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Original Article

THE STUDY OF ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY IN COLD PRESS RICE BRAN OIL FROM RICE IN THAILAND

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ABSTRACT

Objective: The present study is investigation in the rice bran oil from four rice varieties in term of anti-inflammatory and antioxidant activities.

Methods: This research studied the effects of anti-inflammatory and antioxidant activity from cold press rice bran oil. For anti-inflammatory, their inhibitory activity of nitric oxide (NO) production using RAW267.4 cell lines was evaluated. And two methods for antioxidant activities, DPPH radical scavenging assay (DPPH assay) and Ferric Reducing Antioxidant Power (FRAP) assay were used and compared with gallic acid standard and ferric sulfate ($FeSO_4$), respectively.

Results: For the *Oryza Sativa* L. Khaw-khaw exhibited the highest activity against the NO production with an IC_{50} value of 41.96 µg/ml, followed by *O. Sativa* L. Hom Pathum (46.58 µg/ml), *O. Sativa* L. Hom Mali (53.84 µg/ml) and *O. Sativa* L. Hom Mali Gorkho (59.43 µg/ml). However, the antioxidant activity, DPPH method found *O. Sativa* L. Hom Mali Gorkho displayed the most potent effect with IC_{50} value of 0.08 mg/ml, followed by *O. Sativa* L. Hom Pathum (0.11mg/ml), *O. Sativa* L. Hom Mali (0.12mg/ml) and *O. Sativa* L. Khaw khaw (0.88 mg/ml), respectively. The assay of FRAP showed the highest in *O. Sativa* L. Hom Mali Gorkho with an IC_{50} value 2.27 mg/ml, followed by *O. Sativa* L. Hom Pathum (4.30 mg/ml), *O. Sativa* L. Khaw khaw (6.67 mg/ml) and *O. Sativa* L. Hom Mali (7.68 mg/ml), respectively.

Conclusion: This study indicated that cold press rice bran oil from rice varieties in Thailand is responsible for anti-inflammatory and antioxidant activity. Therefore, this study supports the tradition use of cold press rice bran oil for treatment of inflammatory related diseases though the inhibition of nitric oxide release.

Keywords: Anti-inflammatory**,** Antioxidant activities, *Oryza Sativa* L., Nitic Oxide.

INTRODUCTION

Rice is very important plant in Thailand. It contains many bioactive compounds including phenolic and antioxidants that have the potential to reduce the risk of disease such as inhibiting platelet aggregation [1], reducing the risk of coronary heart disease and cancer [2], and preventing oxidative damage of lipid and low-density lipoproteins [3]. Rice milling yields compose 70% of rice (endosperm) as the major product and byproducts consist of 20% rice husk, 8% rice bran and 2% rice germ [4]. In Rice bran oil (RBO) comprises the rich source of many nutraceutical like oryzanol, tocopherols, vitamin E, ferulic acid, phytic acid, lecithin, inositol wax [5] sterols, higher alcohols, gamma-oryzanol, tocotrienols and phenolic compounds [6]. Gamma-oryzanol is the ubiquitous as a component of primary plant cell walls, offers some benefits [7] such as lowering of blood cholesterol [8] and antioxidant properties [9]. In addition, tocopherols, tocotrienols and several phenolic compounds have potentially beneficial effects [10] such as antioxidative activity [11] and antibacterial properties [12]. The addition of an antioxidant is required to retard lipid peroxidation and preserve the flavor, color and vitamin of food during storage.

Nitric oxide (NO) is one of the inflammatory mediators causing inflammation in many organs. This inorganic free radical has been implicated in physiological and pathological process such as vasodilation, non-specific host defence and acute or chronic inflammation. NO acts as a host defence by damaging pathogenic DNA and as a regulatory molecule with homeostatic activities [13]. However, excessive production of this free radical is pathogenic to the host tissue itself, since NO can bind with other superoxide radicals and acts as a reactive radical which directly damages the function of normal cells [14]. Reactive Oxygen Species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide play a crucial role in the development of various ailment such as arthritis, asthma, dementia, mongolism, carcinoma and parkinson' s disease. The free radicals in the human body are generated through aerobic respiration or from exogenous sources [15]. Some of the *in vivo* free radicals play a positive role in phagocytosis, energy production and regulation of cell growth. However, free radicals may also be damaging. Free radicals produced in the body react with various biological molecules namely lipids, proteins and deoxyribonucleic acids resulting in the imbalance between oxidants and antioxidants. Even though our body is safeguarded by natural antioxidant defense, there is always a demand for antioxidants from natural sources [16]. 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 compared the IC_{50} of rice bran oil across four rice varieties in order to explore their effects on the anti-inflammatory and antioxidant activities from *Oryza Sativa* L. (Hom-Pathum rice, Hom-Mali rice, Hom-Mali Gorkho rice, and Khaw-Khaw).

MATERIALS AND METHODS

Chemical

Roswell Park Memorial Institute (RPMI) 1640 Medium and trypsin-EDTA was obtained from LifeTechnologies (Grand Island, NY, USA). Fetal bovine serum (FBS), penicillin-streptomycin and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) were obtained from Invitrogen (Grand Island, NY, USA). Lipopolysaccharides from *Salmonella enterica* (LPS), Nω-Nitro-L-arginine (L-NA), dimethyl sulfoxide (DMSO), 1,1- diphenyl-2-picryl-hydrazyl (DPPH), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), gallic acid and ferric sulfate ($FeSO_4$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals and reagents were of analytical grade.

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, namely *Oryza Sativa* L. CV. Hom-Pathum; *O. Sativa* L. CV. Hom-Mali; *O. Sativa* L. CV. Hom-Mali Gorkho and *O. Sativa* L. CV. 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 kept in sterile bottle and stored at room temperature for future use.

Assay for NO inhibitory effect using RAW264.7 cell

Inhibitory effect on NO production by murine macrophage like RAW264.7 cell was evaluated using a method by Banskota [17]. Briefly, the cell lines were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 units/ml penicillin and 100 µg/ml streptomycin (completed medium). The cell were harvested with trypsin-EDTA and diluted to suspension in a fresh medium. The cells were seed in 96 -well plate with $1x10^6$ cells/well and allowed to adhere for 2 h at 37° C in humidified chamber with 5% CO_2. After that, the medium was replaced with a fresh medium containing 100 ng/ml of LPS, together with the test samples at various concentrations and was then incubated for 24 h. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent. The cytotoxic activity was determined using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric method. Briefly, after 24 h of incubation with the test samples, MTT solution (5mg/ml in PBS) was added to the well. After 2 h of incubation, the medium was removed, and DMSO was added to dissolve the formazan crystal and measured with microplate reader at 570 nm. The test compounds were considered to be cytotoxic when the optical density of the sample-treated group was less than 80% of that in the control (vehicle-treated) group. L-NA was used as positive control. The inhibition (%) of the test sample was calculated by the following equation and 50% inhibitory concentration, IC_{50} values were determined graphically:

$$\text{NO Inhibition (\%)} = \left[\frac{(OD_C - OD_{Bc})}{(OD_S - OD_{Bs})} \right] \times 100$$

Where C = control (RPMI+ LPS)

Bc = blank of control (RPMI)

S = Sample (Sample + LPS)

Bs = Blank of sample (RPMI + Sample)

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

DPPH radical-scavenging activity of rice bran extracts were determined according to the method reported by Brand and Williams [18]. 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. DPPH free radical-scavenging ability was calculated by using the formula: scavenging ability (%) = [Absorbance of control - Absorbance of sample/absorbance of control] x100. The scavenging activity of rice bran extract was expressed as IC_{50} (mg/ml) and was obtained by interpolation from linear regression analysis. Gallic acid was used for comparison.

Determination of ferric reducing ability power

The ferric reducing antioxidant power (FRAP) assay measures the reducing ability of extracts. The FRAP assay is a method of measuring the ability of reductants (antioxidants) to reduce Fe^{3+}–Fe^{2+}. The formation of blue colored Fe^{2+}-TPTZ complex (Fe^{2+} tripyridyltriazine) increases the absorbance at 593 nm. This method was established by Benzie and Strain [19]. The FRAP reagent was freshly prepared by mixing together of 10 mM of 2, 4, 6-tripyridyl triazine (TPTZ) in 40 mM of hydrochloric acid, 20 mM of ferric choride in distilled water and 300 mM of acetate buffer pH 3.6. The test sample/$FeSO_4$ (30 µL) was added in a 96 well plate followed by 270 µL of FRAP reagent. The absorbance was read at 593 nm after 30 minutes incubation at room temperature against a blank. The reducing power ability was calculated by using the formula: reducing power (%) = [absorbance of sample - absorbance of control/absorbance of sample] x100. Ferric reducing ability power was expressed as IC_{50} (mg/ml) and was obtained by interpolation from linear regression analysis. $FeSO_4$ was used for comparison.

RESULTS AND DISCUSSION

The rice varieties from rice bran oil were examined for their inhibitory activities against nitric oxide (NO) production. Inhibition of NO production from rice varieties on LPS-induced NO release from RAW264.7 cells was shown in Fig. 1. The result shown that Khaw-khaw exhibited the highest activity against NO production with an IC_{50} value of 41.96 µg/ml followed Hom Pathum, Hom Mali and Hom Mali Gorkho as shown in Table 1. The NO inhibitory activity of rice bran oil (except Hom Mali Gorkho) were stronger than that of L-NA (IC_{50} = 54.68 µg/ml). In rice bran oil, it composts of ferulic acid, ɣ-oryzanol, inositol hexaphosphate, campesterol, β-sitosterol, linoleic acid, α-tocopherol, tocotrienol, salicylic acid, caffeic acid, coumaric acid and tricin. The ferulic acid, ɣ-oryzanol, linoleic acid and salicylic acid are anti-inflammatory [20]. This constituent is a group of ferulic acid esters being increasingly focused as an ingredient for drugs, nutraceuticals foods, as well as cosmetics. The ɣ-oryzanol can inhibit tumor promotion, reduce serum cholesterol levels and also be used to treat nerve imbalance and menopause disorders [21]. Several properties have been attributed to them such as anti-inflammatory, antitumor like bactericidal and fungicidal. Nevertheless, their best scientically characterized effect is the hypocholesterolemic [22].

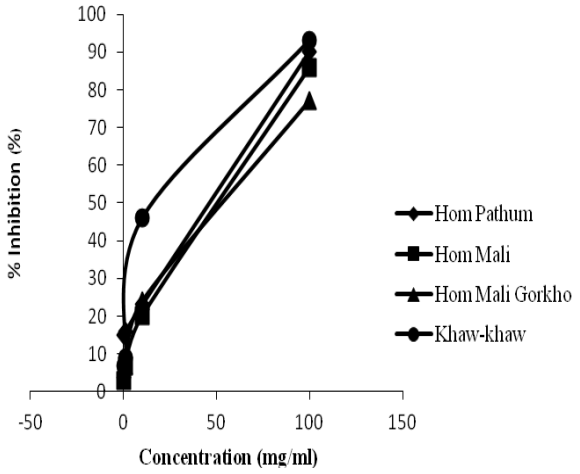

Fig. 1: Inhibition of NO production from rice varieties on LPS-induced

NO release from RAW264.7 cells

Table 1: IC_{50} of anti-inflammatory from rice varieties on LPS-induced NO release from RAW264.7 cell

Rice bran oil varieties	IC_{50} (µg/ml)
Hom Mali Gorkho	59.43
Khaw-khaw	41.96
Hom Pathum	46.58
Hom Mali	53.84
L-NA	54.68

Various mechanisms, such as free radical-scavenging and reducing ability power, have been studied to explain how rice bran extracts could be used as effective antioxidants. The DPPH free radical method has been used extensively to evaluate reducing substances, based on the reduction of ethanol DPPH· solution in presence of

proton-donating substance, resulting in the formation of diamagnetic molecules [23]. The scavenging effects of all extracts on DPPH radicals increased with increasing of concentration (Table 2) and DPPH·radical scavenging at IC_{50} values of rice bran extract is shown in Table 2. With consider to IC_{50} values of DPPH- scavenging activity, the highest DPPH· radical scavenging of rice bran extracts was found in Hom Mali Gorkho (0.08 mg/ml) and lowest activity was in Khaw-Khaw (0.88 mg/ml).

According to other researches, DPPH free radical-scavenging of Pakistani rice bran extracts [24], rice hulls [25] and unsaponifiable matter from rice bran [26] produced similar results. However, DPPH free radical-scavenging of all the rice bran cultivars was weaker than gallic acid, a synthetic antioxidant. As rice bran extracts have a high ability to donate hydrogen atoms, the results of DPPH free radical-scavenging might be due to hydrogen donation ability [27].

Table 2: Antioxidant activity by DPPH method from rice bran oil varieties

Rice bran oil varieties	% Inhibition at varieties of various concentration						
	0.25	**0.5**	**1**	**2**	**3**	**5**	**IC_{50} (mg/ml)**
Hom Pathum	48.66±0.04	55.61±0.03	62.59±0.02	69.40±0.00	76.12±0.03	83.87±0.05	0.11
Hom Mali	49.94±0.03	50.76±0.01	57.00±0.01	60.76±0.01	65.13±0.04	76.47±0.01	0.12
Hom Mali Gorkho	42.47±0.02	54.33±0.00	82.13±0.06	94.54±0.04	117.94±0.01	132.75±0.02	0.08
Khaw-Khaw	43.79±0.01	45.87±0.02	53.19±0.03	58.18±0.00	66.75±0.16	76.61±0.03	0.88
Gallic acid							14.57 µg/ml

The ferric reducing ability power of extracts of rice varieties expressed in inhibition and IC_{50} value are shown in Table 3. The different varieties rice showed significant ($\rho < 0.05$) differences in IC_{50} value, indicating that the varieties rice significantly influenced the ferric reducing power evaluation. $FeSO_4$ was used to compare the reducing power of these extracts. The highest reducing power out of these varieties was observed in Hom Mali Gorkho, which was in agreement with DPPH radical scavenging activity. However, for all varieties the reducing power of Hom Mali Gorkho was higher than that for $FeSO_4$. Literature reports [28] are evident that the reducing power of bioactive compounds is associated with antioxidant activity. Thus, the relation should be located between reducing power and the antioxidant effect. Although some researchers [29] report that the antioxidative effect is concomitant with the development of reducing power. As results for all the varieties were in agreement with scavenging activities. Therefore, reducing power evaluation may be taken as an important parameter for the assessment of antioxidant activity.

Table 3: Antioxidant activity by FRAP method from rice bran oil varieties

Rice bran oil varieties	% Inhibition at varieties of various concentration						
	0.25	**0.5**	**1**	**2**	**3**	**5**	**IC_{50} (mg/ml)**
Hom Pathum	-4.83±0.00	-4.70±0.00	-4.30±0.00	22.03±0.00	36.90±0.02	55.90±0.04	4.30
Hom Mali	1.38±0.00	4.44±0.00	12.63±0.00	21.80±0.00	42.98±0.00	66.80±0.00	7.68
Hom Mali Gorkho	8.93±0.00	8.93±0.00	28.87±0.01	50.40±0.01	72.10±0.02	98.5±0.02	2.27
Khaw-Khaw	0.70±0.00	3.10±0.01	2.50±0.00	9.60±0.00	22.37±0.00	37.47±0.00	6.67
Ferric sulphate							3.57

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 [30].

The ability of rice bran phenols such as ferulic, salicylic, caffeic, and coumaric acids and α-tocopherol (a methylated phenol), to scavenge free radicals, alter enzymes, affect biochemical pathways and interfere with gene expression has attracted the attention of researchers in search of cancer-fighting agents [31].

The efficacy of ferulic acid, which remains in the bloodstream longer than do other known antioxidants and therefore may provide more protection, is dependent on its bioavailability and the dosage [32].

CONCLUSION

Thailand has varieties rice producer of rice bran oil which contain ɣ-oryzanol and another. This high value compound can be isolated from physical refined oil and from the residual husk. This study supports the tradition use of cold press rice bran oil for treatment of inflammatory related diseases though the inhibition of nitric oxide release. And the present data suggests that given a good source of nutrition and function food ingredient for the future.

CONFLICT OF INTERESTS

Declared None

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