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Research Article

POTENT *IN VITRO* COLLAGEN BIOSYNTHESIS STIMULATING AND ANTIOXIDANT ACTIVITIES OF EDIBLE MUSHROOM *VOLVARIELLA VOLVACEA* AQUEOUS EXTRACT

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ABSTRACT

Beside Thai herbs and spices, several mushrooms are used in Thai cuisine such as straw mushrooom(*Volvariella volvacea*) in Tom Yam Kung.

Objective: To investigate the *in vitro* collagen biosynthesis stimulating and antioxidant activities of edible mushrooms which are common use as Thai food ingredient including *V. volvacea* collected from northern Thailand.

Methods: The 11 selected mushrooms were extracted by three extraction methods including the hot (HW) and sonicated (SW) aqueous and the macerated ethanolic (ME) processes. These extracts were tested for antioxidative activity (including DPPH radical scavenging, metal chelating and lipid peroxidation) and *in vitro* collagen biosynthesis stimulating activities.

Results: The sonicated aqueous extracts of *V. volvacea* (VV SW) not only showed the highest total phenolic (6.68 mg GAE) and polysaccharide contents (0.069 mg GLU) but also gave the highest DPPH radical scavenging, lipid peroxidation inhibition and collagen biosynthesis stimulating activities with the relative amount of the collagen biosynthesis stimulation of 146.77±13.20 % of negative control which was significantly higher than standard ascorbic acid at about 1.14 times.

Conclusion: This present study has suggested the sonicated aqueous extracts of *V. volvacea* to be developed as a functional food or cosmeceutical product with the potent *in vitro* collagen biosynthesis stimulating and antioxidant activities which can be promoted the traditional ingredient for Thai cuisine recipes as well.

Keywords: Antioxidative activity, *In vitro* collagen biosynthesis stimulating, Mushroom, *Volvariella volvacea*

INTRODUCTION

The global market values of anti-oxidant products which help the body fights off the damage caused by aging are increasing continuously. Furthermore, the largest group of compounds in anti-aging products is antioxidants. Free radicals are highly reactive molecules with the unpaired electrons, which can cause cellular damage to cell membranes, lipids, proteins, and DNA. Damage to DNA eventually results in collagen breakdown. Although antioxidant defense and repair systems are available in humans and other organisms to protect them against oxidative damage, these systems are insufficient to prevent the damage [1].

Mushrooms are widely consumed and have been valued as an edible and medical resource. Many studies have found that some species of mushrooms are having therapeutic properties such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immuno stimulatory effects [2, 3]. They accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids [4]. Mushrooms are traditional in Thailand and also commonly used as food ingredients. In addition to common mushrooms and commercial mushrooms such as *Pleurotus ostreatus*, *Flammulina velutipes*, *Pleurotus eryngii*, *Volvariella volvacea*, *Hypsizygus marmoreus*, *Lentinus edodes*, *Agrocybe cylindracea* and *Pleurotuso streatus*. *V. volvacea* (Family: Phuteaceae) which also known as paddy straw mushroom or straw mushroom or "Hed Fang" in Thai is a specie of edible mushroom cultivated throughout East and Southeast Asia and used extensively in Thai cuisines. *V. volvacea* is a popular variety among people because of its distinct flavor, pleasant tastes, higher protein content and shorter cropping duration compared to other cultivated mushrooms. Presently, this mushroom is available in most of the supermarkets. It originally grows in rice straw stack in tropical zone that have high temperature and a rainy climate especially in Chiang Mai, Thailand.

Although, there are many studies on cultivated and biological activities of commercial and edible mushrooms in many countries, there is little information available about antioxidant properties of edible mushrooms in Thailand, and this is the study on the antioxidant activities of edible mushrooms collected from the Northern Thailand. Our objective was to evaluate the bioactive compound contents (total phenolic and polysaccharide), antioxidant properties of aqueous and ethanolic extracts of twelve edible mushroom species including DPPH radical scavenging, metal chelating and lipid peroxidation activities as well as *in vitro* collagen biosynthesis stimulating activity in order to evaluate their anti-oxidant and the stimulation of collagen biosynthesis activities of the mushroom extracts.

MATERIALS AND METHODS

Materials

L-(+)-Ascorbic acid (vitamin C), α-tocopherol, butylatedhydroxytoluene (BHT), 2,2-diphenyl-1-picryhydrazyl radical (DPPH), ammonium thiocyanate (NH_4SCN), Ethylenediamine tetraacetic acid (EDTA), ferrozine and ferric chloride ($FeCl_2$) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were analytical grade.

Preparation of the mushroom extracts

Eleven mushrooms including *V. volvacea* were collected from Chiang Mai Province in Thailand, during January to June in 2013 (**Table 1**). The specimen was authenticated by a botanist at Faculty of Pharmacy, Chiang Mai University, Thailand and deposited at Faculty of Pharmacy, Chiang Mai University in Thailand. Then, the mushrooms were washed, cut into pieces, dried at 40±2°C in a hot air oven, ground to powder and kept in an airtight plastic bag at 4±2°C until use. For the extraction process, 100 g of the dried mushroom powder were extracted using three different conditions. For HW (hot-water), the powder was heated for 2 h with 1000 ml distilled water at 100±2°C and then cooled to room temperature (27±2°C). For SW (sonicated-water) and ME (macerated-ethanol), 100 g of the powder were sonicated in 1000 ml of distilled water for 2 h at room temperature (27 ± 2°C) or macerated in 1000 ml of ethanol for 24 h. The mixtures were filtered through Whatman No. 1

filter paper and the plant residues were re-extracted twice under the same conditions. The filtrates were pooled and concentrated under vacuum by a rotary evaporator (R-124 Buchi, Switzerland), and lyophilized. The dried extracts were stored at 4±2°C prior to use. Thirty three extracts were obtained and the percentage yields were calculated on a dry weight basis.

Determination of bioactive compounds

Determination of total phenolic contents

The Folin-Ciocalteu method [5] was used to determine the total phenolic content of the extracts. Briefly, 500 µl of the extract dissolved in distilled water at the concentration of 10 mg/ml was mixed with an equal volume of 1N Folin-Ciocalteu reagent and 1 ml of 20% sodium carbonate (Na_2CO_3) and incubated for 2 h at room temperature (25 ± 2°C). The reaction mixture was then centrifuged at 5000 rpm for 10 min. The absorbance of the supernatant was measured at 730 nm using a spectrophotometer. The experiments were done in triplicate. The total phenolic contents were calculated from the calibration curve of the standard gallic acid (Gallic acid (mg) = 0.1910× absorbance −0.0316; R^2=0.9863). The average of three experiments was used and the total phenolic content was expressed in mg of gallic acid equivalents (GAE)/ g of the extract.

Determination of total polysaccharide contents

Soluble polysaccharide assay was conducted with the phenol-sulfate method [6] with a modification. Briefly, boil dried mushroom powder in 95± 2°C for 2 h, with filtrate dialyzed under 4± 2°C for 12 h. The obtained extracts were analyzed with results compared to the data shown on the glucose standard curve at 730 nm spectrophotometrically [7].

Antioxidative assays

DPPH radical scavenging activity

The DPPH radical scavenging activity of all mushroom extracts was determined by a modified method previously described [8]. Briefly, 50 µl of the five serial concentration extracts (0.001-10 mg/ml dissolved in distilled water) and 50 µl of ethanolic solution of DPPH were put into each well of a 96-well microplate (Nalge Nunc International, NY, USA). The reaction mixture was allowed to stand for 30 min at 27±2°C, and the absorbance was measured at 515 nm by a well reader (Bio-Rad, model 680 microplate reader, USA) against a blank (distilled water). Ascorbic acid (0.001-10 mg/ml) was used as positive controls. The experiments were done in triplicate. The IC_{50} value which was the concentration of the sample that scavenged 50% of the DPPH radical was determined. The percentages of DPPH radical scavenging activity were calculated [Scavenging (%) = ($Abs_{control}$ − Abs_{sample})/$Abs_{control}$× 100%]. The histogram of the percentages of DPPH radical scavenging activity of the extract at 0.5 mg/ml was presented.

Chelating activity

The Fe^{2+} chelating ability of the mushroom extract was measured by the ferrous iron–ferrozine complex method [9]. Briefly, the reaction mixture containing 2 mM $FeCl_2$ (10 µl) and 5 mM ferrozine (10 µl) and 100 µl of the five serial concentration extracts (0.001-10 mg/ml dissolved in distilled water) were mixed in a 96-well plate and incubated for 10 min at 27±2°C. The absorbance was recorded by a well reader at 570 nm. The absorbance of the control was determined by replacing the extract with methanol. EDTA (0.001-10 mg/ml) was used as a positive control. The experiments were done in triplicate. The IC_{50} value which was the concentration of the sample that chelated 50% of the ferrous iron was determined. The ability of the sample to chelate ferrous ion was calculated [Chelating effect (%) = [($Abs_{control}$ − Abs_{sample})/$Abs_{control}$× 100%]. The histogram of the percentages of chelating effect of the extract at 0.5 mg/ml was presented.

Lipid peroxidase inhibition activity

The lipid peroxidase activity of mushroom extract was assayed by the modified Ferric-thiocyanate method [10, 11]. An amount of 50 µl of five serial concentrations of the extract at 0.001-10 smg/ml dissolved in distilled water was added to 50 µl of linoleic acid in 50% (v/v) DMSO in each well of a 96-well microplate. The reaction was initiated by the addition of 50 µl of NH_4SCN (5 mM) and 50 µl of $FeCl_2$ (2 mM). The mixture was incubated at 37±2°C for 1 h. During the oxidation of linoleic acid, peroxides are formed leading to the oxidation of Fe^{2+} to Fe^{3+}. The latter ions form a complex with thiocyanate which can be detected at 490 nm. The dissolving solution without the sample was used as negative control. α-Tocopherol (at 0.001-10 mg/ml) was used as a positive control. All experiments were performed in triplicate. The inhibition percentages of lipid peroxidation of linoleic acid were calculated as the following: Inhibition of lipid peroxidation (%) =(Abs_{sample}/$Abs_{control}$) × 100%. The sample concentration providing 50% inhibition of lipid peroxidation (IC_{50}) was calculated from the graph plotted between the percentages of lipid peroxidation inhibition and the sample concentrations. The histogram of the percentages of Inhibition of lipid peroxidation of the extract at 0.5 mg/ml was presented.

Stimulation of collagen synthesis

Cell culture

The human fibroblasts (ATCC, Virginia, USA) were cultured under the standard conditions in the DMEM (Gibco BRL, Gaithersburg, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco BRL, Gaithersburg, USA), 100 IU/ml of penicillin and 100 mg/ml of streptomycin (Gibco BRL, Gaithersburg, USA). The cells were incubated at 37°C under 5% CO_2 for 24 hr.

Cytotoxicity on human fibroblast of selected mushroom extracts

The mushroom extracts which gave the best antioxidant activity were selected for the stimulation of collagen synthesis study. Cytotoxicity assay of the selected mushroom extracts at various concentrations (0.001-10 mg/ml) on human fibroblasts at 24 hr was determined by the SRB assay [12] to evaluate for the appropriate concentration that gave more than 80% cell viability in order to use in the collagen assay.

Collagen biosynthesis stimulating activity

Collagen biosynthesis stimulating activity assay is based on the binding of Sirius red dye (Direct Red 80, Sigma, USA), to the triple helical collagen following the previously described [13] with some modifications. A monolayer of 5 x 10^5 cells in 6-well plate was maintained in the culture medium without FBS for 24 hr, treated with the mushroom extracts at the selected concentration from SRB assay and incubated for 24 hr. Then, the cells were scrapped and 500 µl of 0.1% Sirius red in saturated picric acid was added into each well. The reaction was performed by incubation at room temperature for 1 hr. The cell suspensions were transferred into a microfuge, and centrifuged at 15,000 rpm for 5 min. The plates were rinsed with 500 µl of 10 mM HCl for 5 times, then dissolved with 0.1M NaOH. The absorbance was read at 492 nm. The experiments were done in triplicate (n=3). The amounts of collagen were compared to the standard curve of type I bovine collagen (Sigma, USA) samples.

Statistical analysis

All assays were performed in triplicate in three independent and separate experiments. The data were presented as means±standard deviations (SD) from three independent analyzes, separated experiments. ANOVA was used for the analysis of the test results (LSD test) at the significance level of *p*-value < 0.05 and 0.01.

RESULTS AND DISCUSSION

Percentage yields of the mushroom extracts prepared by different processes

The percentage yields of the 33 mushroom extracts prepared by three different extraction processes (HW, SW and ME) of the 11 selected edible mushrooms were presented in **Table 1.** Aqueous extracts of

most plants gave higher percentage yield than the ethanol extracts. The mushroom may contain more water soluble than water insoluble constituents. The highest percentage yields were from *P. ostreatus* Kummer (POK) by SW and HW at 66.26 and 63.33%, while *V. volvacea* (VV) and *A. cylindracea* (AC) from the sonication and hot water process gave 45.25, 14.66, 46.98 and 57.11 %, respectively. Sonication with water (SW) process of almost mushroom extracts gave higher yields than the hot water process (HW). Most mushrooms may contain heat labile and water soluble components [14]. Thus, the aqueous with sonication (SW) which was the cold process appeared to be the superior process for water soluble components.

Total phenolic and polysaccharide contents

Figure 1 showed the total phenolic and polysaccharide contents of the 33 extracts from 11 edible mushrooms. The total phenolic contents (mg of GAE/g ± SD) varied from 2.48 to 6.68 mg of GAE/g, whereas the total flavonoid contents (mg of GLU/g ± SD) varied from 0.013 to 0.069.

Table 1: Comparison of percentage yields of the 33 mushroom extracts from the 11 selected edible mushrooms including *V. volvacea* prepared by aqueous and ethanol processes

Scientific name	Extract code	% Yield		
		HW	SW	ME
Agaricusbisporus (langes) Sing.	AB	32.56	45	34.5
Agrocybecylindracea	AC	57.11	46.98	21.91
Flammulinavelutipes (Curt,ex Fr.) Sing	FV	43.6	62.45	39.6
Hericiumerinaceus	HE	40	41.8	16.97
Hypsizygusmarmoreus (Peck)Bigelow	HM	47.68	49.23	5.53
Lentinusedodes (Berk.) Sing.	LE	24.26	32.25	8.34
Lentinuspolychrous Lev.	LP	25.6	51.5	9.53
*Pleurotuseryngii*Quel.	PE	33.26	41.8	16.28
Pleurotusostreatus (Fr.)Guel	POG	32.68	29.76	6.72
Pleurotusostreatus (Fr.) Kummer	POK	63.33	66.26	17.24
Volvariella volvacea (Bull. Ex.Fr.) Sing	VV	14.66	45.28	40.4

Note: HW = hot water process; SW = sonicatedwater process; ME = macerated ethanol process

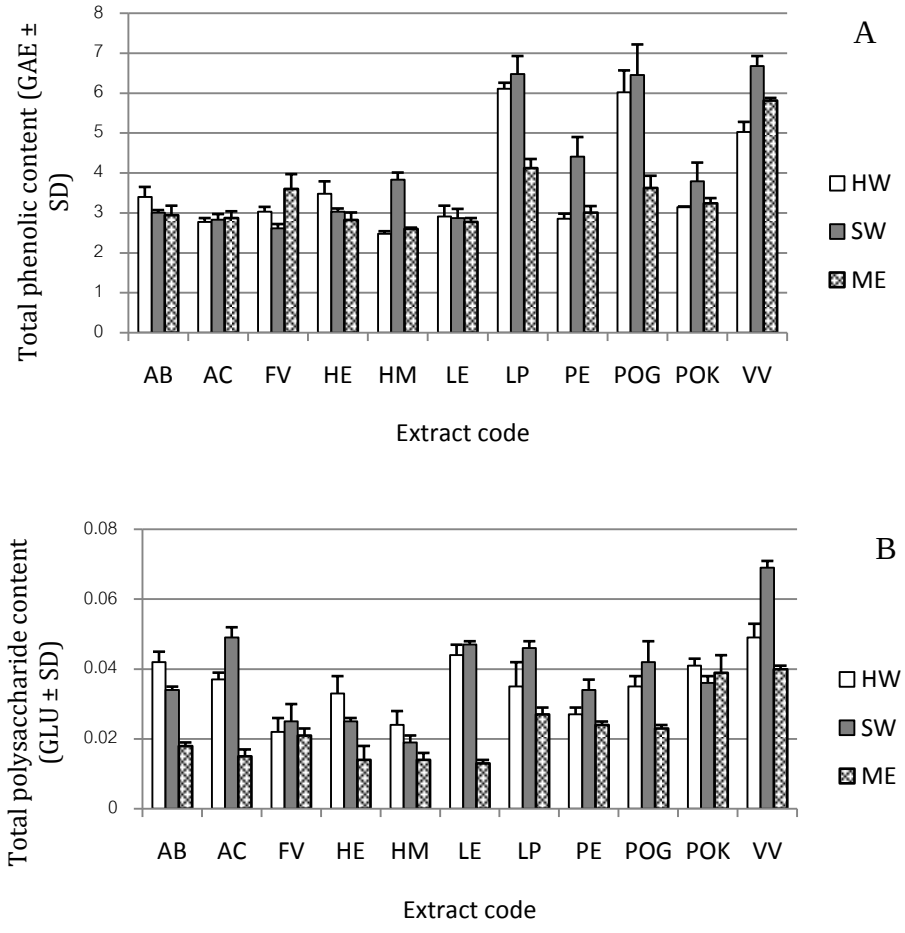

Fig. 1: Comparison of bioactive compound (A= total phenolic and B= total polysaccharide) contents in the 33 mushroom extracts from 3 extraction process (HW = hot water process; SW = sonicated water process; ME = macerated ethanol process)

The highest total phenolic and polysaccharide contents were observed in sonicated water (SW) of *V. volvacea* (VV) with GAE and GLU of 6.68±0.25 and 0.069±0.002, respectively. All aqueous mushroom extracts (HW and SW) showed slightly higher total phenolic and polysaccharide contents than ethanolic extracts (ME). For the extraction temperatures of aqueous extracts, the extracts from the cold process (SW) gave significant higher (p<0.05) total phenolic and total polysaccharide content (summation of GAE at 45.99 and GLU at 0.426) than that from the hot temperature (HW) process (summation of GAE at41.22 and GLU at 0.389). This might be due to the higher heat labile substances obtained from mushroom extracts [14] resulting in the higher content of phenolic and polysaccharide contents in the extracts from the cold process (SW). Mushrooms have developed chemical defence mechanisms (against insects and microorganisms) analogous to those in plants, such as the production of phenolic compounds. In fact, phenolic compounds have been shown to protect the plant cell wall during ultraviolet, salt, or pathogenic stress [15]. Indeed, other authors [16] reported the presence of caffeic, p- coumaric and ellagic acids.

DPPH radical scavenging activity

Table 2 showed the IC_{50} values of the DPPH radical scavenging assay of the 33 mushroom extracts. **Figure 2** demonstrated the percentages of DPPH radical scavenging activity of the 33 extracts at 0.5 mg/ml. The standard antioxidants, ascorbic acid at 0.5 mg/ml, gave the scavenging activity of 90.93±2.16% with the IC_{50} values of 0.09±0.01 mg/ml. The aqueous extract of most mushrooms exhibited higher activity than the ethanolic extracts. The phenolic compounds which have been reported to scavenge DPPH include flavonoids, anthraquinones, anthocyanidins, xanthones, and tannins. They also scavenged superoxide and hydroxyl radical by the single electron transfer [17, 18]. Aqueous solvent has been commonly employed to extract phenolic compounds from plants, because of obtaining high yield although they were not highly selective for phenols [19]. Extracts from *V. volvacea* (VV) prepared by all processes especially by sonicated water process (SW) exhibited higher DPPH radical scavenging activity than those of other mushrooms.

Table 2: IC_{50} values of the 33 extracts from the 11 selected edible mushrooms including *V. volvacea* determined by the DPPH radical scavenging, chelating and lipid peroxidase inhibition assays.

Extract code	IC_{50} of DPPH radical scavenging (mg/ml)			IC_{50} of chelating (mg/ml)			IC_{50} of lipid peroxidation inhibition (mg/ml)		
	HW	SW	ME	HW	SW	ME	HW	SW	ME
AB	3.16±0.34	2.34±1.63	10.84±13.10	-	-	5.58±4.0	13.82±2.71	26.69±3.28	-
AC	2.16±1.3	2.41±0.05	2.87±1.34	-	-	12.41±1.48	5.84±0.65	2.41±1.48	14.86±0.29
FV	5.15±1.54	5.11±1.11	3.90±0.82	-	6.14±0.87	3.07±1.00	-	10.84±0.29	-
HE	3.95±0.96	3.82±0.21	4.48±0.52	7.75±1.15	4.18±1.65	2.74±1.10	-	-	-
HM	3.32±0.16	3.53±0.48	14.55±1.52	-	-	16.54±2.88	9.98±1.44	2.56±1.12	10.77±1.34
LE	4.05±1.10	2.28±0.20	10.59±2.12	2.10±1.39	2.12±0.99	2.13±0.70	8.49±0.26	5.31±0.30	-
LP	10.55±7.29	4.37±0.62	17.50±17.42	-	2.33±1.02	1.90±0.77	7.60±4.81	6.40±8.72	-
PE	4.29±1.47	3.71±0.28	4.57±1.00	2.65±1.05	1.32±0.66	1.00±0.37	8.32±1.88	8.76±2.22	9.94±1.24
POG	5.27±2.08	4.72±3.76	9.50±3.55	-	-	23.21±3.56	-	-	-
POK	5.66±0.81	2.33±0.46	4.36±0.14	2.95±0.39	2.66±2.39	4.17±5.70	14.17±1.07	9.91±0.39	-
VV	2.17±0.22	2.05±0.18	2.30±0.08	2.36±0.62	2.04±0.57	1.75±1.31	4.02±0.98	1.61±0.37	6.98±1.50
Ascorbic acid		0.09±0.01			N/A			N/A	
EDTA		N/A			0.08±0.01			N/A	
α-Tocopherol		N/A			N/A			0.11±0.04	

Note: = No activity; N/A = not available; HW = hot water process; SW = sonicatedwater process; ME = macerated ethanol process

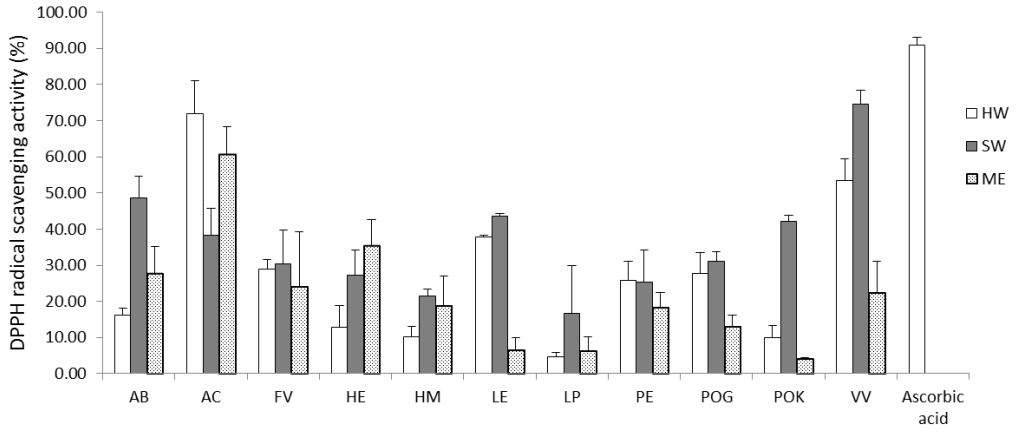

Fig. 2: Comparison of the percentages of DPPH radical scavenging activity of the 33 extracts at 0.5 mg/ml from the 11 selected edible mushrooms including *V. volvacea* and the standard antioxidant (ascorbic acid at 0.5 mg/ml) (HW = hot water process; SW = sonicated water process; ME = macerated ethanol process)

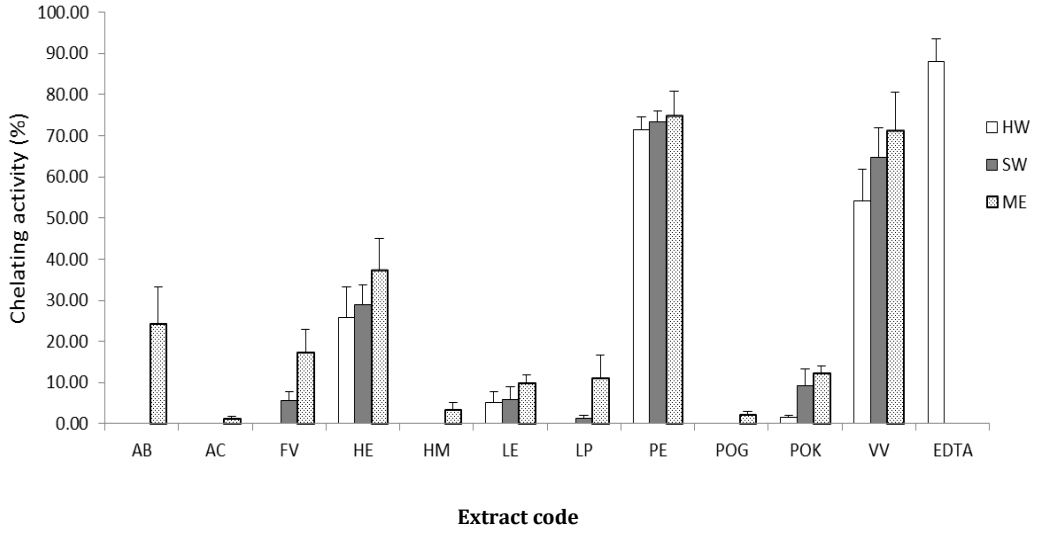

Fig. 3: Comparison of the percentages of the chelating activity (%) by the ferrous iron–ferrozine complex method of the 33 extracts at 0.5 mg/ml from the 11 selected edible mushrooms including *V. volvacea* and the standard chelating agent (EDTA at 0.5 mg/ml) (HW = hot water process; SW = sonicated water process; ME = macerated ethanol process)

The highest DPPH radical scavenging activity of *V. volvacea* extract was 74.47±3.95% with the IC_{50} value of 2.05±0.18 mg/ml. The highest total phenolic content of this extract (**Figure 2**) may be responsible for its activity. The previous report [20] showed the antioxidant activities of the aqueous extract from the mycelia of*V. volvacea*. The extracts were found to possess radical scavenging and antioxidant activities, as determined by scavenging effect on the DPPH, ABTS, DMPD and hydroxyl radicals. They found that the aqueous extract of the mycelia contains substantial amount of phenolics and flavonoids and it is the extent of phenolics present in this extract being responsible for its marked antioxidant activity as assayed through various *in vitro* models.

Chelating activity

Table 2 showed the IC_{50} values of chelating activity assay of the 33 mushroom extracts. **Figure 3** presented the percentages of the chelating activity of 33 extracts at 0.5 mg/ml. None of the mushroom extracts indicated better chelating activity than EDTA at 0.5 mg/ml which gave 87.97±5.46% and the IC_{50} value of 0.08±0.01 mg/ml. The HW, SW and ME extracts of *V. volvacea* gave the IC_{50} values of 2.36±0.62, 2.04±0.57 and 1.75±0.31 mg/ml, respectively. The highest chelating activity of 74.88±5.98% was found in macerated ethanol process (ME) of *P. eryngii* (PE) with the IC_{50} value of 1.00±0.37 mg/ml. The previous study also investigated metal chelating effect of *P. erygnii* and found that the methanolic extract of *P. eryngii* revealed the highest chelating effects [21]. Transition metals are believed to serve as the catalysts for the initial formation of radicals. Chelating agents, on the other hand may stabilize transition metals in living systems and inhibit generation of free radicals, consequently reducing free radical mediated damage [22]. Methanolic extracts of medicinal mushrooms such as *Ganoderma lucidum, Ganoderma tsugae* and *Coriolus versicolor* have been found to chelate ferrous ions[1].

Lipid peroxidation inhibition

Table 2 showed the IC_{50} values of lipid peroxidation inhibition of the 33 mushroom extracts. **Figure 4** presented the percentages of the lipid peroxidation inhibition of 33 extracts at 0.5 mg/ml. None of the mushroom extracts indicated better chelating activity than standard α-Tocopherol at 0.5 mg/ml which gave 82.56±7.65% and the IC_{50} value of 0.11±0.04 mg/ml. The HW, SW and ME extracts of *V. volvacea* gave higher activity than the other mushroom extracts with IC_{50} values of 4.02±0.98, 1.61±0.37 and 6.98±1.50, respectively. The highest lipid peroxidation inhibition of 64.91±8.20% was found in sonicated water process (SW) of *V. volvacea*. The SW extract of *V. volvacea* exhibited not only high DPPH radical scavenging activity but also high lipid peroxidation inhibition activity. The phytochemical especially polyphenolic compounds might be responsible for these activities. In fact, plant foods which are a good source of polyphenols have been reported to be effective radical scavengers and inhibitors of lipid peroxidation [23, 24].

Collagen biosynthesis stimulating activity on human skin fibroblast cell line

The mushroom extracts which gave the highest yield and also antioxidant activity, *V. volvacea* (VV) and *A. cylindracea* (AC) extracts from all 3 extraction processes (SW, HW and ME), were selected for the determination of collagen biosynthesis stimulating activity. The highest collagen biosynthesis stimulating activity was obtained from the sonication with water process extracts (SW) of both extracts, *V. volvacea* (VV) and *A. cylindracea* (AC) extracts. The SW extract of *V. volvacea* showed the highest stimulation collagen synthesis with the collagen content of 0.1001±0.0090 mg/ml which was significantly higher (p<0.01) than the negative control (untreated cells) at about 1.47 times, and also gave higher activity than the positive control (ascorbic acid) at about 1.14 times (**Table 3**). The aqueous extracts from both processes (SW and HW) gave higher collagen biosynthesis stimulating activity than those of ME extracts. This result agreed with the more bioactive compound contents in the aqueous extracts than those of ethanolic extracts from this study. The SW extract of *V. volvacea* (VV SW) showed the highest relative amount of the collagen biosynthesis stimulation, followed by the SW extract of *A. cylindracea* (AC SW) and the HW extract of *A. cylindracea* (AC HW) of 146.77±13.20, 132.40±22.14 and 121.99±13.78 % of negative control, respectively**(Figure 5).** The superior collagen biosynthesis stimulating activity of the SW extract of *V. volvacea* (VV SW) might be from the highest total polysaccharide content. This results agreed with the previous study showed that the polysaccharides played an important role in collagen biosynthesis stimulating activity of *Grifola frondosa* mushroom [25].

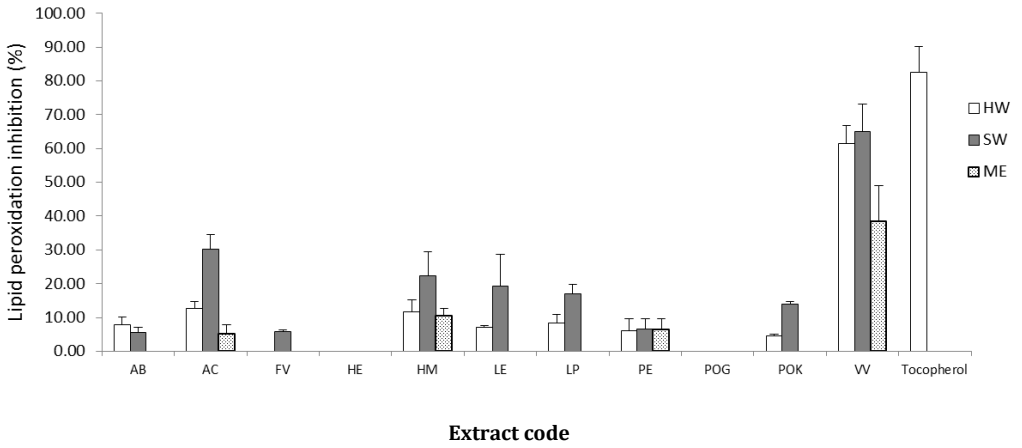

Fig. 4: Comparison of the percentages of the lipid peroxidation inhibition (%) of the 33 extracts at 0.5 mg/ml from the 11 selected edible mushrooms including *V. volvacea* and the standard α-Tocopherol at 0.5 mg/ml (HW = hot water process; SW = sonicated water process; ME = macerated ethanol process)

Table 3: Collagen contents from collagen biosynthesis stimulating activity of the 6 selected mushroom extracts (at 0.1 mg/ml) on human skin fibroblasts cell line

Extract code	Collagen contents (mg/ml)
VV SW	0.1001 ± 0.0090
VV HW	0.0791 ± 0.0031
VV ME	0.0756 ± 0.0093
AC SW	0.0903 ± 0.0151
AC HW	0.0832 ± 0.0094
AC ME	0.0824 ± 0.0085
Negative control (untreated cell)	0.0682 ± 0.0097
Positive control (0.1 mg/ml ascorbic acid)	0.0878 ± 0.0026

Note: HW = hot water process; SW = sonicated water process; ME = macerated ethanol process

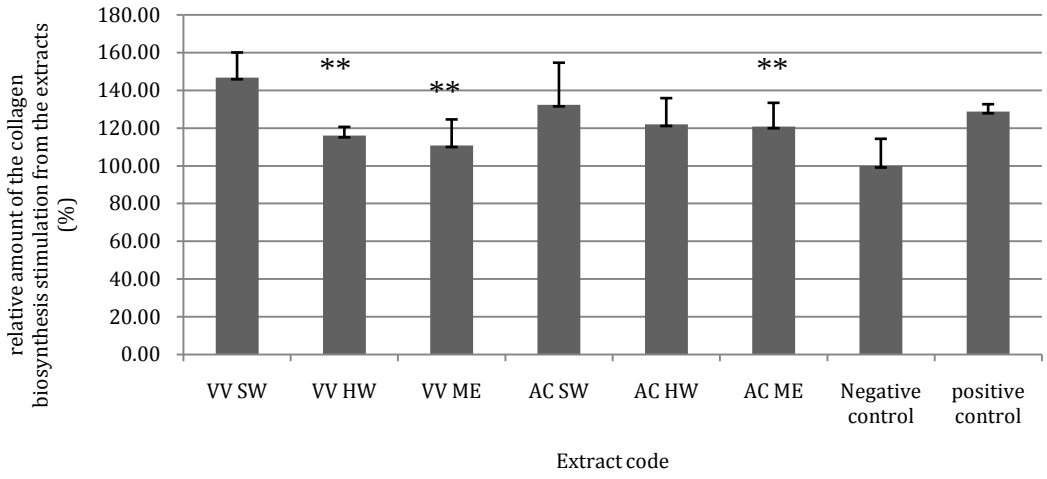

Fig. 5: Influence of 0.1 mg/ml of *V. volvacea* (VV) and *A. cylindracea* (AC) extracts from 3 extraction processes (HW = hot water process; SW = sonicated water process; ME = macerated ethanol process) in collagen biosynthesis stimulating activity on human skin fibroblast cell line as determined by ELISA. NC: negative control (untreated cells) and PC: positive control (0.1 mg/ml of ascorbic acid)

CONCLUSION

This present study has demonstrated *in vitro* collagen biosynthesis stimulating and antioxidant activities of *V. volvacea* in comparing to the 11 edible mushrooms which were the common ingredient of Thai cuisine recipes. The antioxidant activities including DPPH radical scavenging, chelating and lipid peroxidation activities were used to evaluate the 33 mushroom extracts prepared by hot water (HW) or sonicated aqueous (SW) and macerated ethanolic (ME) processes. For all 11 mushrooms, the cold processes which were sonicated aqueous (SW) process gave higher yields, total phenolic and polysaccharide contents than the hot aqueous process (HW). The aqueous extract either by the hot (HW) or cold process (SW) of most mushroom extracts exhibited higher DPPH radical scavenging and lipid peroxidation inhibition activities than the ethanolic

extracts, whereas the macerated ethanolic process (ME) gave better chelating activity than the aqueous processes. The sonicated aqueous extracts of *V. volvacea* not only showed the highest total phenolic and polysaccharide contents but also gave the highest DPPH radical scavenging, lipid peroxidation inhibition and collagen biosynthesis stimulating activities. This present study has suggested the sonicated aqueous extracts of *V. volvacea* to be developed as a functional food or cosmeceutical product with the potent *in vitro* collagen biosynthesis stimulating and antioxidant activities and can be promoted the traditional ingredient for Thai cuisine recipes as well.

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