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Antioxidant properties and cytotoxicity of crude polysaccharides from *Lentinus polychrous* Lév.

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ABSTRACT

Crude polysaccharides of mature fresh and dried fruit bodies and dried mycelia of *Lentinus polychrous* Lév. were evaluated for their antioxidant properties and cancer cell line cytotoxicity. The crude polysaccharides were in yields of 39.0–97.5 mg/g dry weight of sample. Trolox equivalent values in scavenging abilities of those crude polysaccharides against both 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid ($ABTS^{\cdot+}$) radical and 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) radicals were in the range of 53.4–131 and 6.4–38.8 μmol trolox/g dwt of extract, respectively, whereas values of reducing power were in the range 27.6–54.9 μmol trolox/g dwt of extract. Scavenging ability and reducing power of these crude polysaccharide extracts were in the descending order of mycelia > dried fruit bodies > fresh fruit bodies. *In vitro* cytotoxicity, determined with the crude polysaccharides of fresh and dried fruit bodies of the fungus at a concentration of 1 mg/ml, by the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) method, showed cytotoxic effects of about 38% and 45%, respectively, against the human breast adenocarcinoma cell line (MCF-7). This mushroom might be a good source of bioactive compounds for cancer prevention.

1. Introduction

Free radicals are highly reactive molecules, having one or more unpaired electrons (Wu & Hansen, 2008). They are released during oxidative stress (Xu et al., 2009). Free radicals are able to damage numerous biological substances, including DNA, protein, and lipid membranes (Tsai, Song, Shih, & Yen, 2007), resulting in various diseases and disorders, such as cancer, cardiovascular diseases, impaired immunofunction, atherosclerosis, and aging (Wu & Hansen, 2008; Xu et al., 2009). Antioxidants can scavenge free radicals and provide protection against diseases. Synthetic antioxidants have been used in preservation of foods, for example butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylated hydroxyquinone (TBHQ), but several studies suggest that they could promote tumour formation (Cheung, Cheung, & Ooi, 2003). Several components of fruits and vegetables have been shown to have antioxidant activity, e.g. ascorbic acid, tocopherol, β-carotene, flavonoids, and phenol compounds (Wu & Hansen, 2008; Xu et al., 2009).

Polysaccharides are widely found in animals, plants, and microorganisms, to exhibit varied bio-activities, e.g. antitumour, anticancer, antiviral, anticoagulant, and immunological activities (Xu et al., 2009). Recently, polysaccharides from mushroom extracts have been reported to have antioxidant activity, e.g. a polysaccharide from the fruit bodies of *Ganoderma atrum* (Chen, Xie, Nie, Li, & Wang, 2008), a crude polysaccharide from *Lentinus edodes* (Wu & Hansen, 2008) and polysaccharides produced from submerged culture of the edible Basidiomycete *Grifola frondosa* (Lee et al., 2003).

The mushroom, *Leninus polychrous* Lév., is edible and commonly consumed in northeastern and northern regions of Thailand. There is research on laccase enzyme production from this mushroom mycelia for a decolorization study of some synthetic dyes (Khammuang & Sarnthima, 2007). In the present study, the crude polysaccharides from hot water extraction of *L. polychrous* Lév. were used to investigate antioxidant activity and several properties, including total phenol content, amount of protein, total carbohydrate, reducing sugar, as well as cancer cell line cytotoxicity.

2. Materials and methods

2.1. Chemicals and apparatus

Absolute ethanol, ferrous sulphate heptahydrate, ferric chlorine hexahydrate, and sodium chloride were purchased from Merck

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(Germany). Methanol, sulphuric acid, and sodium dodecyl sulphate (SDS) were from BDH (England). Sodium carbonate (anhydrous), Folin–Ciocalteu's reagent, bromophenol blue, and phenol crystal were obtained from Carlo Erba (France). 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$) and 2,2-diphenyl-1-picryhydrazyl ($DPPH^{\cdot}$) were products of Sigma (Germany). Gallic acid, 3,5-dinitrosalicylic acid (DNS), and 2,4,6-tripyridyl-*s*-triazine, Coomassie Brilliant Blue R250, *N*,*N*′-methylenebisacrylamide and *N*,*N*,*N*′,*N*′-tetramethylenediamine were purchased from Fluka (Germany). Protein assay dye reagents were a product of Bio-Rad (USA). Potato dextrose agar, potato dextrose broth, malt extract powder, yeast extract, and tryptone Type-I were purchased from Himedia (India). Ammonium persulphate and acrylamide were obtained from Sigma (USA). Cell culture media and antibiotics were purchased from Gibco (Grand Island, NY, USA). Fetal bovine serum (FBS) was purchased from Hyclone (UT, USA). [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was obtained from Sigma (USA). All other chemicals were of analytical grade.

2.2. Fungal cultivation

Fruit bodies of *L. polychrous* Lév. were obtained from Rujira Mushroom Farm in Ka La Sin province (northeast Thailand), and were maintained at 4 °C on potato dextrose agar (PDA) plates with periodic subculture. The fungus was cultured on a PDA agar plate for 5–7 days at ambient temperature. Then, plugs of active growing mycelium (diameter 0.4 cm) were inoculated into a 250 ml flask containing slightly modify submerged medium of Hwang, Kim, Choi, and Yun (2003) including 2.4% potato dextrose broth, 1% malt extract, and 0.1% tryptone, and cultured for 14 days at ambient temperature under shaking conditions at 125 revolution per minute (rpm).

2.3. Crude polysaccharides extraction

Mycelia of the cultured fungus were washed three times with distilled water and filtered through a filter paper (Whatman No. 4). Then, the mycelia were oven-dried at 60 °C. The dried fungal biomass was boiled for 4 h. After centrifugation at 6000 rpm for 30 min, the supernatant was extracted for crude polysaccharide with four volumes of absolute ethanol (4:1 v/v) at 4 °C overnight, as described by Lee et al. (2003). The supernatant was discarded after centrifugation at 6000 rpm for 20 min. Pellet was washed with absolute ethanol, then centrifuged, and the ethanol washing step was repeated twice. The crude polysaccharide was re-dissolved in distilled water for determination of total carbohydrate content, reducing power, total phenol content, protein content, and antioxidant activity.

Fresh fruit bodies and air-dried fruit bodies of *L. polychrous* Lév. were extracted for crude polysaccharides by the same method as for mycelia extraction.

2.4. Properties of crude polysaccharides

2.4.1. Determination of total carbohydrate content

The carbohydrate contents were determined with a slightly modified phenol–sulphuric acid method according to Masuko and co-workers (2005). The colour reaction was initiated by mixing 50 μl of crude polysaccharide solution with 150 μl of concentrated sulphuric acid, followed immediately with 30 μl of 5% phenol, and the reaction mixture was kept at 90 °C for 5 min. After cooling to room temperature, the absorbance of the mixture was measured at 490 nm. The total carbohydrate content was calculated with D-glucose as standard.

2.4.2. Determination of reducing sugar

The reducing sugar was determined by the modified method of Miller (1959). Briefly, 0.5 ml of 1% 3,5-dinitrosalicylic acid (DNS) was added to an aliquot of sample (20–500 μl), and the volume adjusted to 5 ml with distilled water. After shaking, the mixture was heated in boiling water for 5 min and cooled to room temperature; 2.5 ml of distilled water were added to the mixture. The absorbance was measured at 540 nm, and the total reducing sugar was calculated with D-glucose as a standard reducing sugar. Total polysaccharide was the subtraction of reducing sugar from the total carbohydrate.

2.4.3. Determination of total phenol content

The total phenol content of the crude polysaccharide was estimated by the Folin–Ciocalteu colorimetric method, based on the procedure described by Singleton and Rossi (1965) and Oliveira et al. (2008) with some modifications. Briefly, a sample (0.5 ml) was mixed with 0.5 ml of Folin–Ciocalteu reagent (10× dilution). Three minutes later, 0.5 ml of 35% (w/v) Na_2CO_3 was added, and the mixture was brought up to 5 ml with distilled water. After being kept in the dark for 90 min, the absorbance of the mixture was read at 725 nm. The quantification was based on a standard curve of gallic acid. The total amount of phenol contents was expressed as GAE (mg/g sample).

2.4.4. Determination of protein content and protein pattern analysis

The procedure used in determining the total protein content was adapted from the Bradford (1976) method. An aliquot of sample (10–50 μl) was mixed with 200 μl of Bio-Rad Protein Assay kit solution, and brought up to 1 ml with distilled water. After shaking and incubating in room temperature, the absorbance was measured at 595 nm, and the protein content was calculated with bovine serum albumin (BSA) as standard protein.

Protein profile analyses of each polysaccharide fractions were performed by Tris–tricine SDS–PAGE, with a slightly modified method of Schägger and von Jagow (1987), using 15% separating gel and 5% stacking gel. The gel was applied at an initial voltage of 30 V and then a constant voltage of 200 V. The gel was visualised by silver staining.

2.5. Antioxidant activity assay

2.5.1. $ABTS^{\cdot+}$ radical-scavenging activity

$ABTS^{\cdot+}$ was prepared by the laccase reaction, as described previously (Khammuang & Sarnthima, 2008). Laccase was removed from the $ABTS^{\cdot+}$ radicals by centrifugation with a selection membrane molecular weight cut-off of 10 kDa before using the radicals for the antioxidant activity experiment. $ABTS^{\cdot+}$ scavenging activity was modified from Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, and Byrne (2006). The stock $ABTS^{\cdot+}$ radical solution was diluted in distilled water to give an initial absorbance (A_{734}) of 0.7 before use. An aliquot of samples (5–25 μl) was added to 975–995 μl of $ABTS^{\cdot+}$ radical solution. The absorbance was measured at 734 nm by a spectrophotometer after 30 min of incubation. Total antioxidant capacity was calculated relative to the reactivity of trolox in a parallel experiment and the result was reported as μmol trolox/g dwt of polysaccharide sample.

2.5.2. $DPPH^{\cdot}$ radical-scavenging

$DPPH^{\cdot}$-scavenging activity was determined by the modified method of Thaipong et al. (2006). $DPPH^{\cdot}$ radical was prepared by dissolving in methanol (2.4 mg/ml). Before use, the stock of $DPPH^{\cdot}$ radical solution was diluted in methanol to give the initial A_{515} reading at 0.7. An aliquot of samples (5–25 μl) was added to 975–995 μl of $DPPH^{\cdot}$ solution. The absorbance was measured at its maximum (λ_{max} 515 nm) after 30 min of incubation at room

temperature. Total antioxidant capacity was calculated relative to the reactivity of trolox in a parallel experiment and the results were reported as μmol trolox/g dwt of polysaccharide sample.

2.5.3. Ferric reducing antioxidant power (FRAP)

The FRAP assay was previously described by Vasco, Ruales, and Kamal-Eldin (2008) and Soong and Barlow (2004). Fresh FRAP reagent was prepared daily, by mixing 10 volumes of acetate buffer (300 mM, pH 3.6), one volume of TPTZ (2,4,6-tripyridyl-*s*-triazine) solution (10 mM TPTZ in 40 mM HCl), and one volume of $FeCl_3 \cdot 6H_2O$ solution (20 mM). The reagent was warmed to 37 °C. An aliquot of samples (10–50 μl) was mixed with 950–950 μl of FRAP reagent. The change in absorbance was measured at 593 nm after initial mixing and up to 90 min. Aqueous solutions of $FeSO_4 \cdot 7H_2O$ were used for calibration of the FRAP assay and antioxidant power was reported as μmol trolox/g dwt of polysaccharide sample.

2.6. In vitro cancer cell lines cytotoxicity assay

The A549 non-small cell lung adenocarcinoma cell line, SK-Hep-1 hepatocellular carcinoma cell line and MCF-7 human breast adenocarcinoma cell line from ATCC were used for the cytotoxicity test. The cells were grown in RPMI 1640 (A549 and SK-Hep-1), or DMEM (MCF-7) supplemented with 10% fetal bovine serum (FBS), 100 U/ml of penicillin, 100 μg/ml of streptomycin and 125 ng/ml of amphotericin B. All cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2.

Cytotoxicity assays of crude polysaccharides from fresh fruit bodies and dried fruit bodies, on SK-Hep1 and MCF-7, were evaluated *in vitro* using the MTT assay. Briefly, cell suspensions in culture medium were seeded in 96-well plates (1×10^4 cells/well), and incubated at 37 °C in a humidified atmosphere of 5% CO_2. After 24 h, additional medium (100 μl) containing test sample was added to each well, followed by further incubation for 48 h. Then, the wells were replaced and incubated with fresh culture media containing MTT (0.5 mg/ml) for 2 h at 37 °C. Finally, the media were removed and DMSO was added to the wells (100 μl/well), and absorbance was measured at 550 nm in a microtitre plate reader and subtracted from the absorbance at 650 nm. The number of viable cells was determined from the absorbance. Assays were performed in quadruplet wells. Data were expressed as percent viability compared with control (mean ± SD).

3. Results and discussion

3.1. Crude polysaccharide yields

Extraction of crude polysaccharides from several forms of *L. polychrous* gave the yields in descending order of: mycelia (5.9%) > dried fruit bodies (5.7%) > fresh fruit bodies (5.2%), as shown in Table 1. The hot water extraction yield of polysaccharides from *Ganoderma tsugae* showed a higher yield from mycelia than did fruit bodies, as in our result (Tseng, Yang, & Mau, 2008). However, yields of hot water-extracted crude polysaccharides from mycelia in the present work were a bit less than those from *G. tsugae* reported by Tseng et al. (2008), which were 8.3%, whereas the yields of polysaccharide from fruit bodies in our work were clearly higher than those from fruit bodies of other species, such as *Pleurotus ostreatus*, 3.32% (Tong et al., 2009), *Russula virescens*, 1.94% (Sun, Zhang, Zhang, & Niu, 2010) and *G. tsugae*, 1.5–1.7% (Tseng et al., 2008). The crude polysaccharide yields from mycelia of *L. polychrous* Lév. were far less than the yield reported by Mau, Tsai, Tseng, and Huang (2005) which was 32%, whereas they were in the same range as those from fruit bodies, 6.2–9.9%. High yields of polysaccharides were extracted from both the natural and cultured mycelia of *Cordyceps sinensis*, (30.46% and 39.11%, respectively) (Dong & Yao, 2008). These differences might depend on the species and extraction methods used.

3.2. Properties of crude polysaccharides

3.2.1. Total carbohydrate content and total polysaccharide

The total carbohydrate content varied from 228 to 523 mg/g dwt of extract (22.8–52.3%), as shown in Table 1. The crude polysaccharide of mycelia had the lowest total carbohydrate, whereas the crude polysaccharide of dried fruit body had the highest value (52.3%). Mycelium was found to give the highest yield of crude polysaccharide, but with the lowest content of total carbohydrate. This might be because other compounds, apart from polysaccharide, reducing sugar and protein, are easier to solubilise from the fungal mycelium by hot water extraction.

The β-glucan fraction from the mushroom fruit bodies of the same genus, *L. edodes* yielded different amounts of β-glucan that varied from strain to strain, ranging from 3.5% to 10.0% (Surenjav, Zhang, Xua, Zhang, & Zeng, 2006). Total polysaccharides of *L. polychrous* from the present work are far higher than those reported in *L. edodes* (Table 1, 18.4–45.5%). The resulting values are in the same range as polysaccharide from the fruit bodies of *Ganoderma lucidum*, which was approximately 57.9% (YouGuo, Zongji, & Xiaoping, 2009). However, the total polysaccharide content of fruit bodies of cultured *Cordyceps militaris* is above 95% (Yu et al., 2007).

3.2.2. Reducing sugar and total phenol content

Crude polysaccharides of dried and fresh fruit bodies had the highest and the lowest amounts of reducing sugar, respectively (Table 1). The total phenol contents in the crude polysaccharides from the fungal mycelia and fresh fruit bodies were similar (58.4 and 58.0 mgGAE/g of polysaccharides, respectively) and almost two times higher than those in the dried fruit bodies' fraction (33.3 mgGAE/g of polysaccharides) as shown in Table 1. Dried fruit bodies yielded a lower total phenol content than did fresh fruit bodies. This might be due to the greater difficulty of hot water solubilisation of phenol compounds from the dead, dried sample or there might be some structural or chemical change during the drying process. Total phenol contents of crude polysaccharides in this present work are far higher than the values for polysaccharide isolated from the fruit bodies of *G. lucidum* (YouGuo et al., 2009), which was only 153 mgGAE/100 g of polysaccharides.

3.2.3. Protein content and protein pattern analysis

Among three tested samples, the highest amount of proteins, as judged by the Bradford method, was found in dried fruit bodies, whereas the lowest was in fresh fruit bodies (Table 1). The different protein patterns of each fraction were shown in Fig. 1. It can be seen that dried fruit bodies having high molecular weight proteins showed a high amount of proteins at the top of the gels. Fresh fruit bodies showed a clear protein band at a molecular weight lower than 14.4 kDa and two bands around 14.4 kDa. The polysaccharide fraction of the fungal mycelia also showed major proteins with molecular weights below 14.4 kDa. Smear band patterns might be due to the carbohydrate portion included in the crude polysaccharide fractions making its natural viscosity similar to the crude polysaccharide fraction of *Lentinus* sp. RJ-2 (Thetsrimuang, Khammuang, & Sarnthima, 2011).

The polysaccharide fractions of the same genus, *L. edodes*, also contained protein (ranging from 4.6% to15.2%) depending on the strain (Surenjav et al., 2006). The *G. lucidum* polysaccharide (GLP) was proven to be a glycopeptide with the ratio of polysaccharides to peptides of about 90.2%:6.71%, as reported by Jia et al. (2009). Dong and Yao (2008) also reported that the hot water extracts of

Table 1
The yield of crude polysaccharides, amount of proteins, total polysaccharides, reducing sugar, and total phenol content of crude polysaccharides of *L. polychrous* Lév. ($n = 3$).

Crude polysaccharide	Yields[a] (%)	Protein contents[b] (mg/g)	Total carbohydrate[b] (mg/g)	Total polysaccharide[b] (mg/g)	Reducing sugar[b] (mg/g)	Total phenol contents[b] (mgGAE/g)
Mycelia	5.89	50.8 ± 3.2a[c]	228 ± 0.4a	184 ± 1.78a	44.2 ± 1.4a	58.4 ± 0.5a
Fresh fruit bodies	5.17	39.0 ± 4.7b	459 ± 3.8b	420 ± 2.91b	39.0 ± 0.9b	58.0 ± 0.8a
Dry fruit bodies	5.74	97.5 ± 11.2c	523 ± 12.3c	456 ± 12.96c	67.7 ± 0.7c	33.3 ± 0.4b

[a] Yield per a hundred gramme dry weight of sample.
[b] g dwt of crude polysaccharide.
[c] Means with different letters within a column are significantly different ($P < 0.05$).

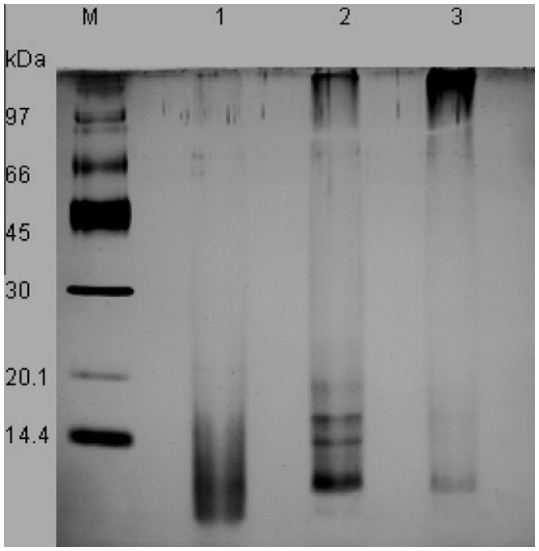

Fig. 1. Tris–tricine SDS–PAGE (15%) of crude polysaccharides from *L. polychrous* Lév. M, molecular weight markers; 1, mycelia crude PS; 2, fresh fruit bodies PS; 3, dried fruit bodies PS.

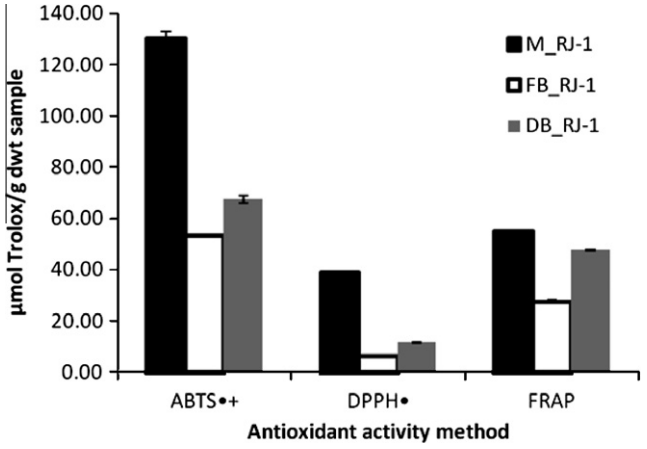

Fig. 2. The antioxidant capacity of crude polysaccharides measured as trolox equivalents (µmol trolox/g dry weight of extract) assay. Error bars indicate mean ± standard deviation ($n = 3$). TEAC value of BHA and vitamin C by $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radical-scavenging equals to 2.43, 1.53 and 0.47, 0.54 µmol/µg, respectively. Each column in each group has p-value <0.05.

both natural and cultured mycelia of *C. sinensis* contained proteins as well as polysaccharides. Protein contents in both polysaccharide extracts were high with the ratios of polysaccharides to proteins of 18.4%:18.4% and 28.4%:15.1% from natural and cultured mycelia, respectively (Dong & Yao, 2008). The protein contents in our three crude polysaccharide extracts, considering the ratios of polysaccharides to proteins, were relatively low (<20%). The exact protein content should be further investigated in the purified fractions. Therefore, purification and structural elucidation of polysaccharides from *L. polychrous* Lév. are still needed in order to understand more about properties and potential uses. Other bioactive polysaccharides have been reported as polysaccharide–peptide complexes (Chan & Yeung, 2006; Eo, Kim, Lee, & Han, 1999).

3.3. Antioxidant activities

The antioxidant activities of the crude polysaccharides from *L. polychrous* Lév. were evaluated as trolox equivalent antioxidant capacity (TEAC) calculated from $DPPH^{\cdot}$-scavenging, $ABTS^{\cdot+}$ scavenging, and FRAP assays (Fig. 2). The analysed crude polysaccharides showed a wide range of antioxidant capacities, 6.4–131 µmol trolox/g dwt of extract. Comparison of response to the antioxidant capacity value among three assays revealed that $ABTS^{\cdot+}$ assay exhibited the highest value (compared to $DPPH^{\cdot}$ and FRAP assays). In addition, the crude polysaccharides of the fungal mycelia have stronger antioxidative activity than have those from fruit bodies. Scavenging ability and reducing power of these polysaccharides were in the descending order: mycelia > dried fruit bodies > fresh fruit bodies. These results were supported by the IC_{50} values of each polysaccharide fraction (Table 2). With regard to scavenging ability on DPPH radicals, the IC_{50} value of polysaccharide from fungal mycelia was less than 0.5 mg/ml whereas those from mature fruit bodies were 1.66 and 3.08 mg/ml for dried and fresh fruit bodies, respectively. The IC_{50} value of mycelia extract from *L. polychrous* Lév. in our work was lower than those of both cultural and natural mycelia extracts from *C. sinensis* (0.93 and 1.23 mg/ml, respectively), as reported by Dong and Yao (2008). Our results are not similar to the results of *G. tsugae* polysaccharides reported by Mau et al. (2005) or those of Tseng et al. (2008) where polysaccharides from fruit bodies had higher antioxidant activity, as well as reducing power, as that from mycelia. Together with the results from Table 1, this suggested that the antioxidant activities might be due to both reducing sugars and phenol compounds. The results from Table 2 showed that antioxidant activities of crude polysaccharide extracts were far smaller than those of the reference phenol compounds (BHA, vitamin C and trolox), which indicates that there might be radical-scavenging by different mechanisms or compounds. Crude polysaccharide fractionation and deproteinization might be explored in the future to elucidate the key bioactive material(s).

3.4. In vitro cancer cell line cytotoxicity assays

The crude polysaccharides of the *L. polychrous* Lév. strain of both fresh fruit body and dried fruit body were subjected to *in vitro* cytotoxicity assay in certain cancer cell lines, including non-small cell lung adenocarcinoma (A549), hepatocarcinoma (SK-Hep1), and human breast adenocarcinoma cell line (MCF-7). The results showed that both crude polysaccharides had slight toxicity effects

Table 2
Half maximum inhibition concentrations (IC_{50}) for radical-scavenging activity of polysaccharides from *Lentinus polychrous* Lév. and comparison with the reference antioxidant compounds.

	IC_{50}[a] (μg extract/ml)					
	Mycelia	Fresh fruit bodies	Dried fruit bodies	BHA	Vitamin C	Trolox
$ABTS^{\cdot+}$ scavenging	123 ± 2.0	432 ± 15	237 ± 5.0	1.65 ± 0.01	8.51 ± 0.05	4.00 ± 0.03
$DPPH^{\cdot}$ scavenging	497 ± 6.0	3078 ± 234	1660 ± 41	3.14 ± 0.02	8.84 ± 0.00	4.82 ± 0.06

IC_{50} value was obtained by interpolation from linear regression analysis.
Each value is expressed as mean ± standard deviation ($n = 3$). Means within a column and a row for crude polysaccharides are significantly different ($P < 0.05$).
[a] IC_{50} value: the inhibition concentration at which the antioxidant activity was 50%.

on the cancer cell line, as shown in Fig. 3. It was found that the higher the crude polysaccharide concentrations, the lower were the cell viability percentages. However, at the highest concentration tested (1 mg/ml), the half inhibition concentration (IC_{50}) could not be determined which means that these crude polysaccharides show a low cytotoxicity toward those tested cancer cell lines. The summary of the lowest viability percentages of cancer cell lines, for the crude polysaccharide concentration of 1 mg/ml, is shown in Table 3.

Most cancer cells, including human endometrial epithelial cells (HEC-1B), murine melanoma (B16F10), A549, human stomach carcinoma (KATO-III), kidney adenocarcinoma (SW156), and human ovarian adenocarcinoma (SK-OV3), are not directly affected by the endo-polysaccharide from cultivated mycelia of *Inonotus obliquus* treatment, even at a high concentration (200 μg/ml), with the exception of human hepatoma (Hur7) and MCF-7 (Kim et al., 2006). These results are not similar to the results obtained in our work, as a high concentration of *L. polychrous* Lév. polysaccharide (250 μg/ml) showed approximately 43.9% and 22.6% inhibition

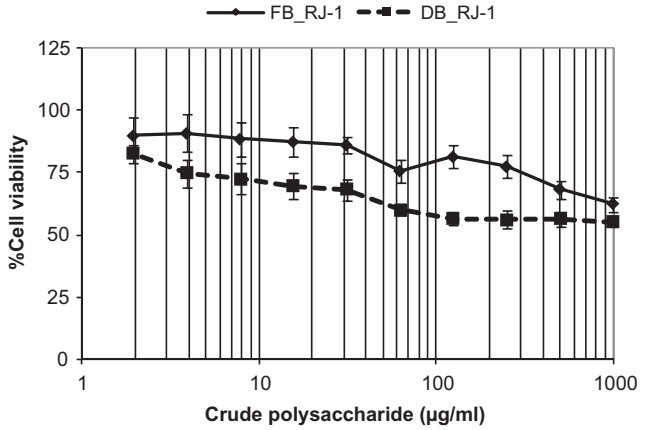

Fig. 3. Cell viability (%) of human breast adenocarcinoma cell line (MCF-7) after treatment with crude polysaccharide of *L. polychrous* Lév. Dried fruit bodies (a) and fresh fruit bodies (b). Error bars indicate means ± standard deviation ($n = 3$).

Table 3
Viability percentages of cancer cell lines[a] treated with crude polysaccharides from *L. polychrous* Lév.

Crude polysaccharide[b]	% Cell viability[c]		
	A549	SK-Hep 1	MCF-7
Fresh fruit bodies	76	65	62
Dried fruit bodies	85	60	55

[a] Cancer cell lines: A549, non-small cell lung adenocarcinoma; MCF-7, human breast adenocarcinoma cell line; SK-Hep1, hepatocarcinoma (liver cancer).
[b] Crude polysaccharide samples: fresh fruit bodies and dried fruit bodies of *L. polychrous* Lév. at a concentration of 1 mg/ml ($n = 3$).
[c] Cell viability is reported as percentage compared with the control group without the crude polysaccharide fraction.

on MCF-7 for dried fruit bodies and fresh fruit bodies polysaccharide extracts, respectively (Fig. 3). The crude polysaccharides from fruit bodies of the fungus tended to exhibit anti-proliferative activity in a dose-dependent manner.

β-Glucan from the same genus, *L. edodes*, named *Lentinan*, has been reported as an antitumour polysaccharide by Maeda and Chihara (1973) as well as having other biological activities, such as antioxidative stress and immunity activity (Yu, Lihua, Qian, & Yan, 2009), tumour-inhibitory effects (Shen et al., 2009), and anti-fatigue effect (Li, Zhang, & Xua, 2009). Fourier transform infrared (FTIR) spectroscopy and thin-layer chromatography (TLC) results of the crude polysaccharides of *Lentinus* sp. RJ-2 suggested that it contained a monosaccharide of six carbon atoms in a pyranose ring and mannose was the major monosaccharide unit (Thetsrimuang et al., 2011). Therefore the crude polysaccharide from the *L. polychrous* in this study might have the β-glycan of a different type (or amount), which requires more investigation.

A water-soluble carboxymethylated β-glucan (CMPTR), partially synthesised from an insoluble native glucan isolated from the sclerotia of *Pleurotus tuber-regium*, induced anti-proliferative activity dose-dependently, with an IC_{50} of 204 μg/ml (Zhang, Cheung, Chiu, Wong, & Ooi, 2006). Hot water extracts from both mycelia and sclerotia (coded as Mh and Sh) of *P. tuber-regium*, extracted by hot water, exhibited significant anti-tumour activity toward solid tumour sarcoma 180 cells *in vivo* and direct cytotoxity toward HL-60 tumour cell lines *in vitro* (Zhang, Zhang, Cheung, & Ooi, 2004). These results also showed a dose–response relationship in inhibiting the HL-60 cells proliferation. The Mh exhibited the highest inhibition ratios of 83%, 69.9% and 56% at concentrations of 200, 100 and 50 μg/ml, respectively.

The growth of Hela cells could be inhibited by crude polysaccharide from *P. ostreatus* (POPS) and a purified fraction (POPS-1) at a concentration as low as 50 μg/ml (Tong et al., 2009). The inhibition ratios of POPS and POPS-1 are 23.1% and 33.7%, respectively. In their report, a dose-dependent manner of the cancer cell line inhibition was observed up to 200 μg/ml with the strongest inhibition ratio against Hela cell proliferation, reaching 45.6% and 63.3% for POPS and POPS-1, respectively (Tong et al., 2009). There are some reports of bioactive polysaccharides consisting of 1 → 3-β-D-glucan as a backbone chain and protein bound polysaccharide complexes (Chan and Yeung, 2006; Eo et al., 1999; Surenjav et al., 2006).

4. Conclusions

Hot water extraction of fruit bodies and mycelia of *L. polychrous* Lév. yielded varying amounts of crude polysaccharides from strain to strain. All crude polysaccharides of *L. polychrous* Lév. revealed different antioxidant activities. The three different antioxidant activity assays, including radical-scavenging activity ($ABTS^{\cdot+}$ and $DPPH^{\cdot}$) and FRAP assay, revealed strong positive correlations. These crude polysaccharide fractions also consisted of proteins and phenol compounds. Even though their antioxidant activities are weak and non-comparable to the synthetic antioxidant (BHA), or known

compounds, such as vitamin C and trolox, the crude polysaccharides of fruit bodies of *L. polychrous* Lév. had certain cytotoxic effects on cancer cell lines MCF-7, SK-Hep1, and A549. Therefore, supplementation of *L. polychrous* Lév. polysaccharide might serve as possible antioxidant or immunity agent to aid cancer prevention. Further investigation, with details about glycan structures and other biological activities, such as immune modulation and anti-inflammatory activities, are of interest.

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