EXAMINATION OF ANTIOXIDANT ACTIVITY AND DEVELOPMENT OF RICE BRAN OIL AND GAMMA-ORYZANOL MICROEMULSION

Suwanna Vorarat[1,*], Chittima Managit[1], Lukkhana Iamthanakul[1], Wikalda Soparat[1], and Narisa Kamkaen[1,2]

[1]Faculty of Pharmacy, Srinakharinwirot University, Ongkharak, Nakhorn-nayok, 26120, Thailand
[2]Research and Development Institute, Suan Dusit Rajabhat University, Bangkok 10300, Thailand

ABSTRACT: The aims of this study were to examine the antioxidant activity and formulate rice bran oil and gamma-oryzanol product. Two methods of antioxidant activity examination were DPPH radical scavenging assay and Ferric Reducing Antioxidant Potential (FRAP) assay. According to our study, the outcome of free radical scavenging properties of gamma-oryzanol was demonstrated in term of Trolox equivalent antioxidant capacity (TEAC). For DPPH assay, TEAC values were of 0.0015-0.0206 mmol/g when the concentrations were 0.0625-1.0000 mg/ml. For FRAP assay, TEAC values were of 0.0054-0.0272 mmol/g when the concentrations were 0.0680-1.0910 mg/ml. While the linear correlation between TEAC and log concentration was determined as R^2 = 0.9929 and 0.9975 respectively. The outcomes of free radical scavenging properties of rice bran oil, for DPPH assay, TEAC values were of 0.0059-0.0214 mmol/g when the concentrations were 8-40 mg/ml. While FRAP assay, could not examine the antioxidant activity because of the immiscibility between reagent and rice brain oil. Then microemulsion was formulated using rice brain oil and gamma-oryzanol as an active antioxidant, Cremophor and Span 80 as a surfactant and absolute ethanol as a co-surfactant. The developed formulation had high antioxidant activity with no skin irritation.

Keywords: Rice bran oil, Gamma-oryzanol, Microemulsion, Antioxidant

INTRODUCTION: Rice is an important economic crop and export product of Thailand. The by-products of rice are consisting of bran, germ, and grain. Rice bran has high nutritional value maximize to 60% such as amino acids. Rice bran oil (RBO) is the oil extracted from the germ and inner husk of rice. Rice bran oil contains a range of oils, with 18-23% of the unsaturated oil including monounsaturated and polyunsaturated oil. The fatty acids compositions of RBO are linolenic acid (omega-3, omega-6) and oleic acid (omega 9). This natural oil is rich in essential vitamin E complex; tocotrienols and gamma oryzanol[1)]. These compounds play an important role in preventing heart attack and reducing bad cholesterol level (LDL-C) in the blood[2,3)]. RBO is an interesting natural antioxidant and very popular as a cooking oil in several countries.

Gamma-oryzanol (Figure 1) is an extract from RBO that has high anti-oxidant activity. It is a mixture of sterol esters of ferulic acid and triterpene alcohols. The chemical structure serves as an important anti-oxidation effect caused by part of ferulic acid similar to cholesterol that effected on blood glucose levels and serum lipid parameters[2,4-6)]. It also protects the skin from Ultra-violet radiation and increase moisture to the skin as well. Therefore, the idea for application in the cosmetics is both antiwrinkle and moisturizer for the skin.

Microemulsion is a novel drug delivery system for topical use[7-9)]. This system is suitable for lipophilic substances including RBO and gamma-

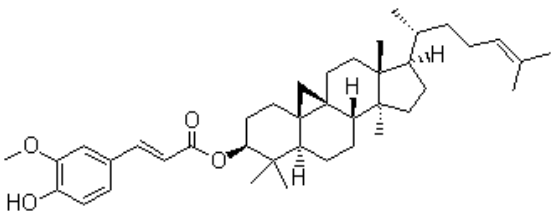

Figure 1 Chemical structure of gamma-oryzanol

*To whom correspondence should be addressed.
E-mail: suwannav@swu.ac.th,
Tel. +66 3739 5095, Fax. +66 3739 5096

oryzanol. Moreover, the surfactant and co-surfactant in microemulsion effect on the skin permeation and penetration through stratum corneum[10].

In this work, the free radical scavenging activities of RBO and gamma-oryzanol were followed via their reaction with the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical and their ferric ions reducing antioxidant activity potential (FRAP) assay. Results from this study would provide a better evidence of the antioxidant properties and development into value-added cosmeceuticals.

MATERIALS AND METHODS:

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), Iron (III) chloride hexahydrate ($FeCl_3 \bullet 6H_2O$) and Trolox ($C_{15}H_{20}O_4$) were from Sigma-Aldrich (St. Louis, USA). Gamma-oryzanol was purchased from Wako Pure Chemical Industries, Co.Ltd, Japan. Rice bran oil was from Namseng Co.Ltd, Thailand. Absolute ethanol, isopropranol, hydrochloric acid, glacial acetic acid and sodium acetate trihydrate were from Merck (Darmstadt, Germany). All other reagents were analytical grade available.

Antioxidant activity of rice bran oil and gamma-oryzanol

The DPPH radical scavenging and Ferric Reducing Antioxidant Potential (FRAP) assay were used to evaluate the antioxidant properties[11-13].

DPPH radical scavenging assay

The 96-wells of a microtiter plate were divided into 3 sets (set A-C) as follows: each well of set A (test sample) contained 100 µl of ethanolic test sample (concentration 25 – 400 µg/ml) and 100 µl of the ethanolic DPPH radical; each well of set B (blank of test sample) contained 100 µl of ethanolic test sample and 100 µl of ethanol; each well of set C (control) contained 100 µl ethanol and 100 µl of the ethanolic DPPH radical. After filling and mixing the solutions in the well, the plate was incubated at 25°C for 30 min. The absorbance was measured at a wavelength of 520 nm by using a microplate reader (Anthos Labtech Instrument, Zenyth 200). %AA was calculated from the equation below by comparing with the standards Trolox. IC_{50} was obtained from the calibration curve between % Inhibition of free radical DPPH and the concentration of sample. Percent Inhibition of free radical DPPH was calculated according to the formula:

$$\% \text{ Inhibition} = ((A_{control} - A_{sample})/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the difference of absorbance of test sample and the absorbance of blank of test sample

Ferric-reducing antioxidant power (FRAP) assay

The FRAP reagent was prepared from acetate buffer (300 mM, pH 3.6), 10 mmol TPTZ solution in 40 mM HCl and 20 mM ferrous chloride solution in the proportion of 10:1:1 (v/v), respectively. The FRAP reagent was fresh daily prepared and was warmed to 37°C in a water bath prior to use. The 96-wells of a microtiter plate were divided into 3 sets (set A-C) as follows: each well of set A contained 20 µl of isopropanolic RBO (concentration 6.25 – 50 mg/ml) and 200 µl of the FRAP reagent; each well of set B contained 20 µl of isopropanolic gamma-oryzanol (concentration 0.15625 – 2.5 mg/ml) and 200 µl of the FRAP reagent; each well of set C (control) contained 20 µl of isopropanolic Trolox (concentration 0.01 - 0.05 mg/ml) and 200 µl of the FRAP reagent. The absorbance of the reaction mixture was then recorded at 595 nm after 5 min. The standard curve was constructed using Trolox (concentration 0.01-0.05 mg/ml), and the results were expressed as TEAC mmol equivalents per gram of the sample. All measurements were taken in triplicate and the mean values were calculated.

Preparation of rice bran oil and gamma-oryzanol microemulsion

The microemulsion was prepared by using rice bran oil and gamma-oryzanol as an active antioxidant, cremophor and span 80 as the surfactant and absolute ethanol as a co-surfactant. The oil phase was mixed with the surfactant and co-surfactant. The mixture was

titrated with water until it turned turbid. The water titration was continued until it turned clear. The pseudoternary phase diagrams (Figure 2 and 3) were constructed by plotting the amounts of water phase, oil phase, and surfactant:co-surfactant phase used in the experiment. The corresponding microemulsion regions were identified as shown as three red spots in Figure 2 and six red spots in Figure 3.

Skin irritation studies

The skin irritation studies were performed in 12 healthy volunteer in accordance with the guidelines of the Consumer Product Safety Commission. The study was approved by the Institutional Ethics Committee at Faculty of Pharmacy, Srinakharinwirot University. The selected formulations; microemulsion of RBO and RBO plus gamma-oryzanol were applied at a dose of 0.5 g to the desired area (3 x 3 cm^2). The erythema index was measured by using Mexameter™ at 0, 30, 60 min, and 24 h after use.

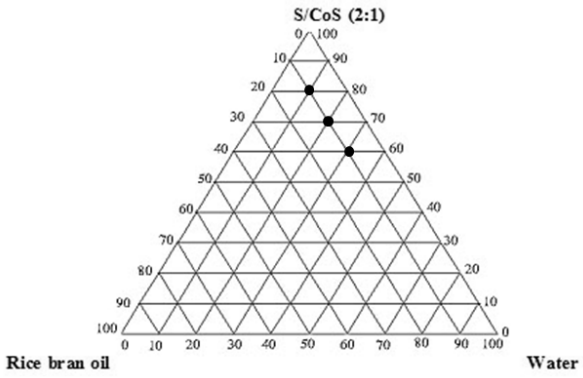

Figure 2 The phase diagram of microemulsion of RBO and RBO plus gamma-oryzanol using surfactant and co-surfactant (2:1).

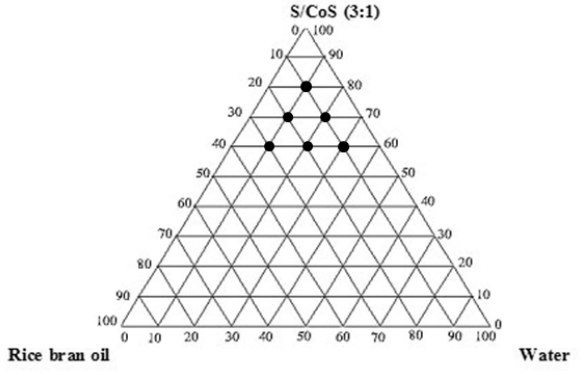

Figure 3 The phase diagram of microemulsion of RBO and RBO plus gamma-oryzanol using surfactant and co-surfactant (3:1).

RESULT:

Antioxidant activity of rice bran oil and gamma-oryzanol

DPPH radical scavenging assay

The standard curve between concentration (mg/ml) and UV absorbance of Trolox was performed by DPPH assay as shown in Figure 4. The linear correlation between % inhibition and concentration was determined as:

$$Y = 3084.8X + 9.0124 \text{ and } R^2 = 0.9982$$

IC_{50} of Trolox was calculated to be 0.0133 mg/ml.

The antioxidant activity of gamma-oryzanol was performed by DPPH assay as shown in Figure 5. The linear correlation between TEAC and log concentration was determined as:

$$Y = 0.0071 \ln (X) + 0.0205 \text{ and } R^2 = 0.9929$$

TEAC values were of 0.0015-0.0206 mmol/g when the concentrations were 0.0625-1.0000 mg/ml.

The antioxidant activity of RBO was performed only by DPPH assay as shown in Figure 6. The linear correlation between % inhibition and concentration was determined as:

$$Y = 1.4404 X + 18.485 \text{ and } R^2 = 0.9844$$

IC_{50} of rice bran oil was calculated to be 21.8793 mg/ml.

Ferric-reducing antioxidant power (FRAP) assay

The standard curve between concentration (mg/ml) and UV absorbance of Trolox was performed by FRAP assay as shown in Figure 7. The linear correlation between % inhibition and concentration was determined as:

$$Y = 101.86X + 0.094 \text{ and } R^2 = 0.9962$$

IC_{50} of Trolox was calculated to be 0.0133 mg/ml.

The antioxidant activity of gamma-oryzanol was performed only by FRAP assay as shown in Figure 8. The linear correlation between TEAC and log concentration was determined as:

$$Y = 0.0078 \ln (X) + 0.0263 \text{ and } R^2 = 0.9975$$

TEAC values were of 0.0054-0.0272 mmol/g when the concentrations were 0.0680-1.0910 mg/ml.

The antioxidant activity in term of TEAC values could not be examined by FRAP assay because of the immiscibility between FRAP reagent and rice brain oil.

Preparation of rice bran oil and gamma-oryzanol microemulsion

The microemulsion of RBO and RBO plus gamma- oryzanol were light-yellowish in color, odorless, and clear.

Antioxidant activity of rice bran oil and gamma-oryzanol microemulsion

The antioxidant activities of two types of microemulsion were evaluated by using two different assays (DPPH and FRAP assay). In Table 1 the results of the monitoring of antioxidant capabilities are present. It can be seen that the antioxidant activity of microemulsion contained rice bran oil plus gamma-oryzanol was higher than microemulsion contained rice bran oil alone. In addition, the antioxidant activity of micro-emulsion was associated with the ratio of the surfactant and co-surfactant. The tube number 6/3 (S:CoS = 2:1) and the tube number 6/2 (S:CoS = 3:1) have the highest percent of inhibition of oxidation (more than 94%).

Skin irritation studies

The safety of the products was observed in term of the skin irritation (edema and erythema). The developed formulation showed non-irritant to the skin with no erythema or edema in all subjects.

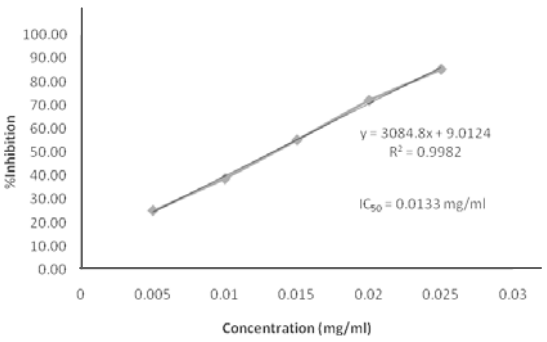

Figure 4 The correlation between concentration (mg/ml) and UV absorbance of Trolox by DPPH assay

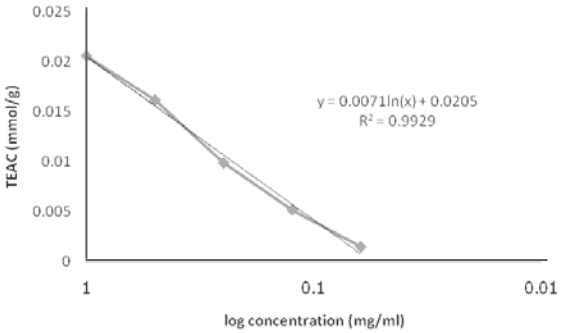

Figure 5 The correlation between log concentration and TEAC (mmol/g) of Gamma Oryzanol by DPPH assay

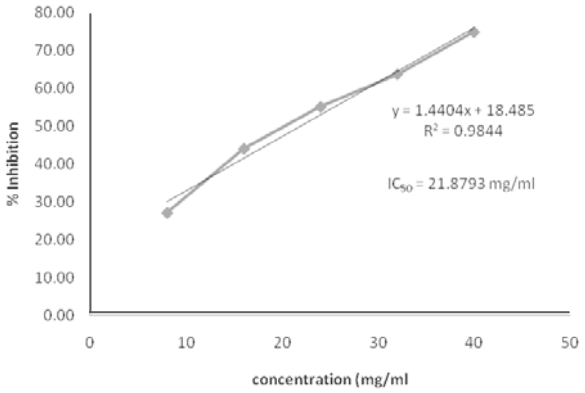

Figure 6 The correlation between concentration (mg/ml) and percent of inhibition of RBO by DPPH assay

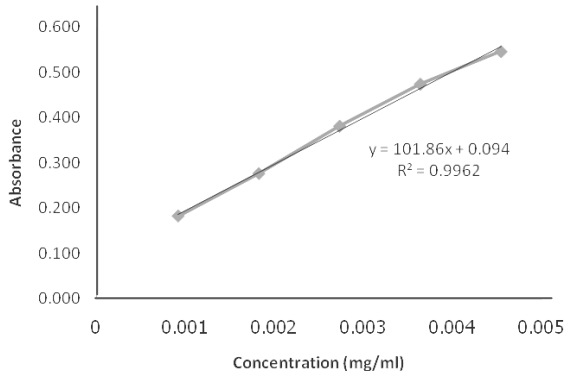

Figure 7 The correlation between concentration (mg/ml) and UV absorbance of Trolox by FRAP assay

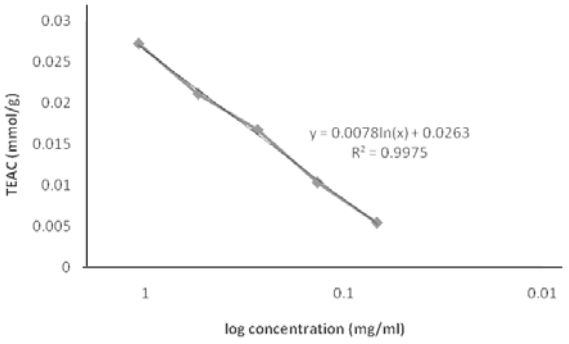

Figure 8 The correlation between log concentration and TEAC (mmol/g) of Gamma Oryzanol by FRAP assay

Table 1 The correlation between concentration of RBO and TEAC (mmol/g) of Microemulsion of RBO and RBO plus gamma-oryzanol by DPPH and FRAP assay

Sample	S/CoS Ratio	Plane/ Tube No.	RBO (%w/w)	% inhibition DPPHassay	TEAC (mmol/g)	
					DPPH assay	FRAP assay
Microemulsion of RBO	2:1	6/3	10	54.14	0.0146	0.0141
		7/2	10	64.37	0.0179	0.0157
	3:1	6/2	20	83.40	0.0241	0.0013
		6/3	10	78.10	0.0224	0.0041
		7/1	20	80.17	0.0231	0.0040
		7/2	10	81.09	0.0234	0.0058
		8/1	10	74.11	0.0211	0.0101
Microemulsion of RBO plus gamma-oryzanol	2:1	6/3	10	94.57	0.0277	0.0216
		7/2	10	89.92	0.0262	0.0245
	3:1	6/2	20	94.13	0.0276	0.0126
		6/3	10	84.82	0.0246	0.0053
		7/1	20	86.36	0.0251	0.0218
		7/2	10	87.70	0.0255	0.0062
		8/1	10	88.97	0.0259	0.0125

DISCUSSION:

The generation of radical oxidative species involves either radical preocesses or different potential redox systems. The soluble properties of antioxidant compounds determine their effective antioxidant activities in either aqueous or lipid systems[11]. Therefore, the microemulsion was chosen to assess the antioxidant activity of RBO and RBO plus gamma-oryzanol, one measuring radical-scavenging activities, and the other measuring total reducing power. DPPH radical scavenging assay was estimated from their ability to eliminate free radicals of DPPH• to DPPH-H. The color was changed from purple to colorless. While FRAP assay was estimated from their power to reduce the TPTZ-Fe (III) complex to TPTZ-Fe (II) complex which is simple, fast, and reproducible[12]. The color was changed from colorless to violet. The measured absorbance by the DPPH and FRAP methods were represented as the percent of inhibition. The comparison with standard trolox was reported in term of a Trolox Equivalence Antioxidant Capacity (TEAC) value. The anti-oxidation examination of gamma-oryzanol demonstrated that TEAC value of FRAP assay were higher than those of DPPH assay. The FRAP assay was versatile and could be readily applied to both aqueous and alcohol extracts[12]. The limitation of this method was the false negative reaction with the SH-group compounds, such as Glutathione (GSH)[14].

The formulation of microemulsion was developed by using three phases diagram: oil, water and surfactant. The ratio of surfactant: co-surfactant was varied from 2:1 to 3:1. When using S/CoS ratio 2:1, the microemulsion was performed in plane/tube number 6/3, 7/2 and 8/1. While using S/CoS ratio 3:1, the micro-emulsion was performed in plane/tube number 6/1, 6/2, 6/3, 7/1, 7/2 and 8/1. The developed microemulsions were light-yellowish in color, odorless, and clear. After mixing all three phases together and observed the physical properties. Then the anti-oxidation activity was performed by DPPH and FRAP assay. The results of both methods showed that microemulsion of RBO plus gamma-oryzanol gave higher TEAC value than RBO microemulsion. The highest antioxidant activity of microemulsion of tube number 6/3 was detected by DPPH assay and that of tube number 7/2 was detected by FRAP assay. The ratio of RBO, surfactant, and water was 10:60:30 and 10:70:20 in the tube number 6/3 and 7/2, respectively.

CONCLUSION:

In conclusion, the antioxidant activity of RBO and RBO plus gamma-oryzanol microemulsion were evaluated by DPPH and FRAP assay. The RBO, in general, showed high antioxidant activities and vitamin E complex particularly gamma-oryzanol. Relatively, the antioxidant activity both assays showed the highest in gamma-oryzanol.

Moreover, there were relative between antioxidant activities and gamma-oryzanol contents in microemulsion. The RBO plus gamma-oryzanol microemulsion showed higher antioxidant activity than RBO microemulsion. It could be concluded that gamma-oryzanol contribute to antioxidant activity in RBO plus gamma-oryzanol micro-emulsion.

ACKNOWLEDGEMENT

This research work was supported by research budget from Srinakharinwirot University in academic year 2552.

REFERENCES:

1. Chotimarkorn C, Benjakul S, Silalai N. 2008. Antioxidant components and properties of five long-grained rice bran extracts from commercial available cultivars in Thailand. Food Chem 111: 636-41.

2. Tsuji E, Sugano M. 1997. Rice bran oil and cholesterol metabolism. J Nutr 127: 521S-4S.

3. Xu Z, Hua N, Godber JS. 2001. Antioxidant activity of tocopherols, tocotrienols, and γ-Oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2'-Azobis(2-methylpropionamidine) dihydrochloride. J Agric Food Chem 49: 2077-81.

4. Graf E. 1992. Antioxidant potential of ferulic acid. Free Radic Biol Med 13: 435-48.

5. Qureshi A, Samai S, Khan F. 2002. Effect of stabilized rice bran, its soluble and fiber fraction on blood glucose levels and serum lipid parameters in humans with diabetes mellitus type I and II. J Nutr Biochem, 13: 175-87.

6. Juliano C, Cossu M, Alamanni MC, Piu L. 2005. Antioxidant activity of gamma-oryzanol: Mechanism of action and its effect on oxidative stability of pharmaceutical oils. Int J Pharm 299: 146-54.

7. Kreilgaard M, Kemme MJ, Burggraaf J, Schoemaker RC, Cohen AF. 2001. Influence of a microemulsion vehicle on cutaneous bio-equivalence of a lipophilic model drug assessed by microdialysis and pharmacodynamics. Pharm Res 18: 593-9.

8. Rhee Y, Choi J, Park E, Chi S. 2001. Transdermal delivery of ketoprofen using micro-emulsions. Int J Pharm 228: 161-70.

9. Puranajoti P, Patil RT, Sheth PD, Bommareddy G 2002. Design and development of topical microemulsion for poorly water-soluble antifungal agents. J Appl Res 2: 1-12.

10. Lehmann L, Keipert S, Gloor M. 2001. Effects of microemulsion on the stratum corneum and hydrocortisone penetration. Eur J Pharm Biopharm 52: 129-36.

11. Huang D, Ou B, Prior RL. 2005 The chemistry behind antioxidant capacity assays. J Agric Food Chem 53: 1841-56.

12. Wong CC, Li H, Cheng K, Chen FA. 2006. Systemic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem 97: 705-11.

13. Norhaiza M, Maziah M, Hakiman M. 2009. Antioxidative properties of leaf extracts of a popular Malaysian herb, *Labisia pumila*. J Med Plant Res 3: 217 – 23.

14. Cao G, Prior RL. 1998. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. Clin Chem 44: 1309 -15.