Biological Activities and Chemical Content of Sung Yod Rice Bran Oil Extracted by Expression and Soxhlet Extraction Methods

Sakuntala Uttama MS*, Arunporn Itharat PhD**.***,
Rujiluk Rattarom MS*, Sunita Makchuchit MS*,
Sumalee Panthong BSc*, Intouch Sakpakdeejaroen MS**

** Student of Doctor of Philosophy in Medical Sciences (Nutraceutical), Faculty of Medicine, Thammasat University, Klongluang, Pathumthani, Thailand*
*** Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Klongluang, Pathumthani, Thailand*
**** Center of Excellence on Applied Thai Traditional Medicine Research (CEATMR), Faculty of Medicine, Thammasat University, Klongluang, Pathumthani, Thailand*

Background: *Sung Yod rice is a red-violet pigmented rice and grown in the southern part of Thailand. Its rice bran oil has attracted the attention of scientists who have described anti-oxidant properties of some ingredients in Sung Yod rice bran oil. Normally, extraction methods of commercial product from rice bran oil are by expression or soxhlet extraction with hexane. Thus, biological activities of Sung Yod rice bran oil related to health and chemical content of rice bran oil from the two methods should be studied.*

Objective: *The objectives of this research were to investigate for biological activities and chemical content of Sung Yod rice bran oil obtained from expression or soxhlet extraction method.*

Material and Method: *Biological activities such as cytotoxic, anti-inflammatory and antioxidant activities were investigated. Sulphorhodamine (SRB) assay was used to test cytotoxic activity against four human cancer cell lines: lung (COR-L23), cervical (HeLa), prostate (PC-3) and breast (MCF-7) and normal human lung cells (MRC-5). The inhibitory effect on lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264.7 cell lines was used for the determination of anti-inflammatory effect. DPPH, TEAC and FRAP assay were carried out for antioxidant activity. Total phenolic compound was determined by Folin-Ciocalteu reagent. γ-oryzanol and vitamin E content were determined by HPLC. Sung Yod rice bran oil was produced by expression method (EX) or by soxhlet extraction method using hexane (SXH).*

Results: *The percentage of yield of Sung Yod rice bran oil by EX and SXH were 2.16 and 15.23 %w/w, respectively. Only EX showed the selective cytotoxicity against prostate cancer cells (PC-3), (IC_{50} = 52.06±1.60 μg/ml). It also exhibited high inhibitory effects on NO production (IC_{50} = 30.09 μg/ml). In contrast, SXH had no anti-inflammatory effect and cytotoxic activity against any of the cancer cells. EX showed higher antioxidant activity determined using DPPH compared to SXH. It also showed higher amount of γ-oryzanol and vitamin E than that of SXH (3.09±1.04 and 1.35±1.56 mg % w/w of extracts, respectively). Yet, SXH exhibited higher antioxidant power determined by FRAP assay and higher total phenolic content compared with EX.*

Conclusion: *Sung Yod rice bran oil, produced by expression method, had better benefit for health regarding cytotoxicity against prostate cancer cells (PC-3), anti-inflammatory effect and antioxidant (using DPPH) than that produced by soxhlet method extracted with hexane.*

Keywords: *Sung Yod rice bran, Cytotoxicity against cancer cells, Anti-inflammatory, Antioxidant, Gamma oryzanol, Total phenolic content*

J Med Assoc Thai 2014; 97 (Suppl. 8): S125-S132
Full text. e-Journal: *http://www.jmatonline.com*

Rice is a popular health food in Thailand and has been a main food of Thai people since long time ago. It is a highly nutritious food[1] and used for reducing blood cholesterol[2], antioxidative properties[3-5], preventing Alzheimer's disease[6], natural antioxidant to improve the stability of foods[7], decreasing the incidence of atherosclerosis disease[8], proposed as a UV-A filter in sunscreen[9] and anti-inflammatory in pigmented rice[10,11]. Rice bran has also long been used as health food products. Rice bran oil in commercial

Correspondence to:
Itharat A, Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, 95 Moo 8 Paholyothin Road, Klongluang, Pathumthani 12120, Thailand.
Phone & Fax: 0-2926-9749
E-mail: iarunporn@yahoo.com

production is produced by many methods such as expression or by extraction continuously by soxhlet apparatus using hexane. It is an oil that is extracted from the germ and inner husk of rice. It is notable for its high smoke point of 232°C (450°F) and its mild flavor, making it suitable for high-temperature cooking methods such as stir frying or deep frying. It is popularly used as a cooking oil in several Asian countries, including Japan and China[12]. Sung Yod rice is pigmented rice from the southern part of Thailand and supported by Her Majesty Queen Sirikit[13]. Sung Yod rice contains various nutritive substances, including vitamin B1, B2, B6, carbohydrate, fibers and protein. It is also rich in various minerals such as iron, calcium and phosphorus[14]. Its bran is used in many health food products. Oil from this rice bran is also produced by people from southern part of Thailand, but there is no report about its biological activities relating to health such as cytotoxicity, anti-inflammatory and antioxidant activities of Sung Yod rice bran oil obtained by expression or by soxhlet extraction with hexane. Their chemical compounds such as total phenolic compound, gamma oryzanol and vitamin E (Tocopherol) content were also determined and compared between oils, which is obtained from these two different extraction methods.

Material and Method

Materials

Plant materials and extraction methods

Sung Yod rice bran was collected from the Patthalung Province, Thailand (organic rice) on October 2011. It was kept in a freezer before use, then left at the room temperature before extraction. Two extraction methods were performed as follows.

Expression method, the plant material (100 g) was put into expression machine, which was made in Thailand, then oil and residue was separated. The extract was kept at room temperature for 24 hours and filtered.

Soxhlet extraction method using hexane, 30 g plant material was put in a thimble and extracted by using a soxhlet apparatus using 1,000 ml of hexane at a temperature 60°C for 8 hours, filtered and dried using an evaporator. The samples were stored at -20°C prior to analysis.

Methods

In vitro cytotoxicity testing by SRB assay

The cytotoxicity assay was carried out using Sulphorhodamine B (SRB) assay[5]. The target cell lines were four types of human cancer cells i.e. lung (COR-L23), prostate (PC-3), breast (MCF-7), and cervical (HeLa) cancer cell lines and one type of human normal lung cell (MRC-5). The 100 µg/ml from each of the two extracts were tested against all cell lines by SRB assay. The culturing of the cancer cells or MRC-5 cells was as described by Itharat et al[15].

The monolayer culture of each cell line in a 96-well micro titer plate was treated with each extracts, each with six replications. The plates were incubated for an exposure time of 72 hours, and then the medium was removed and washed. The plates were incubated for a recovery period of three days. The percentage toxicity was measured calorimetrically using SRB assay.

Anti-inflammation by nitric oxide inhibitory assay

RAW 264.7 murine macrophage leukemia cell lines used in this study were obtained from Assoc. Prof. Dr. Supinya Tewtrakul. Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkhla University. RPMI-1640 medium, Fetal bovine serum (FBS) and Penicillin-streptomycin (P/S) were purchased from Gibco. Lipopolysaccharide (LPS) and 3-(4,5-dimethyl 1-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma. 96-well microplates were purchased from Costar Corning.

Inhibitory effects on NO production by murine macrophages, RAW 264.7 cells, were evaluated by the following method[16]. The RAW 264.7 cells were cultured in RPMI-1640 medium supplemented with 10% FBS and 1% P/S in 96-well plates with 1 x 10^5 cells/well for 1 h. Cells were stimulated with 5 µg/ml LPS together with the test samples at various concentration for 48 h. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent. Cytotoxicity was determined using the MTT colorimetric method. The absorbance at 570 nm was measured. Indomethacin was used as positive control.

The inhibition of NO production was calculated using the following equation and IC_{50} values was calculated using the prism program.

$$\text{Inhibition (\%)} = \frac{(A-B) \times 100}{(A-C)}$$

A: LPS (+), sample (-); B: LPS (+), sample (+); C: LPS(-), sample (-)

Determination of 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity (DPPH method)

DPPH radical-scavenging activity of rice bran

extract was determined according to the method reported[17] with some modification. An aliquot of 0.5 ml of sample solution in methanol was mixed with 2.5 ml of a 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm against a blank, using a UV-vis spectrophotometer (model Lambda EZ201 UV/vis spectrophotometer, Perkin Elmer, USA). DPPH free radical-scavenging ability was calculated by using the formula: scavenging ability (%) = [Absorbance 517 nm of control-Absorbance 517 nm of sample/Absorbance 517 nm of control] x 100. BHT was used for comparison.

Determination of ferric reducing anti-oxidant power (FRAP method)

The FRAP assay[18] is a method of measuring the ability of reductants (antioxidants) to reduce Fe^{3+} to Fe^{2+}. The formation of blue colored Fe^{2+}-TPTZ complex (Fe^{2+} tripyridyltriazine) increases the absorbance at 593 nm. This assay is used with some modifications. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 6H_2O$ solution. The fresh working solution was warmed at 37°C before use. Rice bran oil (300 µl) was allowed to react with 1.7 ml of the FRAP solution. The absorbance at 593 nm of the mixture was measured after 60 min of reaction. The results were calculated using standard curves prepared with known concentrations of $FeSO_4$, and were expressed as µmol $FeSO_4$/g fresh weight.

Determination of total phenolic content

Total phenolic content of the rice bran oil was measured according to the method reported by Chotimakorn et al[19] by using Folin-Ciocalteu reagent with some modification. Rice bran oil (10 mg) was dissolved in methanol (1 ml). An aliquot of rice bran extract (250 µl) at appropriate dilution of sample was mixed with 500 µl of the freshly prepared Folin-Ciocalteu reagent and 6.0 ml of distilled water. The mixture was shaken vigorously, 2.0 ml of sodium carbonate (15% w/v) was added, and the mixture was further shaken vigorously for 2 min. The final volume was made up to 10.0 ml with distilled water. The mixture was left to stand for 2 h at room temperature; the absorbance at 750 nm was measured using a UV-vis spectrophotometer (model Lambda EZ201 UV/vis spectrophotometer, Perkin Elmer, USA). The results of total phenolic content were expressed as µg gallic acid equivalent per g of rice bran weight.

Determination of γ-oryzanol content in rice bran oil

γ-oryzanol content in rice bran oil was measured using reverse phase high performance liquid chromatography (RP-HPLC)[19]. Rice bran oil 100.0 mg was dissolved in 1.0 ml of methanol before filtering through a syringe filter with PTFE (0.2 µm; Ascorbic syringe filter). The RP-HPLC consisted of an Agilent 1100 series (Palo Alto, CA, USA), including auto sample and column oven equipped with Hypersil ODS (4.0 x 250 mm, 5 µm, Agilent Technologies, Palo Alto, CA, USA), and a variable wavelength UV-vis detector (model G1379A) at 330 nm using a mixture of methanol: acetonitrile: dichloromethane: acetic acid (50:44:3:3 v/v/v/v) as a mobile phase at a flow rate of 1.0 ml/min. The content of γ-oryzanol was calculated from the peak area of standard γ-oryzanol (r^2 = 0.997) and reported as mg/g of oil.

Determination of tocopherol (vitamin E) content in rice bran oil

Tocopherol (vitamin E) content in rice bran extract was measured using reverse phase high performance liquid chromatography (RP-HPLC)[20] with some modification. Rice bran oil 10.0 mg was dissolved in 1.0 ml of methanol before filtering through a syringe filter with PTFE (0.2 µm; ascorbic syringe filter). The RP-HPLC consists of an Agilent 1100 series (Palo Alto, CA, USA), including auto sample and column oven equipped with Phenomenex C18 Column (100 x 4.6 mm, 3 micron, Phenomenex, CA, USA), and a variable wavelength UV-visible detector (model G1379A), at 292 nm using a mixture of methanol: water (98: 2 v/v) as a mobile phase at flow rate 1.5 ml/min in isocratic mode using 10 µl per injection. The content of tocopherol (vitamin E) was calculated from the peak area (r^2 = 0.999) of standard tocopherol (vitamin E) and reported as mg/g of oil.

Statistical analysis

The experimental data were expressed as mean ± SEM (replication = 3).

Results and Discussion

Percentage of yield of Sung Yod rice

Sung Yod rice bran oil from expression method (EX) showed less percentage of yield than soxhlet extraction (SXH) using hexane as solvent (2.16 and 15.23%, respectively). This result showed that soxhlet method can produced more oil, about 7 times greater than that of expression method. This is because in

soxhlet extraction, use of heat helps extraction and including the fact that hexane is a good solvent to extract oil, whereas expression through compression using machine which breaks oil cells from rice bran gives less yield.

Cytotoxic activity

The results of cytotoxic activity screening is shown in Table 1. Sung Yod rice bran oil from expression method showed higher cytotoxicity activities than oil from soxhlet extraction using hexane as solvent. EX showed the highest inhibition at concentration 100 µg/ml against prostate cancer cells (PC-3) (84.55%) (IC_{50} = 52.06 µg/ml) and followed by breast cancer cells (MCF-7) (54.21%), but it had no cytotoxic activity against lung cancer. However, it showed higher cytotoxicity against normal lung cells than lung cancer cell. In addition, EX has selective cytotoxic activity against only hormone-dependent human cancer cells such as prostate (PC-3) and breast (MCF-7) cells (72.01 and 80.53%, respectively). SXH also showed highest cytotoxic activity against prostate cancer, however oil from SXH promotes cervical cancer and normal lung cell growth (survival as 110.78 and 126.6%, respectively). Thus, Sung Yod rice bran oil from expression should be used for prostate cancer treatment but it should not be used in lung cancer because it is toxic against normal lung cells. Oil from SXH should not be used with cervical cancer cells but it can be used for lung cancer treatment because SXH has good inhibitory effect against lung cancer and helps promoting normal lung cell growth.

There should be further studies on free fatty acids in Sung Yod rice bran oil from the two extraction methods because from the previous report, free fatty acid from rice bran oil exhibited cytotoxicity against P388 mouse leukemia cells[21] and against endometrial adenocarcinoma cells (Sawano)[22].

However, oil from these two methods showed low cytotoxicity against all types of cancer cells because % inhibition by these oils is less than 50% at concentration 100 µg/ml on all cancer cell types. The benefit of this study is that oil from different extraction methods showed different cytotoxicity against different cancer cells. Thus, selecting method for extracting Sung Yod rice bran oil to use against cancer cell is important. Since oil from expression method is appropriate for prostate cancer treatment, while rice bran oil from soxhlet extraction with hexane is good to promote lung normal cell growth.

Anti-inflammatory activity

Sung Yod rice bran oils were investigated for anti-inflammatory activity measured as inhibitory activities against LPS induced NO production in RAW 264.7 cell lines and measurement of nitrite accumulation in the culture medium was used to determine NO production. The nitrite concentration was measured by Griess reaction. The anti-inflammatory effect of the two rice bran oils on inhibition of production of inflammatory mediator macrophages can be mediated through oxidative degradation of products from phagocytes. Rice bran oil from Sung Yod by expression method exhibited the highest inhibitory activity against NO production with IC_{50} value of 30.09±3.12 µg/ml, where that of SXH was >100 µg/ml (Table 2). However, they showed less anti-inflammatory effect than Indomethacin as a positive anti-inflammatory compound. As the previous studies reported that γ-oryzanol rich extracts from Thai glutinous purple rice bran showed high anti-inflammatory activity as inhibitory effect on NO production. In addition, rice from northern part of Thailand called GAM BOUNG exhibited the highest inhibitory effects on NO production (IC_{50} = 29.32±2.21 µg/ml)[10]. In the previous report, it was rice bran extract, but this is the first time

Table 1. Cytotoxic activity (% inhibition and IC_{50}) Sung Yod rice bran against cancer cells of lung (COR-L23), cervical (HeLa), prostate (PC-3) and breast (MCF-7) and normal human cells line (MRC-5) (Mean ± SEM, n = 3)

Type of cells	EX		SXH	
	% inhibition	IC_{50}	% inhibition	IC_{50}
COR-L23	8.47±3.22	>100	16.95±3.22	>100
HeLa	5.54±1.69	>100	-10.78±1.44	>100
PC-3	84.55±2.54	52.06±1.60	27.99±2.75	>100
MCF-7	54.21±2.67	>100	19.47±2.14	>100
MRC-5	58.73±3.12	>100	-26.6±1.85	>100

that Sung Yod rice bran oil from different extraction method is reported. However, oils from the two extraction methods exhibited anti-inflammatory activity with no toxic effect against RAW 264.7 cell (% Cytotoxicity <20 by MTT assay).

Antioxidant activities by DPPH method and FRAP method

The free-radical scavenging activity of oils from two extraction methods were evaluated using DPPH method and FRAP method. The ability to scavenge DPPH radical by rice bran oil from expression method was greater than by oil from soxhlet extraction method. EX gave the highest percentage inhibition by DPPH method, which was 4.02±1.68% at concentration 100 µg/ml and BHT as 62.48±3.45% (Table 3). According to other research, it was found that rice bran extract from Pakistan rice and Hom Mali rice showed DPPH free radical-scavenging[23,24]. Pakistan rice can reduce DPPH radical, where the remaining amount of DPPH radical was 20.6-30.6% at 5 min after initiating the reaction[24]. However, DPPH free-radical scavenging of oil from two extracts was less than that from BHT, a synthetic antioxidant, at the same concentration. Oil of the two extracts also had less antioxidant activity than rice bran extract from previous reports[23,24].

The results from FRAP assay found that SXH gave the highest TEAC and FRAP i.e. 45.14±4.88 mg trolox/g and 63.55±5.69 mg Fe(II)/g , respectively, but none of the extracts were as good as BHT (TEAC is 252.43±5.86 Trolox/g and FRAP is 369.36±2.50 mg Fe(II)/g) (Table 3). However, SXH showed higher antioxidant activity by FRAP assay than previous report (63.55 compared to 32.2 mg Fe(II)/g, respectively)[23].

Total phenolic compound, γ-oryzanol and vitamin E content

The total phenolic content of two extracts, as determined by the Folin-Ciocalteu reagent, SXH gave higher total phenolic content value than EX (13.04 and 7.52 mg gallic acid eq/g rice bran extract, respectively) (Table 4). This result corresponds to the previous report, Hommali rice bran oil by soxhlet extraction with hexane gave the total phenolic content much more than oil from the expression method[23]. This results of Sung Yod rice bran oil from two extracts can support the findings of antioxidant compound from rice bran extracts from previous studies such as rice bran from Pakistan[24], defatted rice bran[25], rice from Iran[26], five long grains of Thailand[19] and rice varieties in Thailand[27].

The γ-oryzanol content of two oils is shown in Table 4. EX gives higher content of γ-oryzanol than SXH (3.09±1.04 and 1.35±1.56 mg/g of extract, respectively). This result corresponds with the four previous reports[1,12,19,28]. It showed that γ-oryzanol content in rice bran was very high and that the expression method gave higher γ-oryzanol content than that of soxhlet extraction by hexane.

The vitamin E content of two extracts is shown in Table 4. The content of vitamin E in EX (156±3.04 mg/100 g of extract, respectively) also related with γ-oryzanol content. EX had higher vitamin E content than SXH (1.56 and 1.09 mg/g of extract). They correspond to the previous rice bran reports which found that the vitamin E content in rice bran oil was very high[3,19].

The present study showed that rice bran oil from expression method showed higher cytotoxic and anti-inflammatory activities than that from soxhlet extraction. Oil from expression also showed higher γ-oryzanol and vitamin E content than oil from soxhlet method by hexane but oil from soxhlet extraction by hexane gives higher total phenolic content and antioxidant measured by TEAC and FRAP. It is possible

Table 2. Anti-inflammatory activity showed as % inhibition at concentration 50 µg/ml and IC_{50} (µg/ml) by Griess reaction and MTT assay in mouse leukaemia macrophage cell lines (RAW 264.7) (Mean ± SEM, n = 3)

Rice extract/ pure compound	% inhibition at concentration 50 µg/ml (Mean ± SEM)	IC_{50}
EX	67.74±1.22	30.09±3.12
SXH	51.48±5.28	>100
Indomethacin	81.20±1.02	20.01±1.21

Table 3. Percentage inhibition by DPPH method (% inhibition ± SD), TEAC (mg trolox/g) and FRAP [mgFe(II)/g]

Extract	% inhibition ± SEM	TEAC	FRAP
		(mg trolox/g) ± SEM	(mg Fe(II)/g) ± SEM
EX	4.02±1.68	20.25±3.84	26.21±1.83
SXH	-12.75±2.08	45.14±4.88	63.55±5.69
BHT	62.48±1.23	252.43±5.86	369.36±2.50

Table 4. Total phenolic content reported as mg Gallic acid equivalent/g extracts ± SD, gamma oryzanol content reported as mg: 100 g of extracts ± SD and vitamin E reported as mg: 100 g of extracts ± SD

Extract	mg Gallic acid equivalent/g of extracts ± SEM	Gamma oryzanol mg/100 g of extracts ± SEM	Vitamin E mg/100 g of extracts ± SEM
EX	7.52±1.29	309±1.04	156±3.04
SXH	13.04±2.54	135±1.56	109±3.28

that oil from SXH has high polarity compounds much more than oil from expression that is the real oil from plant oil cells.

This finding is the first report which compare Sung Yod rice bran oil extracted by the two different extraction methods. The extraction method to obtain Sung Yod rice bran oil should be expression method rather than soxhlet apparatus using hexane which is a toxic solvent for extraction. The expression method for extraction of Sung Yod rice bran oil helps breaking down oil cells and obtaining real virgin oil therefrom. However, it gave 7 times less yield than oil from soxhlet extraction, yet with higher biological activities better for good health and with higher rice essential compounds such as antioxidant compounds (γ-oryzanol and vitamin E).

Conclusion

Sung Yod rice bran oil extracted by expression or soxhlet extraction method gave percentage yield of 2.16 and 15.23, respectively. EX showed the highest selective cytotoxicity against prostate cancer cells (PC-3), exhibited a high anti-inflammatory activity as inhibitory effects on NO production and antioxidant activities by DPPH method. SXH showed higher antioxidant activity by FRAP assay having TEAC and FRAP values of 45.14 and 63.55 mg Fe(II)/g, respectively. EX gave the highest gamma oryzanol and vitamin E content, but SXH had higher total phenolic compound. This study showed that EX is of greater benefit than SXH. Oil from expression should be suggested as a source of natural effective antioxidant compound for good health.

What is already known on this topic?

Sung Yod rice contains various nutritional components which is beneficial for good health. So far, there was no report on biological activity of Sung Yod rice. In fact, it may have many other benefit on health in addition to its various nutritional components. Sung Yod rice bran oil was extracted by expression or soxhlet extraction method but there was no report on the different chemical content of oils extracting by these two different methods.

What this study adds?

The present study showed comparison of the biological activities and chemical compounds of Sung Yod rice bran oil from expression with oil obtained by soxhlet extraction with hexane. This knowledge is the first report and is new knowledge for consumers who eat Sung Yod rice bran oil. We found that Sung Yod rice bran oil from expression showed greater benefit for good health than oil from soxhlet extraction. However, the yield of soxhlet extraction is higher than oil from expression method.

Acknowledgement

The authors wish to thank the National Research Council of Thailand (NRCT), the National Research University Project of Thailand, Office of Higher Education Commission, Center of Excellent in Applied Thai Traditional Medicine and Thammasat University for their financial support.

Potential conflicts of interest

None.

References

1. Boonsit P, Pongpiachan P, Julsrigival S, Karladee D. Gamma Oryzanol Content in Glutinous Purple Rice Landrace Varieties. CMU J Nat Sci 2010; 9: 151-7.
2. Qureshi AA, Sami SA, Salser WA, Khan FA. Synergistic effect of tocotrienol-rich fraction (TRF(25)) of rice bran and lovastatin on lipid parameters in hypercholesterolemic humans. J Nutr Biochem 2001; 12: 318-29.
3. Xu Z, Godber JS. Antioxidant activities of major components of γ-oryzanol from rice bran using a linoleic acid model. J Am Oil Chem Soc 2001; 78: 645-9.
4. Juliano C, Cossu M, Alamanni MC, Piu L. Antioxidant activity of gamma-oryzanol:

mechanism of action and its effect on oxidative stability of pharmaceutical oils. Int J Pharm 2005; 299: 146-54.
5. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 1990; 82: 1107-12.
6. Alloul K, Sauriol L, Kennedy W, Laurier C, Tessier G, Novosel S, et al. Alzheimer's disease: a review of the disease, its epidemiology and economic impact. Arch Gerontol Geriatr 1998; 27: 189-221.
7. Nanua JN, McGregor JU, Godber JS. Influence of high-oryzanol rice bran oil on the oxidative stability of whole milk powder. J Dairy Sci 2000; 83: 2426-31.
8. Saunders RM. Rice bran: Composition and potential food uses. Food Rev Int 1985; 1: 465-95.
9. Coppini D, Paganizzi P, Santi P, Ghirardini A. Capacita protettiva nei confronti delle radiazioni solari di derivati di origine vegetale. Cosmetic News 2001; 136: 15-20.
10. Saenjum C, Chaiyasut C, Chansakaow S, Suttajit M, Sirithunyalug B. Antioxidant and anti-inflammatory activities of gamma-oryzanol rich extracts from Thai purple rice bran. J Med Plants Res 2012; 6: 1070-7.
11. Schussheim Y, Aschner M, Brodsky B, Proscura E, Erlanger-Rosengarten A, Feldman R, et al. Anti-inflammatory effects of peptide fragments of H2A histone and Oryza Sativa Japonica protein. Peptides 2011; 32: 125-30.
12. Aguilar-Garcia C, Gavino G, Mosqueda MB, Hevia P, Gavino VC. Correlation of tocopherol, tocotrienol, c-oryzanol and total polyphenol content in rice bran with different antioxidant capacity assays. Food Chem 2007; 102: 1228-32.
13. Rice Department, Ministry of Agriculture and Cooperatives, Thailand. Rice knowledge bank [Internet]. 2006 [cited 2010 Feb 27]. Available from: http://www.brrd.in.th/rkb/
14. Department of Health. Nutrition analysis of Sangyod Phatthalung rice. Nonthaburi: Department of Health, Ministry of Public Health, Thailand; 2004.
15. Itharat A, Houghton PJ, Eno-Amooquaye E, Burke PJ, Sampson JH, Raman A. In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. J Ethnopharmacol 2004; 90: 33-8.
16. Tewtrakul S, Itharat A. Nitric oxide inhibitory substances from the rhizomes of *Dioscorea membranacea*. J Ethnopharmacol 2007; 109: 412-6.
17. Yamasaki K, Hashimoto A, Kokusenya Y, Miyamoto T, Sato T. Electrochemical method for estimating the antioxidative effects of methanol extracts of crude drugs. Chem Pharm Bull (Tokyo) 1994; 42: 1663-5.
18. Kubola J, Sirithon S. Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction extract in vitro. Food Chem 2008; 110: 881-90.
19. Chotimakorn C, Benjakul S, Silalai N. Antioxidant components andproperties of five long-grained rice bran extracts from commercial available cultivars in Thailand. Food Chem 2008; 111: 636-41.
20. Edison B. Analysis of tocopherols by high performance liquid chromatography. E-J Chem 2009; 6: 395-8.
21. Hayashi Y, Nishikawa Y, Mori H, Tamura H, Matsushita Y, Matsu T. Antitumor activity of (1OE, 12Z)-9-hydroxy-10,12-octadecadienoic acid from rice bran. J Biosci Bioeng 1998; 86: 149-53.
22. Fan H, Morioka T, Ito E. Induction of apoptosis and growth inhibition of cultured human endometrial adenocarcinoma cells (Sawano) by an antitumor lipoprotein fraction of rice bran. Gynecol Oncol 2000; 76: 170-5.
23. Butsat S, Siriamornpun S. Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. Food Chem 2010; 119: 606-13.
24. Iqbal S, Bhangera MI, Anwarb F. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. Food Chem 2005; 93: 265-72.
25. Shokrzadeh M, Ebadi AG. Study of antioxidant activity in four kinds of cultivated rice grains of Muzandaran province (Iran). Pakistan J Biol Sci 2006; 9: 2723-5.
26. Renuka DR, Arumughan C. Phytochemical characterization of defatted rice bran and optimization of a process for their extraction and enrichment. Bioresour Technol 2007; 98: 3037-43.
27. Vichapong J, Sookserm M, Srijesadaruk V, Swatsitang P, Srijaranai S. High performance liquid chromatographic analysis of phenolic compounds and their antioxidant activities in rice varieties. LWT-Food Science and Technology 2010; 43: 1325-30.
28. Nam SH, Choi SP, Kang MY, Koh HJ. Antioxidantive activities of bran extracts from twenty one pigment rice cultivars. Food Chem 2006; 94: 613-20.

ฤทธิ์ทางชีวภาพและองค์ประกอบทางเคมีของน้ำมันรำข้าวสังข์หยดที่สกัดด้วยวิธีการบีบเย็นและสกัดแบบซอกห์เลต

ศกุนตลา อุตมา, อรุณพร อิฐรัตน์, รุจิลักขณ์ รัตตะรมย์, ศุณิตา มากชูชิต, สุมาลี ปานทอง, อินทัช ศักดิ์ภักดีเจริญ

ภูมิหลัง: *ข้าวสังข์หยดเป็นข้าวสีแดงม่วงและปลูกในภาคใต้ของประเทศไทย น้ำมันรำข้าวสังข์หยดได้รับความสนใจจากนักวิทยาศาสตร์ ซึ่งได้รายงานถึงฤทธิ์ต้านอนุมูลอิสระของส่วนประกอบบางอย่างในน้ำมันรำข้าวสังข์หยด โดยปกติการสกัดน้ำมันรำข้าวในทางอุตสาหกรรมใช้วิธีบีบเย็นหรือที่สกัดแบบซอกห์เลตโดยใช้เฮกเซน ดังนั้นน้ำมันรำข้าวสังข์หยดควรจะนำมาศึกษาฤทธิ์ทางชีวภาพที่มีผลดีต่อสุขภาพและองค์ประกอบทางเคมีของน้ำมันรำข้าวจากวิธีการสกัดทั้งสองแบบ*

วัตถุประสงค์: *ทดสอบฤทธิ์ทางชีวภาพและองค์ประกอบทางเคมีของน้ำมันรำข้าวสังข์หยด ที่สกัดด้วยวิธีบีบเย็นหรือที่สกัดแบบซอกห์เลต*

วัสดุและวิธีการ: *ทดสอบฤทธิ์ทางชีวภาพได้แก่ การทดสอบความเป็นพิษต่อเซลล์ ฤทธิ์ต้านการอักเสบ และฤทธิ์ต้านอนุมูลอิสระ การทดสอบความเป็นพิษต่อเซลล์ไลน์ของมะเร็งสี่ชนิด: มะเร็งปอด (COR-L23), มะเร็งปากมดลูก (HeLa), มะเร็งต่อมลูกหมาก (PC-3) และมะเร็งเต้านม (MCF-7) และเซลล์ปอดปกติ (MRC-5) ใช้เทคนิค sulphorhodamine ฤทธิ์ยับยั้งการหลั่งไนตริกออกไซด์ของเซลล์ไลน์ RAW 264.7 ที่ถูกกระตุ้นด้วย LPS ถูกนำมาใช้ทดสอบฤทธิ์ต้านการอักเสบใช้เทคนิค DPPH, TEAC และ FRAP ในการทดสอบฤทธิ์ต้านอนุมูลอิสระ การวิเคราะห์ปริมาณสารกลุ่มฟีนอลิค ทั้งหมดตรวจวัดโดยใช้สาร Folin-Ciocalteu ปริมาณสารแกมม่าออไรซานอลและวิตามินอี ถูกตรวจวัดโดยใช้เทคนิค HPLC ส่วนวิธีการสกัดน้ำมันรำข้าว สังข์หยดคือ การสกัดด้วยวิธีบีบเย็นและวิธีซอกห์เลตโดยใช้เฮกเซน*

ผลการศึกษา: *ปริมาณผลผลิตของน้ำมันรำข้าวสังข์หยดที่สกัดด้วยวิธีบีบเย็นและวิธีซอกห์เลตคือ 2.16 และ 15.23 %w/w น้ำมันรำข้าวที่สกัดด้วยวิธีบีบเย็นเท่านั้นที่มีฤทธิ์ต้านเซลล์มะเร็งต่อมลูกหมาก (IC_{50} เท่ากับ 52.06±1.60 μg/ml) และยังมีฤทธิ์ดีในการต้านการหลั่งไนตริกออกไซด์ (IC_{50} เท่ากับ 30.09 μg/ml) ในทางกลับกันน้ำมันรำข้าวที่สกัดด้วยวิธีซอกห์เลตไม่มีฤทธิ์ต้านการอักเสบและไม่มีความเป็นพิษต่อเซลล์มะเร็งทุกชนิด น้ำมันรำข้าวที่สกัดด้วยวิธีบีบเย็นมีฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH สูงกว่าน้ำมันรำข้าวที่สกัดด้วยวิธีซอกห์เลตและมีปริมาณสารแกมม่าออไรซานอลและวิตามินอีสูงกว่าน้ำมันรำข้าวที่สกัดด้วยวิธีซอกห์เลตอีกด้วย (13.04±2.54 mg GAE/g, 3.09±1.04 และ 1.35±1.56 mg %w/w ของสารสกัดตามลำดับ)*

สรุป: *น้ำมันรำข้าวสังข์หยดที่สกัดด้วยวิธีบีบเย็นมีประโยชน์ต่อสุขภาพมากกว่าน้ำมันรำข้าวที่สกัดด้วยวิธีซอกห์เลตโดยใช้เฮกเซนสกัด*