



Original Article

## Investigation of antioxidative, antityrosinase and cytotoxic effects of extract of irradiated oyster mushroom

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Received: 20 May 2015; Accepted: 12 August 2015

### Abstract

Oyster mushroom (*Pleurotus ostreatus* Fries.) is rich in nutrition and has many medicinal properties such as antioxidant and anticancer activities. It also contains a high amount of ergosterol which can be converted to vitamin D<sub>2</sub> when exposing to UV light. Oyster mushroom powder was irradiated with UV-B for 180 min and extracted with 95% ethanol. Mushroom extract was determined for vitamin D<sub>2</sub> concentration, total phenolic compound, antioxidative activity, tyrosinase inhibitory property and cytotoxicity effect on human keratinocytes (HaCaT) and murine melanoma cells (B16F10) by MTT assay. The results demonstrated that the concentration of vitamin D<sub>2</sub> of irradiated oyster mushroom extract was 153.96 µg/g, which is 13 times higher than that of non-irradiated mushroom extract. Total phenolic content, antioxidative and tyrosinase inhibitory activities of the two mushroom extracts were not significantly different. Neither oyster mushroom extract had a cytotoxic effect on keratinocytes, but on the other hand both inhibited the growth of murine melanoma cells.

**Keywords:** oyster mushroom (*Pleurotus ostreatus*), vitamin D<sub>2</sub>, antioxidant effect, cytotoxic effect, tyrosinase inhibition activity

### 1. Introduction

Oyster mushroom (*Pleurotus ostreatus* Fries.) is a white rot or wood decay fungus. This fungus can grow in a wide range of temperatures and it is cultivated in many parts of the world including Thailand (Abdurrahman *et al.*, 2009). This mushroom is a food with great nutritional value according to its high protein and dietary fiber content. It also provides a valuable source of minerals and vitamins (Gajendra *et al.*, 2014). Vitamin D has been found in mushrooms and can be formed from the plant steroid called ergosterol. Vitamin D has several important functions in the body such

as the regulation of calcium and phosphorus absorption and facilitating normal immune system function (Heaney, 2003). Moreover, vitamin D may play a role in protecting against diabetes, heart disease, high blood pressure, multiple sclerosis and cancer (Rivera *et al.*, 2010).

Cultivated oyster mushrooms are rich sources of ergosterol, the precursor of vitamin D<sub>2</sub>. Ergosterol in mushrooms can be converted to vitamin D<sub>2</sub> after exposure to UV light (Ko *et al.*, 2008). When mushrooms are exposed to UV light, ergosterol undergoes photolysis to yield a variety of photo-irradiation products, principally previtamin D<sub>2</sub>, tachysterol and lumisterol. The previtamin D<sub>2</sub> is spontaneously rearranged into vitamin D<sub>2</sub> (Teichmann *et al.*, 2007). In general, the cultivated mushrooms contain low vitamin D<sub>2</sub>. They may not be exposed to the sunlight, which is essential in the natural production of vitamin D<sub>2</sub> (Mattila *et al.*, 2002).

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Previous research showed that the concentration of vitamin D<sub>2</sub> in cultivated mushrooms could be increased after they were exposed to sunlight, artificial light such as UV light or Pulsed UV light (PUV) (Jasinghe and Perera, 2006). The effects of UV light on vitamin D<sub>2</sub> production among various cultivated mushroom species have been recently reported by a number of authors (Jasinghe and Perera, 2005; Koyyalamudi *et al.*, 2009; Mau *et al.*, 1998).

Oyster mushrooms contain several compounds with antioxidative activity such as vitamin A, C, E, carotenoids, polyphenolic compounds and flavonoids (Nuhu *et al.*, 2011). Oxidative stress caused by excessive free radicals lead to aging and diseases, such as atherosclerosis, diabetes, cancer, and cirrhosis (Ilgaz *et al.*, 2012). Although human body is designed to have its own defense and repair systems to protect against oxidative damage, these systems are insufficient to entirely prevent damages (Nuhu *et al.*, 2010). Food supplements or diets containing antioxidants such as fruit, vegetable and mushrooms may be useful to reduce the oxidative damage (Arbaayah and Umi, 2013). Tyrosinase, a multi-functional copper-containing enzyme, is widely distributed in fungi, plant and animal and it is responsible for melanization in animals and enzymatic browning of fruit. This enzyme catalyzes two distinct reactions involving molecular oxygen: the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to o-quinones (Nuhu *et al.*, 2011). Tyrosinase inhibitors from natural sources were found to inhibit the activities of both monophenolase and diphenolase (Kim and Uyama, 2005), and oyster mushroom was reported to have tyrosinase inhibitory effect (Nuhu *et al.*, 2010; Riani *et al.*, 2012).

Oyster mushrooms have beneficial effects on health and also possess a number of therapeutic properties like anti-inflammatory, immunostimulatory, immunomodulatory and anticancer activity (Iris *et al.*, 2006). Previous reports showed that the ethanol extract of oyster mushroom had high anti-cancer activity on several cell lines such as breast, colon, liver and lung cancer cells. Moreover, the oyster mushroom extracts also showed direct anti-tumor activity against carcinomas possibly through the activation of the body's immune system. (Andrej and Daniel, 2008; Seema and Goyal, 2012).

Although there are many studies on the conversion of ergosterol to vitamin D<sub>2</sub> after UV-B irradiation of oyster mushrooms, there are no reports on the biological activities and cytotoxicity effect of the extract from irradiated oyster mushrooms. Therefore, the objectives of this research were to investigate the content of vitamin D<sub>2</sub> and phenolic compounds, the antioxidative and tyrosinase inhibitory properties, and the cytotoxic effect on keratinocytes and melanoma cells of irradiated oyster mushroom extract.

## 2. Materials and Methods

### 2.1 Chemicals

Ninety-five percent ethanol (analytical grade) was

purchased from Labscan Asia Co.,Ltd. (Bangkok, Thailand). Methanol and acetonitrile (HPLC grade) were obtained from Merck (Darmstadt, Germany). 1,1-diphenyl 2-picryl hydrazyl (DPPH), 3-(4, 5-Dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), ergosterol, ergocalciferol (vitamin D<sub>2</sub>), fetal bovine serum (FBS), Folin-Ciocalteus phenol reagent, kojic acid, L-tyrosine, penicillin, streptomycin, tyrosinase, vitamin C and vitamin E were purchased from Sigma (Steinheim, Germany). Dulbecco's Modified Eagle Medium (DMEM) powder was purchased from Invitrogen corporation. The other chemicals used were of analytical grade.

### 2.2 Irradiation procedure and mushroom extraction

Fresh fruiting bodies of oyster mushrooms obtained from local farms in Khon Kaen province, Thailand were sliced into pieces, dried with hot air at 50°C for 48 hr and finely pulverized. The obtained mushroom powder was stored at -20°C until UV-B treatment. Prior to the treatment, mushroom powder was left at room temperature for 4 hr and placed on an aluminium tray. The samples were placed 15 cm away from the irradiation source and exposed to the UV-B in an irradiation chamber. The UV-B unit was set with 8 UV-B lamps (313 ±12 nm, Philips TL-D 18W) 604 cm in length and the total treatment area was 100x120 cm<sup>2</sup>. Mushroom powder was treated under UV-B lamp, at an irradiation dose of 550.32 J/cm<sup>2</sup> at 25-28°C for 180 min then the irradiated mushrooms powder was stored at -20°C. The un-irradiated mushroom powder was used as a control.

Ten grams of powder samples were extracted with 100 mL of 95% ethanol for 24 hr. The ethanolic extracts were filtered through Whatman No.1 filter paper, rotary evaporated at 40°C then freeze-dried.

### 2.3 Validation of HPLC method

The method validation ensures vitamin D<sub>2</sub> and ergosterol analysis credibility. The criteria of accuracy, precision, linearity, limit of detection (LOD) and limit of quantitation (LOQ) were considered. The accuracy of this HPLC method was determined by using samples spiked with five different concentrations of vitamin D<sub>2</sub> and ergosterol standards and defined in terms of percent recovery. Intra-day and inter-day precision were done to assure the precision of the method. Linearity was tested by eight different concentrations, which were prepared by diluting the standard stock solution. LOD was determined as the lowest concentration of vitamin D<sub>2</sub> and ergosterol giving a response of 3 times the baseline noise defined from the analysis of control sample. LOQ was determined as the lowest concentration giving a response of 10 times the baseline noise (Lloyd *et al.*, 2012).

### 2.4 Analysis of vitamin D<sub>2</sub> and ergosterol

The content of vitamin D<sub>2</sub> was analyzed according to the method described previously (Mattila *et al.*, 1994). Oyster

mushroom extracts (0.5 g) were weighed into a 250 mL round-bottomed flask and mixed with 1 g of L-ascorbic acid, 50 mL of 99% ethanol and 25 mL of 50% potassium hydroxide. The mixture was shaken and saponified under reflux at 85°C for 30 min. It was immediately cooled to room temperature and poured into a separating funnel. The mixture was firstly extracted with 10 mL of de-ionised water and subsequently with 30 mL of *n*-hexane. The organic layers were washed 3 times with de-ionised water until neutralized. The organic layer was transferred into a round bottom flask, rotary evaporated to dryness at 50°C and re-dissolved in 2 mL of a mixed solution of eluent (methanol/acetonitrile = 75:25 v/v) and isopropyl alcohol (2:1 v/v). The sample was passed through a 0.45 µm non-pyrogenic filter. A volume of 20 µl of filtered sample was injected into the HPLC system (LC 20A, Shimadzu, Japan) and eluted through a reversed phase C18 column (Maxsil 5 C18, 250×4.6 mm, Phenomenex, Torrance, CA, USA). The mobile phase was methanol/acetonitrile (75:25 v/v), at a flow rate of 1 mL/min. The UV detection of elute was performed at 264 nm. Ergosterol and vitamin D<sub>2</sub> were qualitatively analyzed by comparing the retention times of standards and their quantifications were done by using a calibration curve.

## 2.5 Scavenging effect on 1, 1-Diphenyl-2-picrylhydrazyl radical

Free radical scavenging is one of the mechanisms in inhibiting lipid oxidation commonly used to estimate antioxidant activity (Pornariya and Kanok-Orn, 2009). The radical scavenging activity of mushroom extracts was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the previous report (Shimada *et al.*, 1992). Various concentrations (0.1-5 mg/mL) of mushrooms extracts were added with 200 µl of 1 mM DPPH radical solutions in methanol, vigorously mixed and allowed to stand for 15 min at room temperature. The DPPH solution without extract solution was used as control. The scavenging effect on the DPPH radical was measured at 515 nm and read using UV/VIS spectrophotometer (UV-1700 PharamSpec). The assay was carried out in triplicate and half maximal effective concentration (EC<sub>50</sub>) value was calculated. The scavenging effect on the DPPH inhibition in term of percent (%) was calculated according to the equation: Percentage (%) of DPPH radical scavenging =  $(Ac_{\text{control}} - As_{\text{sample}} / Ac_{\text{control}}) \times 100$ , where Ac is the absorbance of control blank and As is the absorbance of extract sample. Vitamin C and vitamin E were used as positive controls.

## 2.6 Ferric reducing ability of plasma assay (FRAP)

Ferric reducing ability of plasma (FRAP) assay was also determined to evaluate antioxidant potential. The modified micro-assay method was carried out (Benzie and Strain, 1996; Sripanidkulchai and Junlatat, 2014). The FRAP reagent

containing 10 mM TPTZ, 20 mM FeCl<sub>3</sub> and 300 mM acetate buffer, pH 3.6 in a ratio of 10:1:1 (v/v/v) was freshly prepared. The mushroom extracts were dissolved in 50% ethanol (1-10 mg/mL concentration) and 10 µl of extract solution was mixed with 200 µl of FRAP reagent. The mixture was left to stand at room temperature for 4 min and the absorbance was measured at 600 nm using a UV/VIS spectrophotometer (UV-1700 PharamSpec). The standard ferrous sulfate solution dissolved in 40 mM HCl was tested in a parallel process. The FRAP values were calculated from the standard curve.

## 2.7 Determination of total phenolic content (TPC)

Total phenolic content in extracts was determined using Folin-Ciocalteu reagent based on the method of Singleton *et al.* (1999). Mushroom extracts were dissolved with methanol (0.1-5 mg/mL), then the mushroom extract solution (0.5 mL) was mixed with 0.25 mL of Folin-Ciocalteu reagent and 1.25 mL of 20% sodium carbonate. The sample was incubated for 40 min at room temperature. The absorbance (Abs) was measured at 725 nm in a UV/VIS spectrophotometer (UV-1700 PharamSpec). The standard curve of tannic acid was performed. The total phenolic contents were expressed as mg tannic acid equivalent (TAE)/g dry basis.

## 2.8 Tyrosinase inhibitory activity

Tyrosinase inhibitory activity was measured according to the modified dopachrome method and used L-Tyrosine as a substrate (Momtaz *et al.*, 2008). Mushroom extracts were dissolved in DMSO and diluted with 50 mM phosphate buffer (pH 6.5) (50-5,000 µg/mL).

The mixture solution of each well contained of 70 µL of sample, 100 µL of 2 mM L-tyrosine and 30 µL of tyrosinase (167 units/mL) and was incubated at room temperature for 30 min and the absorbance measured at 492 nm using a Micro-plate reader (Anthos, Austria). Each sample was accompanied by a blank containing all components except L-tyrosine. Kojic acid was used as a positive control. The percentage of tyrosinase inhibition was calculated as follows: % inhibition =  $((Abs_{\text{control}} - Abs_{\text{sample}}) / Abs_{\text{control}}) \times 100$  and the values were expressed as 50% inhibitory concentration (IC<sub>50</sub>).

## 2.9 Cytotoxicity tests

Human keratinocytes (HaCaT) were obtained from CLS-cell lines service (Germany) and murine melanocytes, which are melanoma cells (B16F10) were purchased from ATCC (JR Scientific U.S.A.). The cells were maintained as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 100 µg/mL of streptomycin, and 100 U/mL of penicillin in a 5% CO<sub>2</sub> humidified atmosphere incubator at 37°C. The cells were removed using trypsin-EDTA from their culture flasks twice weekly.

Cell viability was measured using 3-(4, 5-Dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay (Mosmann, 1983). Briefly, each cell line ( $1 \times 10^5$  cells/well) was seeded onto a 96-well plate for 24 hr. The cultivated cells were separately treated with non-irradiated mushroom and UV-B irradiated mushroom extracts (12.5-2,000  $\mu\text{g/mL}$ ) for 24 hr. 50  $\mu\text{L}$  of MTT solution was added and incubated at 37°C for 3 hr in a humidified incubator with 5%  $\text{CO}_2$ . After 3 h of incubation, the formazan crystals were dissolved by adding 100  $\mu\text{L}$  DMSO and the plate was further incubated for 5 min at room temperature. The optical density of the wells was measured at a wavelength of 570 nm using a Micro-plate reader (Anthos, Austria). The data were expressed as the concentration of sample required to kill 50% ( $\text{IC}_{50}$ ) of the cells compared to the controls.

### 2.10 Statistical analysis

All results were expressed as mean  $\pm$  SD (standard deviation). One-Way ANOVA and LSD test were used to analyze significant differences ( $p < 0.05$ ).

## 3. Results

### 3.1 Validation of HPLC analysis

As shown in Table 1, the accuracy (percentage recovery) of ergosterol and vitamin  $\text{D}_2$  were in the range of 99.35-100.98 and 99.01-100.45, respectively. The precision of this method was assessed based on the percent relative standard deviation (%RSD) and values of inter-day and intra-day precision were lower than 2%. By plotting between the peaks area versus concentration (1.25-100  $\mu\text{g/mL}$ ), the linearity of standard ergosterol and vitamin  $\text{D}_2$  was obtained. LOD and LOQ values were 0.08 and 0.27  $\mu\text{g/mL}$  and 0.13 and 0.43  $\mu\text{g/mL}$  for ergosterol and vitamin  $\text{D}_2$ , respectively.

### 3.2 Levels of vitamin $\text{D}_2$ and ergosterol in mushroom extracts

The average yields from two separated extractions of control and irradiated oyster mushroom were 4.23 and 5.66% (w/w), respectively. The chromatographic condition used in this study gave a good resolution for the analysis of vitamin  $\text{D}_2$  and ergosterol (Figure 1). The retention times of vitamin  $\text{D}_2$  and ergosterol were 11.94 and 14.36 min, respectively. As shown in Table 2, irradiated oyster mushroom extract contained significantly higher level of vitamin  $\text{D}_2$  than non-irradiated oyster mushroom extract ( $p < 0.05$ ).

### 3.3 Antioxidative activity and total phenolic compounds

The ethanolic extracts of oyster mushroom were screened for their antioxidative activity by two assays, namely 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and the ferric reducing ability of plasma (FRAP) or reducing power assay. Oyster mushroom extract showed positive antioxidative activity by fading the violet color of DPPH solution to yellow and pale violet. The radical scavenging activities were in direct proportion with the concentrations of the extracts. The inhibition of DPPH radical of vitamin C, vitamin E and mushroom extracts are showed in Figure 2. As the concentration of extract increased, the scavenging activity towards DPPH radicals was elevated. The crude extracts of non-irradiated and irradiated oyster mushrooms showed similar ability to inhibit free radicals from DPPH at half maximal effective concentration ( $\text{EC}_{50}$ ) of 869.95 and 871.3  $\mu\text{g/mL}$ , respectively (Table 3). In addition, both oyster mushroom extracts had significantly weak ability of free radical inhibition as compared with vitamin C and vitamin E. For FRAP assay, both mushroom extracts showed similar appreciable reducing power activities. At a concentration of 10 mg/mL, the greatest ability of reducing power inhibition was 2.12 and 2.15  $\mu\text{M FeSO}_4 \cdot 7\text{H}_2\text{O/g}$  for non-irradiated and irradiated

Table 1. Method validation of HPLC analysis of ergosterol and vitamin  $\text{D}_2$

Standard	Accuracy (%recovery)		Intra-day and inter-day precision			Linear equation	Limit of detection ( $\mu\text{g/mL}$ )	Limit of quantitation ( $\mu\text{g/mL}$ )
	Concentration ( $\mu\text{g/mL}$ )	%Recovery*	Concentration ( $\mu\text{g/mL}$ )	%RSD*				
				Intra-day	Inter-day			
Ergosterol	6.25	99.72 $\pm$ 0.63	1.25	0.64 $\pm$ 0.04	1.46 $\pm$ 0.22	$y = 73718x - 19383$ ( $r^2 = 0.99$ )	0.08	0.27
	12.5	101.26 $\pm$ 0.45	2.5	0.32 $\pm$ 0.15	0.98 $\pm$ 0.12			
	25	100.12 $\pm$ 0.39	5	0.92 $\pm$ 0.08	1.30 $\pm$ 0.18			
	50	99.35 $\pm$ 0.52	10	0.73 $\pm$ 0.14	0.71 $\pm$ 0.34			
	100	100.98 $\pm$ 0.22	20	0.81 $\pm$ 0.09	0.82 $\pm$ 0.10			
Vitamin $\text{D}_2$	1.25	100.55 $\pm$ 0.54	1.25	1.02 $\pm$ 0.18	1.13 $\pm$ 0.10	$y = 58732x + 1273.1$ ( $r^2 = 0.99$ )	0.13	0.43
	2.5	99.71 $\pm$ 0.23	2.5	0.62 $\pm$ 0.23	1.02 $\pm$ 0.07			
	5	99.93 $\pm$ 0.67	5	0.48 $\pm$ 0.35	0.76 $\pm$ 0.23			
	10	99.01 $\pm$ 0.92	10	0.75 $\pm$ 0.09	0.94 $\pm$ 0.12			
	20	100.45 $\pm$ 0.55	20	0.52 $\pm$ 0.11	0.72 $\pm$ 0.21			

\*Values are expressed as means  $\pm$  SD (n=5).

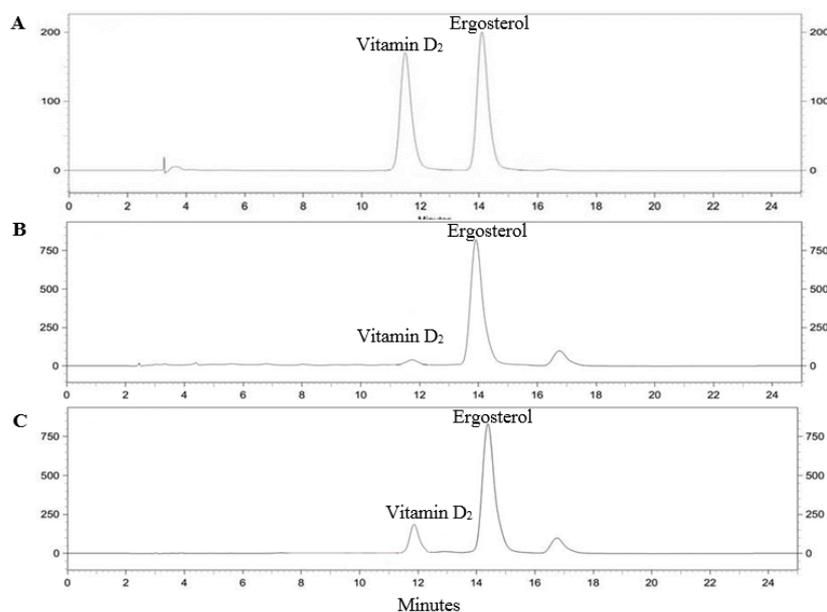


Figure 1. HPLC chromatograms of standard vitamin D<sub>2</sub> (ergocalciferol) and ergosterol (A), control oyster mushroom extract (B) and irradiated oyster mushroom extract (C).

Table 2. The levels of ergosterol and vitamin D<sub>2</sub> in ethanolic extracts of oyster mushroom.

Extract	Ergosterol (mg/g)	Vitamin D <sub>2</sub> (μg/g)
Non-irradiated mushroom extract	23.24±1.65	12.25±2.73
Irradiated mushroom extract	19.79±0.82*	153.96±4.86*

Values are expressed as means ± SD (n=3),

\* Significant difference from non-irradiated mushroom extract ( $p < 0.05$ )

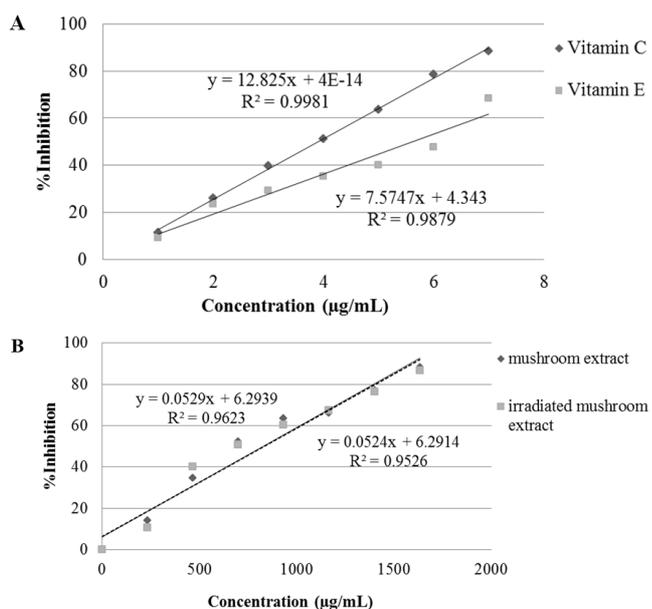


Figure 2. Inhibition curves on DPPH assay of vitamin C, vitamin E (A) and oyster mushroom extracts (B).

mushroom extracts, respectively. The total phenolic contents of non-irradiated and irradiated mushroom were 39.48 and 38.68 mg TAE/g, respectively.

### 3.4 Tyrosinase inhibition

At concentrations of 0.125-5.0 mg/mL, the extracts of non-irradiated and irradiated mushrooms moderately inhibited tyrosinase activity in a concentration dependent manner in the range of 10.23-52.28% and 12.77-53.40%, respectively. The half maximal inhibitory concentrations (IC<sub>50</sub>) of non-irradiated and irradiated mushroom extracts were 2,857.28 and 2,849.07 μg/mL (Table 3). In contrast, at 0.3-5.0 μg/mL of the positive control, kojic acid showed strong inhibition (5.92-78.23%) with IC<sub>50</sub> of 17.16 μg/mL.

### 3.5 Cytotoxicity test of mushroom extracts

Both non-irradiated and irradiated oyster mushroom extracts were subjected to *in vitro* cytotoxicity assay in human keratinocytes (HaCaT) and murine melanoma cells (B16F10) (Figure 3). After 24 h incubation, neither oyster

Table 3. DPPH radical scavenging ability, ferric reducing ability (FRAP), total phenolic content and tyrosinase inhibition activity of oyster mushroom extracts.

Sample	EC <sub>50</sub> of DPPH assay (µg/mL)	FRAP value (µM FeSO <sub>4</sub> ·7H <sub>2</sub> O/g)	Total Phenolics (mg TAE/g)	IC <sub>50</sub> of Tyrosinase inhibitory activity (µg/mL)
Non-irradiated mushroom extract (control)	869.95±4.04	2.12±0.48	39.48±1.24	2,857.28±14.93
Irradiated mushroom extract	871.31±6.68	2.15±0.75	38.68±2.57	2,849.07±16.79
Vitamin C	2.69±0.17*	-	-	-
Vitamin E	7.75±0.24*	-	-	-
Kojic acid	-	-	-	17.16±1.89*

Values are expressed as means ± SD (n=3), \*Significant differences from control at  $p < 0.05$ .

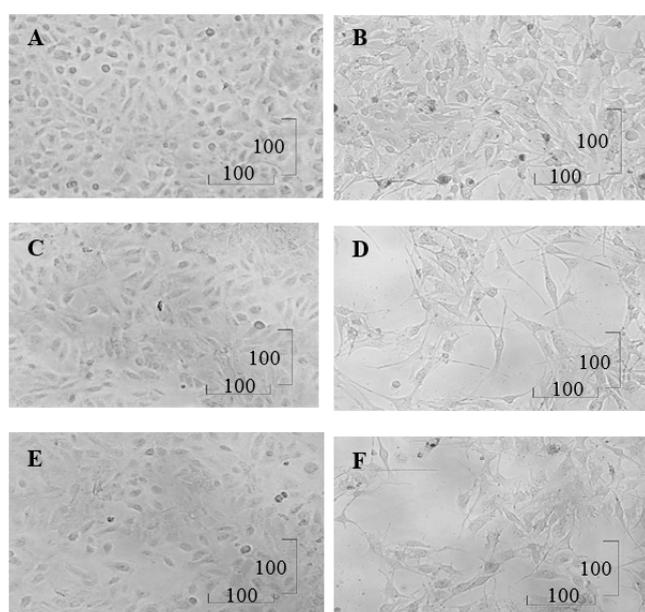


Figure 3. Morphology of keratinocytes (A, C, E) and murine melanoma cells (B, D, F) after incubation for 24 h in DMEM (A, B), with 2,000 µg/mL UV-B irradiated oyster mushroom extract (C, D) and 2,000 µg/mL non-irradiated oyster mushroom extract (E, F).

mushroom extract (12.5-2,000 µg/mL) was toxic to keratinocytes cell line and gave high level of cell viability (more than 90%), therefore the half inhibition concentration (IC<sub>50</sub>) could not be determined (Figure 4A). Both oyster mushroom extracts had slight toxic effects on melanoma cells with the half inhibition concentrations (IC<sub>50</sub>) of 1,556.19 and 1,212 µg/mL for non-irradiated and irradiated mushroom extracts respectively (Figure 4B).

#### 4. Discussion

The result of method validation demonstrated that the HPLC method used for analysis of ergosterol and vitamin D<sub>2</sub> in this study is suitable with the percentage recovery close to 100 percent, with values of inter-day and intra-day pre-

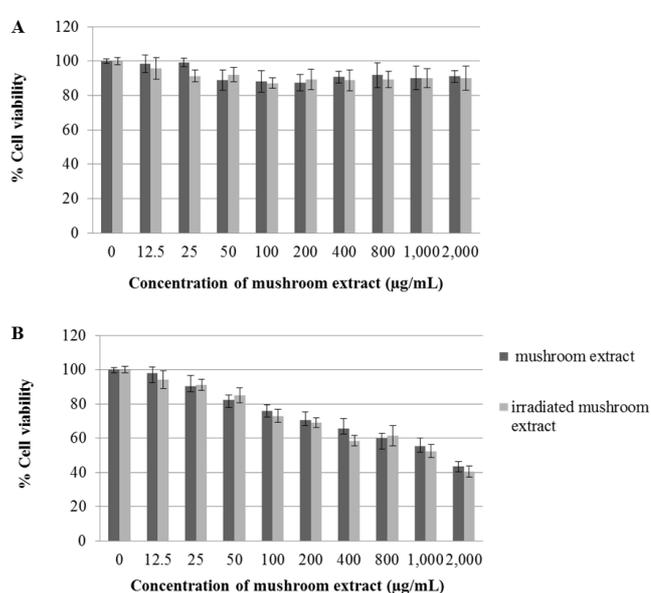


Figure 4. Cell viability (%) of human keratinocytes (A) and murine melanoma cells (B) after treatment with non-irradiated and irradiated oyster mushroom extracts. Error bars indicate means ± SD (n=3).

cision of lower than 2%. Irradiated mushroom extract had higher vitamin D<sub>2</sub> and lower ergosterol values than non-irradiated mushroom extract. Exposing oyster mushrooms to UV light before extraction with ethanol can increase the vitamin D<sub>2</sub> concentration by a multiple of 13 in the mushroom extract. UV light induced the production of vitamin D<sub>2</sub> as it was observed that the level of vitamin D<sub>2</sub> increased, whereas the level of ergosterol decreased. It is suggested that after irradiation some of ergosterols might be partially converted to vitamin D<sub>2</sub> (Jasinghe and Perera, 2005). However, the conversion of ergosterol to vitamin D<sub>2</sub> was rather low of quantity. Even though ergosterol in mushroom extracts was found in terms of milligrams, the yield of vitamin D<sub>2</sub> from this conversion was only in terms of micrograms. The reason for this low vitamin D<sub>2</sub> yield could possibly indicate the limita-

tion of UV penetration into the mushroom tissues as earlier reported that UV-B was able to penetrate through the epidermal layer not deeper than 50  $\mu\text{m}$  approximately from the surface of the mushroom (Teichmann *et al.*, 2007). Therefore, the further modification of UV irradiation method should be conducted to obtain higher vitamin D<sub>2</sub> production.

Oyster mushroom extracts were determined for free radical scavenging efficacy by the DPPH method and reducing power by the ferric reducing-antioxidant power method. Moreover, the total phenolic content and tyrosinase inhibitory effects of oyster mushroom extracts were investigated. Our result showed the low antioxidative activity of the extracts by both DPPH and FRAP assay. The FRAP values (2.12 and 2.15  $\mu\text{M FeSO}_4 \cdot 7\text{H}_2\text{O/g}$ ), were relatively low ( $<10 \mu\text{M Fe}^{2+}/\text{g}$ ) as previously reported by Wong *et al.* (2006) (Wong *et al.*, 2006; Ratchadaporn *et al.*, 2008; Ayub *et al.*, 2010). In overall comparisons of DPPH radical scavenging ability, ferric reducing ability, total phenolic content and tyrosinase inhibitory activity, both non-irradiated and irradiated mushroom extracts gave similar data. This suggests that UV-B irradiation condition did not affect antioxidative activity and tyrosinase inhibitory properties of oyster mushrooms. In contrast, the previous report on *Cordyceps militaris* by Agnes *et al.* (2012) demonstrated that pulsed UV light enhanced more vitamin D<sub>2</sub> production but lower antioxidative properties than UV-B irradiation. The loss in antioxidative properties could be attributed to the thermal damage caused by the use of high intensity. Moreover, our results showed that both oyster mushroom extracts had higher antioxidative activity and total phenolic content than the previous studies on this mushroom (Arbaayah and Umi, 2013; Pornariya and Kanok-Orn, 2009). Our results showing moderate tyrosinase inhibitory effect of the mushroom extracts is similar to that of Riani *et al.* (2012).

The cytotoxicity assay of oyster mushroom extracts on human keratinocytes cells (HaCaT) and murine melanoma cells (B16F10) were measured using MTT assay. Non-irradiated and irradiated oyster mushroom extracts had nearly the same cytotoxic effect. This result revealed that both mushroom extracts could inhibit growth of melanoma cells without significant effect on keratinocytes. Previous studies demonstrating that several derivatives of vitamin D<sub>2</sub>, such as 20(OH)D<sub>2</sub> and 1,2(OH)2D<sub>2</sub> had potent antiproliferative activity in normal and malignant cells (Slominski *et al.*, 2011) and 1,24(OH)2D<sub>2</sub> exerted growth inhibition against breast cancer (Zinser *et al.*, 2005) indicate that the metabolites of vitamin D<sub>2</sub> may play roles in their bioactivities. Our finding that the increasing of vitamin D<sub>2</sub> in the irradiated mushroom extract did not enhance its cytotoxicity to melanoma cells suggests that further study on the bioactivities of vitamin D<sub>2</sub> and its metabolites is necessary.

All the obtained data suggest that UV-B irradiation can induce higher vitamin D<sub>2</sub> production without affecting the mushroom biological activities including antioxidation, tyrosinase inhibition and cytotoxicity. Moreover, the increasing of vitamin D<sub>2</sub> in mushroom extract did not influence the

biological activities of cytotoxicity. However, further studies should consider identifying related mechanisms and investigate other biological activities such as immune modulation and anti-inflammatory activities.

#### Acknowledgements

This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission, through the Food and Functional Food Research Cluster, Applied Taxonomic Research Center and Center for Research and Development of Herbal Health Products, Faculty of Pharmaceutical Sciences, Khon Kaen University.

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