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ORIGINAL ARTICLE

Antioxidant, antibacterial and DNA protective activities of protein extracts from *Ganoderma lucidum*

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Abstract Crude proteins of cultured mycelia and fruiting bodies of *Ganoderma lucidum* were investigated for antioxidant, antibacterial and DNA protective activities. It was found that the half maximal inhibitory concentration (IC_{50}) of the mycelia protein and fruiting bodies protein extracts against 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical ($ABTS^{\bullet+}$) were 2.47±0.01 and 2.77±0.11 μg protein/ml and against 2,2-diphenylpicrylhydrazyl radical ($DPPH^{\bullet}$) were 2.5 ±0.01 and 3.42±0.01 μg protein/ml, respectively. The ferric reducing-antioxidant power (FRAP) values of those samples were 1.73±0.01 and 2.62±0.01 μmole trolox/μg protein respectively. Protein hydrolysates prepared by pronase exhibited a weaker antioxidant activity. Both crude proteins showed antibacterial activity, whereas only the mycelia protein extract could protect DNA damage by hydroxyl ($^{\bullet}OH$) radicals. This protein extract was partial purified by Diethyl amino ethyl (DEAE)-Sepharose column and Sulfopropyl (SP)-Sepharose column, obtained major protein with molecular weight about 45 kilo Dalton (kDa). In conclusion, *G. lucidum* protein extracts have promise potential for applications as antioxidant and antibacterial agents.

Keywords Antioxidant · Mushroom · Reducing power · Protein purification · DNA protection

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Introduction

Free radicals are atoms, molecules or ions with unpaired electrons (Lu et al. 2010). They are highly active to react with biological molecules including DNA, proteins, lipid membranes (Temple 2000), resulting in diseases and disorders, especially degenerative diseases such as cardiovascular diseases and cancers (Wagner et al. 1992). Free radicals can be eliminated by antioxidant molecules. Thus, these molecules have been interested. A wide range of molecules have been reported to possess antioxidant activities such as phenolic compounds, vitamins, polysaccharides, peptides, proteins, organic acids, carotenoids, alkaloids, and nucleotides (Stajić et al. 2013) Recently, proteins and peptides with antioxidant activities have been highly interested. These molecules normally contain amino acids with side chains that can donate electrons such as cysteine and tyrosine (Power et al. 2013; Sarmadi and Ismail 2010).

Mushrooms have been consumed by many cultures for centuries and have long been used in traditional Chinese medicines. Bioactive compounds of mushrooms such as polysaccharides, proteins, polysaccharide-protein complexes have been reported to possess several biological activities (Xu et al. 2011). The mushroom *Ganoderma lucidum* is popular medicinal mushroom and widely used for promote health and longevity. This mushroom also has been reported to have antioxidant activities (Yen and Wu 1999; Mau et al. 2002), cardioprotective effects (Sudheesh et al. 2013) and antidiabetic potency (Teng et al. 2012). However, molecules responses for those activities mostly are not proteins. There are a few investigations reported about proteins or peptides with bioactivities from this mushroom. Immunomodulatory protein derived from this mushroom has been proposed to have therapeutic effects on cancer and autoimmune diseases (Girjal et al. 2012). In the present study, the crude protein extracts from mycelia and fruiting bodies of *G. lucidum* which production in

the Rujira Farm (Kalasin, Thailand) and distribution nationwide, were evaluated for antioxidant and antibacterial activities.

Materials and methods

Fungal cultivation

G. lucidum G2 originated from Department of Agriculture, Thailand were obtained from Rujira Mushroom Farm in Ka La Sin province (Northeast, Thailand). The fungus was cultured and maintained in potato dextrose agar (PDA). After that the fungal active growing mycelia plugs were transferred to aseptic flask containing liquid medium, in which the medium included glucose (10 g/l), yeast extract (5 g/l), peptone Type-I (5 g/l) and $MgSO_4$ (1 g/l). The fungal mycelia were cultured for 14 days at ambient temperature and shaking at 120 revolutions per minute (rpm). Fresh fruiting bodies of the mushroom were obtained from the mushroom farm.

Bacterial strains

The tested bacteria were mostly purchased from Thailand Institute of Scientific and Technological Research (TISTR). The gram positive bacteria included *Bacillus subtilis* TISTR 008, *Bacillus cereus* TISTR 687, *Staphylococus epidermidis* TISTR 518, *Staphyloccus aureus* TISTR 1466. The gram negative bacteria were *Escherichia coli* TISTR 780 and *Pseudomonas aeruginosa* TISTR 781. All bacteria were maintained in LB (Luria-Bertani) medium.

Crude protein extraction

The mycelia of *G. lucidum* were washed with distilled water and homogenized in extraction solution as previously used (0.01 M HCl containing 0.15 M NaCl) (Phansri et al. 2011) in a proportion of 1 g of mycelia to 2 ml of extraction solution. The crude extract solution after filtering through cheesecloth was centrifuged (Hettich Mikro 22R, Germany) at 3,904 × g, 4 °C for 30 min. The fruiting bodies of the mushroom were washed with distilled water and cut into small pieces before homogenization with the extraction solution. Extraction of fruiting bodies was performed similar to the mycelia extraction as described above.

Protein determination

Soluble protein content was determined by the Bradford method (Bradford 1976) using a Bio-Rad protein assay reagent (Bio-Rad, USA). Bovine serum albumin (BSA) was used as a standard protein.

Antioxidant activity assays

$ABTS^{\bullet+}$ radical scavenging activity

The $ABTS^{\bullet+}$ radicals were prepared as described by Khammuang and Sarnthima (2008) with a modification, 0.02 U/ml of crude laccase from *Lentinus polychrous* Lév instead of *Trametes versicolor* laccase, was mixed with 10 mM ABTS. The reaction mixture was incubated at 32 °C for 10 min. The laccase in the reaction was deactivated by heating in boiling water for 10 min and then filtered out using a 10 kDa molecular weight cut off membrane. The filtrate containing $ABTS^{\bullet+}$ radicals was used for the antioxidant activity experiment. The $ABTS^{\bullet+}$ radicals solution was diluted with distilled water to give initial optical density at 734 nm (OD_{734nm}) at around 0.7. The samples (various concentrations) 20 µl was added to 980 µl of $ABTS^{\bullet+}$ radical solution. The antioxidant activity was assayed by measuring the decreasing absorbance at 734 nm after incubation in the dark at room temperature for 5 min. The antioxidant activity was reported as trolox equivalent antioxidant capacity (TEAC) which calculated as shown in Eq. 1.

$$\text{TEAC } (\mu\text{mole Trolox}/\mu\text{g protein of sample}) = \left(\frac{A_0 - A_t}{A_0} * 100\right) * \frac{1}{mX} \quad (1)$$

Where,

A_0 is initial absorbance of reaction,
A_t is final absorbance of reaction
m is slope of a plot between %Inhibition and µmole Trolox
X is µg protein of sample

Half maximum inhibitory concentration (IC_{50}) values were calculated from a linear equation (y = mx) of a plot between %Inhibition (y axis) and concentrations of *G. lucidum* protein extracts (x axis) (Thetsrimuang et al. 2011).

$DPPH^{\bullet}$ radical scavenging activity

Similar to $ABTS^{\bullet+}$ radical scavenging assay, $DPPH^{\bullet}$ radical scavenging activity was measured using a modified Yamaguchi et al. (1998) as previous described (Thetsrimuang et al. 2011). The stock, 10 mM of $DPPH^{\bullet}$ was diluted in ethanol until it gave an initial optical absorbance (OD_{515nm}) of about 0.7 before using. The samples in various concentrations of 20 µl were added to 980 µl of $DPPH^{\bullet}$ solution. The antioxidant capacity was calculated relatively to Trolox under the same conditions and the results were reported as µmol Trolox/µg protein of sample and also as the IC_{50} values as mentioned in "$ABTS^{\bullet+}$ radical scavenging activity" section.

Ferric reducing antioxidant power (FRAP)

The FRAP assay was modified method according to Benzie and Strain (1999) as previously described by Thetsrimuang and co-workers (2011). FRAP reagent was prepared by mixing 1 volume of $FeCl_3$•$6H_2O$ solution (20 mM), 1 volume of TPTZ (2,4,6-tripyridyl-s-triazine) solution and 10 volumes of acetate buffer (0.5 M, pH 3.6). The reagent was warmed to 37 °C. The sample of 20 μl was added to 980 μl of FRAP reagent. The ability of reducing power was measured at absorbance 593 nm after initial mixing and after 90 min of reaction. Antioxidant power was expressed as μmole Trolox/μg protein of sample and $FeSO_4$•$7H_2O$ solution was used to prepare a calibration curve. Calculations were done according to Eq. 2.

$$\text{FRAP}(\mu\text{mole Trolox}/\mu\text{g protein of sample}) = \frac{A593}{m_1 m_2 X} \quad (2)$$

Where,

A_{593} is absorbance of reaction at 593 nm,
m_1 is slope of a plot between A_{593} and μM of Fe^{2+}
m_2 is slope of a plot between μM of Fe^{2+} and μmole Trolox
X is μg protein of sample

Proteolytic digestion by Pronase

Crude protein of mycelia extract and fruiting bodies extract were treated with pronase (Roche, USA) in a ratio of 1 μg pronase: 5 μg of protein in 50 mM Tris–HCl buffer (pH 7.5) containing 10 mM $CaCl_2$ (conditions optimized from the company recommendation protocol). The treatment reactions were incubated at 40 °C for 30 min, 1 h, 2 h and then heated in boiling water for 10 min to deactivate the enzyme. After that, the supernatant was obtained by centrifugation (Hettich Mikro 22R, Germany) before using for antioxidant activity assay.

Antibacterial activity and minimum inhibitory concentration (MIC) assay

Overnight cultures of the reference bacterial strains were grown in LB broth. Then, 50 μl of the overnight culture were added to 50 ml of a new LB broth tube and incubated at 37 °C for 3–4 h with shaking at 180 rpm. Appropriated diluted of one milliliter of this culture containing around 1×10^8 colony was added to 5 ml warm melted LB agar. After mixing briefly, the overlay gel was poured on petri dish plate containing underlay LB agar gel. The sterile discs were then placed on plates. The crude extracts and positive control, 500 μg of kanamycin were applied onto each different disc and extraction solution was used as negative control. The plates were incubated for 5–7 h at 37 °C. After incubation, the diameters of the inhibition zone surrounding the disc were measured. The experiments were carried out in a triplicate (Phansri et al. 2011).

The minimum inhibitory concentration (MIC) determination was also performed. The tested bacteria were first grown in LB broth until an OD_{600} nm diluted with 0.85 % (w/v) NaCl compared to McFaland No. 0.5, in which bacteria concentration 1.5×10^8 cfu/ml were achieved. The 0.45 μm filtrated of mycelia protein extract and fruiting bodies protein extract of *G. lucidum* were prepared in a 2-fold serial dilution by double strength LB broth in a 96-well plate. After that, 50 μl of bacterial suspensions were added and mixed with 50 μl crude protein in 96-well a microtiter plate with a final volume of 200 μl and incubated for 18 h at 37 °C. Kanamycin at final concentration of 125 μg/ml (25 μg/200 μl) was used as positive control (Phansri et al. 2011).

Protective effects against hydroxyl radical induced DNA damage

DNA protection assay was performed as described by Siswoyo et al. (2011). The reaction was carried out in a 1.5 ml microcentrifuge tube at the total volume of 20 μl containing 0.5 μg of pGEX DNA, mixing solution (15 mM H_2O_2, 25 μM ascorbic acid and 40 μM $FeCl_3$) and tested samples at various concentrations. The mixture was then incubated for 15 min at 37 °C. The reactions were then analyzed by 1 % agarose gel electrophoresis and nucleic acid was stained with GelStar™ (Lonza, USA). Trolox was used as a positive control of antioxidant compound.

Total phenol content

Total phenol content (TPC) of the crude protein extracts was assayed according to Singleton and Rossi (1965) with some modification as described previously by Thetsrimuang et al., (2011). Briefly, crude protein 500 μl was mixed with 500 μl of Folin-Ciocalteu reagent (diluted 10×) and incubated in the dark for 3 min. After 3 min, the reaction mixture was added 500 μl of 35 % (w/v) Na_2CO_3, and then diluted with 5 ml of distilled water. After incubation in the dark for 90 min, the reaction mixture was measured at 725 nm by spectrophotometer (Thermo spectronic Genesys 20, Model 4001/4 visible range spectrophotometer, USA). The quantification was determined based on a standard curve of gallic acid and the total phenol content was reported as GAE (mg/g protein).

Partial purification of antioxidant protein from G. lucidum

The crude mycelia protein solution was subjected to ammonium sulfate precipitation (85 % saturation). The precipitant

was collected by centrifugation at 10,000 × g for 30 min at 4 °C, and then re-dissolved in a small volume of distilled water, followed by dialysis (MW cut off 2 kDa) overnight at 4 °C. The retentate was applied onto a DEAE-Sepharose column (ϕ 1.6 cm.×10 cm. height) previously equilibrated with the 10 mM Tris–HCl buffer (pH 7.3) at flow rate 2 ml/min. Un-adsorbed proteins were washed out from the column by the same buffer at flow rate 2 ml/min and collected sample fraction at 2 ml/tube. Adsorbed proteins were eluted with a linear gradient of 0–1 M NaCl in the same buffer. The un-adsorbed proteins and adsorbed proteins were measured at 280 nm by a UV–vis spectrophotometer. The obtained protein peaks of fractions were pooled, concentrated and desalted. After that, each of pooled fractions was used to assay for antioxidant activity. The active pooled fraction was then applied to SP-Sepharose XL column (GE Healthcare, UK) equilibrated with 10 mM ammonium acetate buffer (pH 4.6) at flow rate 2 ml/min. The un-adsorbed proteins were washed out with the same buffer and the adsorbed proteins were eluted with linear gradient of 0–1 M NaCl in 10 mM ammonium acetate buffer (pH 4.6). The pooled fractions after concentrated and desalted were assayed for antioxidant activity and analyzed by a SDS-PAGE staining with silver.

Statistical analysis

Antioxidant activity and antibacterial activity experiments were carried out in triplicate. The data were reported as mean ± standard deviation (S.D.) and statistical analysis was performed with the SPSS version 16 software (SPSS Inc., Chicago, USA). *T*-test was applied for the comparisons and a value of $p<0.05$ was considered as statistically significant.

Results and discussion

Antioxidant activities

The scavenging effect including reducing ability of crude protein from *G. lucidum* were evaluated by $ABTS^{\bullet+}$, $DPPH^{\bullet}$ radical scavenging and FRAP assays and expressed as Trolox equivalent antioxidant capacity (TEAC) (Fig. 1). It was found that mycelia protein extract showed better scavenging activities than fruiting bodies protein extract both in $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical scavenging abilities as observed from the higher TEAC values (Fig. 1). However, fruiting bodies protein extract showed a slightly better reducing ability.

It was possible that in the fruiting bodies extract might contain molecules with a better reducing ability but poorer scavenging radical abilities. The crude protein extracts both from mycelia and fruiting bodies after digestion with pronase, it was observed that higher IC_{50} values of both crude protein extracts were obtained (Table 1 and Fig. 2). These results

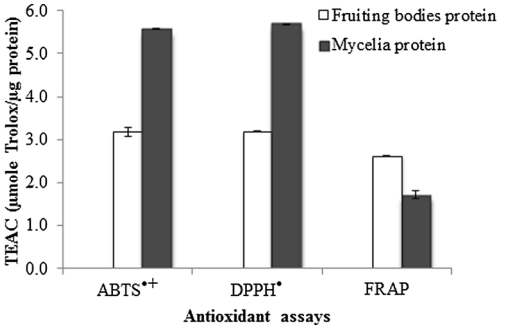

Fig. 1 The antioxidant capacity of crude protein measured as Trolox Equivalents (µmole trolox/µg protein) assay. Each value is expressed as mean ± standard deviation ($n=3$). Mean in each sample is significantly different ($p<0.05$)

suggested that proteins might be key compounds responsible for radicals scavenging activities. Several amino acids compositions in protein have been reported for playing role in those properties. Phe, His and Pro containing peptides have been reported to exhibit antioxidant activity (Girjal et al. 2012).

The very low IC_{50} values of crude protein extract from mycelia and fruiting bodies on $DPPH^{\bullet}$ radicals were obtained in this work, 2.50±0.07 µg protein/ml (or 0.48 mg extract/ml) for mycelia protein extract and 3.42±0.35 µg protein/ml (or 0.60 mg extract/ml) for fruiting bodies extract. Wu and Wang (2009) reported that water soluble glycopeptides isolated from fruiting bodies of *G. lucidum* had the IC_{50} towards $DPPH^{\bullet}$ radicals of about 150 µg/ml. However, it has to keep in mind that such low IC_{50} values from our works are probably due to contributed antioxidant activity of other components apart from proteins presented in the crude protein extracts (i.e. polyphenols etc.). Experimented data from the purified peptide should be obtained. Girjal et al. (2012) reported that purified peptides from *G. lucidum* fruiting bodies had the

Table 1 Half maximal inhibitory concentrations (IC_{50}) for radical-scavenging activity of crude protein undigested and digested from *Ganoderma lucidum*

	IC_{50}^{a}(µg protein/ml)			
	Undigested		Digested	
	Mycelia protein	Fruiting bodies protein	Mycelia protein	Fruiting bodies protein
$ABTS^{+}$	2.47±0.01	2.77±0.11	4.41±0.54	8.49±2.37
DPPH	2.50±0.07	3.42±0.35	10.36±1.54	12.6±0.49

Each value is expressed as mean ± standard deviation ($n=3$). Mean within a column and row for crude protein significantly different ($P<0.05$)

[a] IC_{50} value: the inhibitory concentration at which the antioxidant activity was 50 %

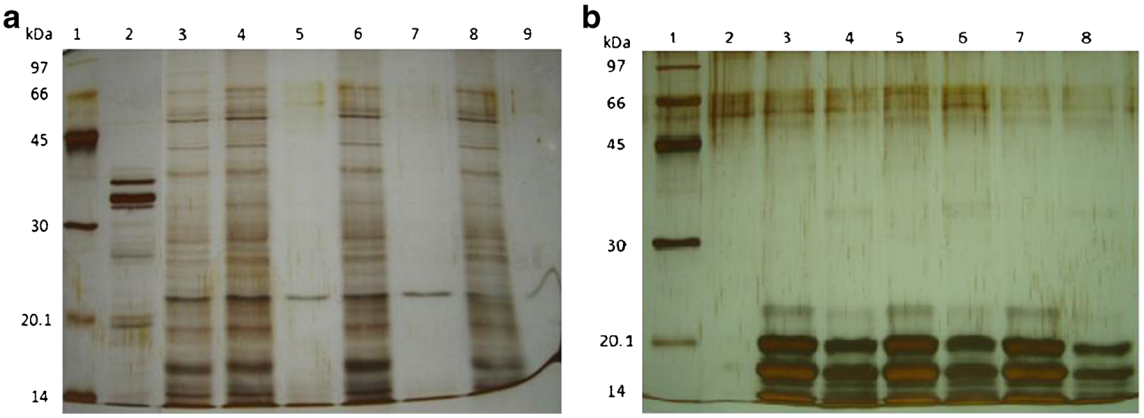

Fig. 2 Twelve percent SDS-PAGE of *Ganoderma lucidum* protein extracts digested with pronase. **a** crude mycelia protein extract, lane 1: molecular weight markers; lane 2: pronase 4 μg; lane 3: crude mycelia protein extract; lane 4; 6; 8: control sample at 30 min, 1 h, 2 h; lane 5; 7; 9: digested sample at 30 min, 1 h and 2 h, respectively, **b** crude fruiting bodies protein extract digested with pronase. Lane 1: molecular weight markers, lane 2: pronase in the reaction, lane 3; 5; 7: control 30 min, 1 h, 2 h, lane 4, 6, 8: treated with pronase at 40 °C for 30 min, 1 and 2 h respectively

ability to inhibit DPPH$^{\bullet}$ radicals 74.21 % at concentration of 40 μg/ml.

For the reducing power of both crude protein extracts as determined by FRAP assay revealed that the fruiting bodies protein extract showed more reducing ability than that of the mycelia protein extract (1.73±0.01 and 2.62±0.01 μmole trolox/μg protein respectively, Fig. 1). This assay is one of the total antioxidant capacity methods which do not detect glutathione and protein thiols. The differences between ferric reducing ability and the anti-radical activities of both crude protein extracts, might probably due to differences in active compositions in the crude extracts. Apart from differences in proteins (as shown in Fig. 2) and phenolic compounds, reducing sugars might be presented differently. Determination of reducing sugar in the crude protein extracts and successfully purified proteins before evaluation its antioxidant activity will be significant evidence. However, this work indicates that crude proteins from *G. lucidum* clearly exhibited antioxidant activity.

Antibacterial activity

Protein extracts of mycelia and fruiting bodies from *G. lucidum* showed interesting antibacterial activities. Since protein concentration from fruiting bodies extract was very low when compared to mycelia protein extract. Thus, protein amounts used for antibacterial tests were different. At the protein amount tested, 25 μg protein of fruiting bodies extract and 115 μg protein of mycelia extract, it was found that the fruiting bodies protein extract could inhibit all tested bacteria except for *S. aureus*. Whilst the mycelia protein extract could inhibit all tested bacteria except for *P. aeruginosa* (Table 2).

The MIC values of crude proteins towards tested bacteria are as shown in Table 2. The ability of fruiting bodies protein extract towards tested bacteria were *S. epidermidis* (20 μg protein/ml) and *B. cereus* (20 μg protein/ml) > *E. coli* (81.5 μg protein/ml). The ability of mycelia protein extract to inhibit tested bacteria were *B. cereus* (81.5 μg protein/ml) and *E. coli* (81.5 μg protein/ml) > *S. epidermidis* (512.5 μg protein/ml).

Table 2 Antibacterial activity of the mycelia protein and fruiting bodies protein extracts of *Ganoderma lucidum*

Protein extract	*S. epidermidis*		*S. aureus*		*B.subtilis*		*B.cereus*		*E. coli*		*P. aeruginosa*	
	Inhibition zone[a]	MIC[b]	Inhibition zone[a]	MIC[b]	Inhibition zone[a]	MIC[b]	Inhibition zone[a]	MIC[b]	Inhibition zone[a]	MIC[b]	Inhibition zone[a]	MIC[b]
Fruiting bodies	+	20.0	−	ND	+	ND	++	20.0	+	81.5	+	ND
Mycelia	+++	512.5	+	ND	+	ND	+++	81.5	++	81.5	−	ND
Kanamycin*	++++++	7.8	+++++	ND	+++++	ND	++++++	15.6	+++++	7.8	++++	ND

Bacterial growth inhibition; − No inhibition, + = inhibition zone ≥ 1 nm <3 mm, ++ = inhibition zone ≥ 3 mm <6, +++ = inhibition zone ≥ 6 mm < 8 mm, ++++ = inhibition zone ≥8 mm < 11 mm, +++++ = inhibition ≥11 mm <14 mm, ++++++ = inhibition zone ≥14 mm <17 mm

[a] inhibition zone (mm); [b] MIC (mg/ml)

*Kanamycin is positive control antibiotics. *ND* not determined

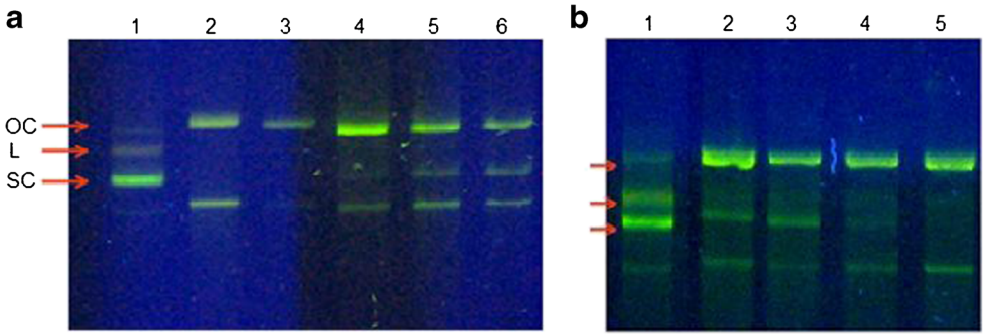

Fig. 3 Inhibitory effect of *Ganoderma lucidum* protein extracts on DNA damage from hydroxyl radicals. **a** mycelia protein extract, lane 1 plasmid DNA (0.05 μg), lane 2: plasmid DNA exposed Fenton reaction, lane 3: plasmid DNA with Fenton reaction plus 200 μM trolox, lane 4; 5; 6: plasmid DNA with Fenton reaction plus mycelia protein extract 25, 100, 200 μg/ml respectively; **b** fruiting bodies protein, lane 1: plasmid DNA (0.05 μg), lane 2: plasmid DNA with Fenton reaction, lane 3; 4; 5: plasmid DNA plus fruiting bodies protein 2.5, 25 and 100 μg/ml respectively. OC: open chain; L: linear; SC: supercoil DNA

Protective effects of DNA damage from Fenton's reaction

Single-strand break in supercoiled plasmid DNA, exposed to hydroxyl radical derived from Fenton's reaction can be occurred, leads to the formation of open circular DNA. The DNA protection assay was used to evaluate protective activity of the crude protein against hydroxyl radical induced DNA damage on pGEX-1 plasmid DNA by an in vitro method. The results revealed that when amounts of the mycelia protein extract were increase, DNA damage by hydroxyl radicals tended to decrease, observed from intensity of supercoiled DNA band in agarose gel seemed to increase in proportional with amount of the mycelia protein extract (Fig. 3a). In contrast, the protective effect of DNA has been observed with the fruiting bodies protein extract better in lower protein concentrations (Fig. 3b). Standard antioxidant, Trolox (Fig. 3a, lane 3), revealed a completely disappeared of supercoiled DNA to be open chain. Even though it exhibited antioxidant activity, this compound has been reported to be a pro-oxidant (Ko et al. 1994). Our results also suggested that it might have certain compounds with pro-oxidant activity presenting in the crude protein extract of *G. lucidum* fruiting bodies. This is probably due to its higher reducing power than the crude protein extract of the fungal mycelia. Predomination of reducing power over scavenging activity resulted in the pro-oxidant effect of aloe-emodin at high concentrations (Tian and Hua 2005).

Table 3 Total phenolic content of crude proteins from *Ganoderma lucidum*

Sample	Total phenolic content (mg GAE/g dw. extract)	Total phenolic content (mg GAE/g of protein)
Fruiting bodies	0.48±0.01[a]	96.0±0.01
Mycelia	0.66±0.01[a]	70.6±0.01

Each value is expressed as mean ± standard deviation (n=3). Mean with the same letters are not significantly different (p>0.05)

The ability of protein extract containing DNA protection property has been reported from Melinjo (*Gnetum gnemon*) seed protein which showed to protect the super coiled DNA from hydroxyl radical induced strand breaks, as reported by Siswoyo et al. (2011). Polysaccharide extracted from *G. lucidum* fruiting bodies has been reported in protecting against hydroxyl radical-induced DNA strand breaks (Kim and Kim 1999). Our investigation found that only protein extract from the fungal mycelia showed such property but not protein extract from its fruiting bodies. The difference of ability in protecting against DNA strand break induced by hydroxyl radicals between previous reported (Kim and Kim 1999) and our investigation may due to differences in other composition of the fruiting bodies protein extract, i.e. polysaccharides, polyphenols and etc..

Total phenolic content

Both types of *G. lucidum* crude protein extracts contained certain amounts of phenol compounds as shown in Table 3. When compared in term of mg GAE/g dry weight extract, it

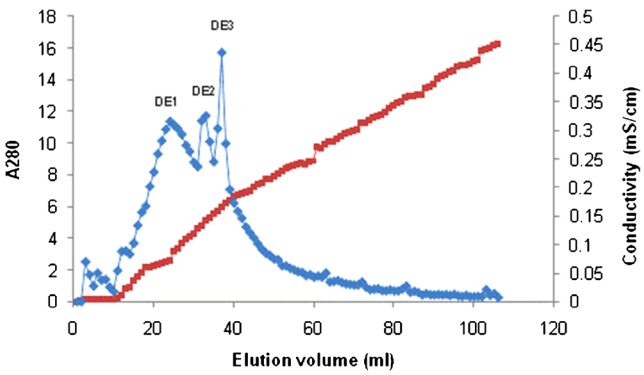

Fig. 4 DEAE-Sepharose profile of the mycelia protein of *Ganoderma lucidum*. The proteins were eluted with a linear gradient of 0–1 M NaCl in 10 mM Tris–HCl buffer (pH 7.3). DE1 showed the highest antioxidant activity

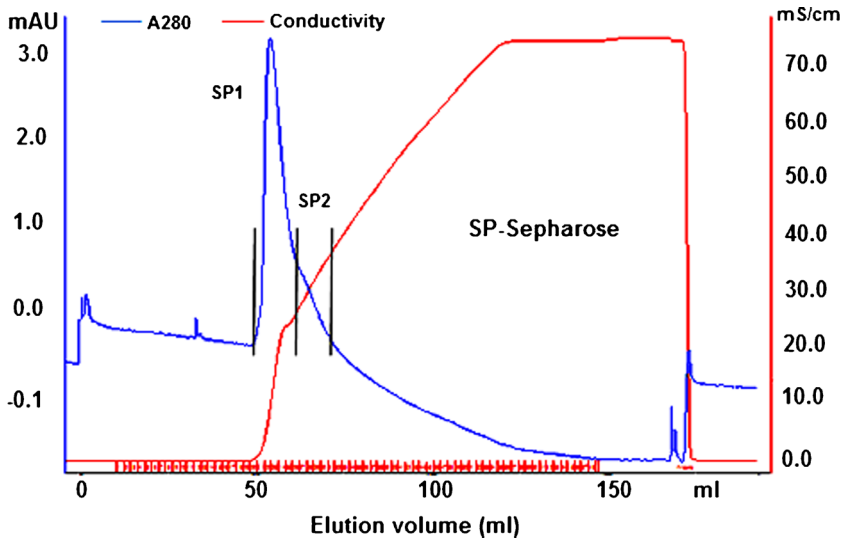

Fig. 5 SP-Sepharose profile of DE1 fraction. SP1 and SP2 were eluted with a linear gradient of 0–1 M NaCl in 10 mM ammonium acetate buffer (pH 4.6). SP2 showed the higher antioxidant activity

was found that TPC in both protein extracts are not significantly different ($p>0.05$). However, the TPC in both extracts were different when compared in term of mg GAE/g of protein due to difference in protein content in each extract. The TPC was found higher in the fruiting bodies extract than that of the mycelia extract. However, the mycelia protein extract exhibited stronger anti-radical abilities (Fig. 1). This supported that anti-radicals of *G. lucidum* crude extracts might be mainly due to proteins presented in the crude extracts. In this work, phenolic compounds presented in the crude extracts might somehow play roles in its antiradical activities, unless the protein with antioxidative property could be successfully purified and characterized. There are reports of *G. lucidum* phenolic extracts contained phenol compounds with antioxidant activity (Mau et al. 2002; Heleno et al. 2012). Some medicinal herbs or plants have been reported potent anti-radical, reducing power and metal chelating activity suggested being due to several polyphenol compounds presented in the extracts (Prathapan et al. 2011a, b).

Partial purification of the antioxidant protein

The mycelia protein of *G. lucidum* was selected for purification due to its easier preparation and bioactivity. It was first precipitated using 85 % saturation ammonium sulfate. Protein pellet was re-suspended in distilled water, dialyzed to remove salt and then applied onto a DEAE-Sepharose column. The elution profile showed three protein peaks according to absorbance at 280 nm (Fig. 4) and pooled fraction DE1 showed the strongest antioxidant activity against $ABTS^{\bullet+}$ radical. This fraction was dialyzed to remove salt and changing buffer and further purified by SP-Sepharose XL column. Adsorbed proteins were assigned as SP1 and SP2 (Fig. 5). Antioxidant activity observed in the adsorbed peak SP1 ($ABTS^{\bullet+}$ radical scavenging at 20.7 %) and SP2 ($ABTS^{\bullet+}$ radical scavenging at 32.2 %). After SDS-PAGE analysis, both pooled fractions showed partially purified with molecular mass about 45 kDa as shown in Fig. 6. Some reports of antioxidant peptides from *G. lucidum* have been reported. Peptide is major component responsible for the antioxidant activity of *G. lucidum* (Sun et al. 2004). Moreover, purified antioxidant peptide from fruiting bodies of *G. lucidum* with molecular mass about 2.8–3.35 kDa had been reported by Girjal et al. (2012).

However, proteins with antioxidant activity have also been reported in plants with higher MW i.e. about 18 kDa purified protein from *Curcuma comosa* Roxb. rhizomes (Boonmee

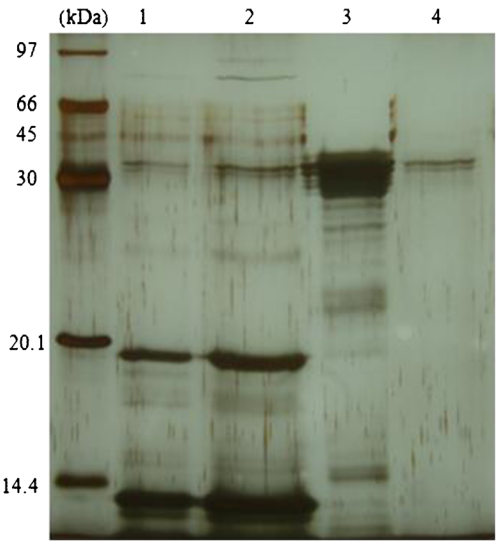

Fig. 6 Fifteen percent SDS-PAGE of the purified *Ganoderma lucidum* mycelia protein after SP-Sepharose XL column. Lane M: molecular weight markers; lane 1: crude mycelia protein; lane 2: un-adsorbed protein; lane 3: SP1; lane 4: SP2. Partial purified protein (SP1 and SP2) had major protein with molecular mass about 45 kDa

et al. 2011) and about 28 kDa purified protein from *Solanum torvum* seeds (Sivapriya and Leela 2007).

Conclusion

The mycelia protein extract from *G. lucidum* G2 had higher potential antioxidant and DNA protection than the fruiting bodies protein extract. However, the fruiting bodies protein extract had stronger reducing power and antibacterial activity. Both crude protein extracts had differences in protein content and pattern. Its protein hydrolysates prepared by pronase digestion, showed a decrease in anti-radicals activity, suggesting that proteins are responsible for anti-radical activity. Successful purification of antioxidant protein from *G. lucidum* is still needed to understand its molecular structure and other biological functions. To obtain fungal mycelia is easier and shorter than to obtain its fruiting bodies. Fermentation technology can be applied in the mass production for application as food supplement.

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