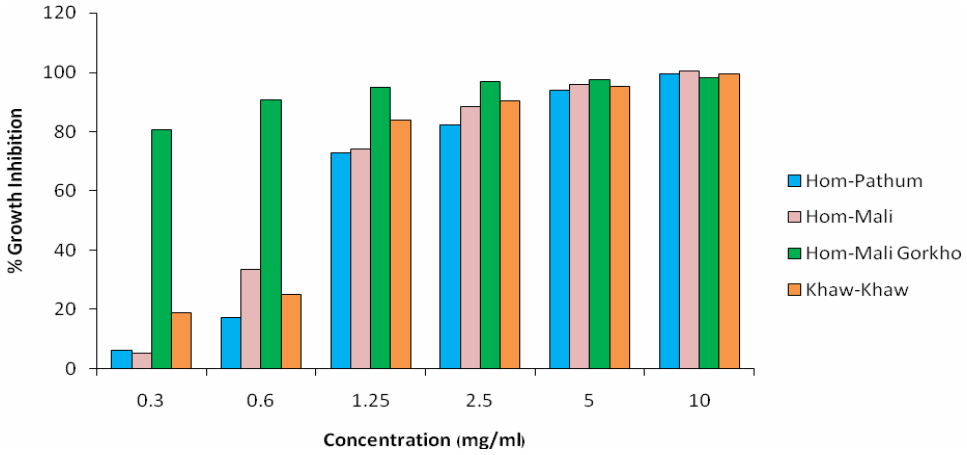

Figure: 1. Cytotoxic evaluation of rice bran oil against COLO 205 cell and different concentration of rice bran oil. Cell viability is expressed as percentage of number of growth inhibition.

Natural antioxidants that are present in herbs are responsible for inhibiting or preventing deleterious consequences of oxidative stress. Among the four rice bran oil varieties of the *in vitro* antioxidant activity using the DPPH method and FRAP assay, the rice bran oil of Hom-Pathum, Hom-Mali, Hom-Mali Gorkho and Khaw-Khaw showed antioxidant activity with IC_{50} values of 0.137, 0.197, 0.136, 3.533 mg/ml, respectively for DPPH method and 7.675, 8.195, 2.273, 6.327 mg/ml, respectively for FRAP assay (Table 1). The results indicated that the antioxidant activity of the Hom-Mali Gorkho rice bran oil was high and consistant in two test methods. The antioxidant activity of rice bran oil varieties is presented in the Figure 2 and Figure 3. The graph showed that the percentage inhibition of 0.25 mg/ml of Hom-Pathum was 60.26% which comparable with other rice bran oil, when increase concentration found that Hom-Mali Gorkho showed the highest percentage inhibition (100%). According to FRAP assay the percentage inhibition of Hom-Mali Gorkho showed the highest inhibition at the 50% at concentration of 2 mg/ml. These free radicals can cause damage to cell walls, certain cell structures and genetic material within the cells. Vitamin E is thought to be the most effective antioxidant due to its abundance in the body. The ɣ-oryzanol is also a potent antioxidant[15]. The nutritional function of ɣ-oryzanol components may be related to their antioxidant property because of the ferulic acid strcture. Ferulic acid is a phenolic acid antioxidant[16]. The free radical scavenging activity of varie cold press rice bran oil was confirmed in the present investigation.

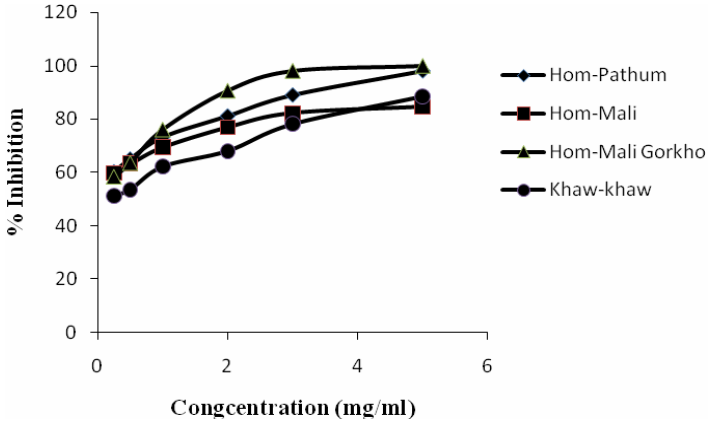

Figure: 2. DPPH free radical scavenging activity of rice bran oil varieties

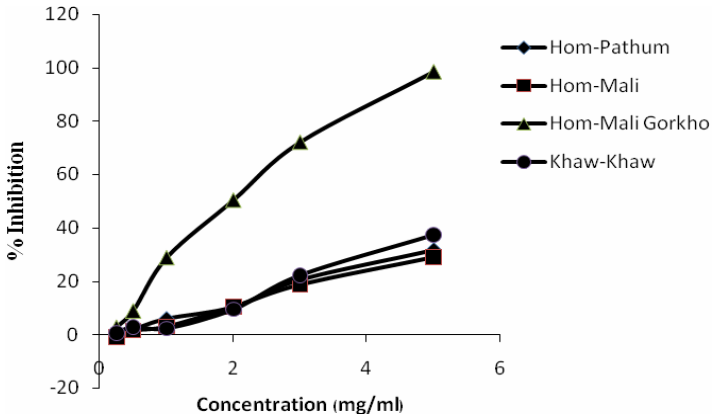

Figure: 3. Determination of FRAP assay on cold press rice bran oil varieties

Conclusion

Four rice bran oil varieties were studied for cytotoxicity and antioxidant potential revealed the rice bran oil bioactive components that inhibit colon cancer cell growth and exhibit high antioxidant activity significantly different across rice varieties. Results from this study support variation in rice bran oil bioactivity with respect to cancer growth inhibition and demonstrates the importance of reporting specific rice varieties used in preclinical and clinical investigations that may enhance rice bran oil as a functional food ingredient.

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