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Evaluation of the cytotoxic effect of crude aqueous and ethanolic extracts isolated from *Lentinus* sp. on human cancer cell lines

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Aims: The aim of this study was to evaluate some chemical properties and the cytotoxic effect of aqueous and ethanolic crude polysaccharides extracted from five *Lentinus* sp. on human cancer cell lines. It was hypothesized that other species of the genus *Lentinus* could show the pharmacological actions as presence in *Lentinus edodes*, especially anticancer properties.
Methodology and results: Crude extracts of dried fruit bodies and mycelia from *L. edodes*, *Lentinus sajor-caju*, *Lentinus swartzii*, *Lentinus squarrosulus* and *Lentinus velutinus* were extracted using two solvents, hot water and 95% ethanol, and evaluated for their total carbohydrates, proteins, reducing sugar, phenol contents, and cytotoxicity. The yield of crude extracts was 33.6-205.3 mg/g dry weight of a sample. Cytotoxicity was determined with 10 mg/mL of crude aqueous and 1 mg/mL of crude ethanolic extracts by using the [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (MTT) method. All extracts showed non-cytotoxicity against the normal cell lines, LLC-MK2 and L929 cells. While, the extracts of *L. edodes*, *L. sajor-caju*, *L. squarrosulus* and *L. velutinus* displayed the cytotoxicity against the human cancer cell lines.
Conclusion, significance and impact study: The crude aqueous and ethanolic extract from fruit bodies of *L. velutinus* and the ethanolic extract from fruit bodies of *L. sajor-caju* and *L. squarrosulus* displayed the adverse effect against the human cancer cell lines. Hence, these extracts are a potential source of bioactive compounds for cancer treatment.

Keywords: Lentinus spp., cytotoxicity, aqueous extract, ethanolic extract, human cancer cell lines

INTRODUCTION

Cancer is a major cause of death in people of various ages and racial background. There are many research efforts and clinical studies in the fight against the disease (Bhanot *et al.*, 2011; Daba and Ezeronye, 2003; Patel and Goyal, 2012; Zong *et al.*, 2012). Nowadays, several options are used for cancer treatments such as surgery, radiotherapy, chemotherapy and hormonal therapy. These methods of cancer treatment and the current anticancer drugs available on the market could pose several side effects for patients (Bhanot *et al.*, 2011; Patel and Goyal, 2012; Zong *et al.*, 2012). Thus, novel effective and less-toxic anticancer agents for cancer therapy are needed.

One of the alternatives for treating cancer is the use of natural products that can be obtained from plants, animals, marine organisms, and microorganisms. They have received increasing attention in recent years because they show lower adverse effects than chemical drugs. They can activate the body's immune system to kill or repress the growth of cancer cells (Bhanot *et al.*, 2011; Mantovani *et al.*, 2008).

Mushrooms are a good source of natural compounds for cancer treatments. Many edible mushrooms and their products have been noted as dietary supplements and sources of medicinal compounds with potential anticancer properties against several types of cancer in human (Ferrari *et al.*, 2012; Yukawa *et al.*, 2012.) *L. edodes* is one of the medicinal mushrooms which was studied and showed numerous pharmacological properties, such as antimicrobial, antiviral, antioxidant, anti-inflammatory, antiatherogenic, hypoglycemic, hepatoprotective, anticancer, and immunomodulating properties (Ferreira *et al.*, 2012; Rai *et al.*, 2005; Zaidman *et al.*, 2005). Several compounds extracted from *L. edodes* such as Lentinan (Chihara *et al.*, 1970; Zhang *et al.*, 2007), the polysaccharide L-II (Zheng *et al.*, 2005), water extracts of fruiting bodies (Israilides *et al.*, 2008), L·E·M (Shen *et al.*, 2009), and LEP (Yukawa *et al.*, 2012), show immunomodulatory and anticancer activity.

Most *Lentinus* species are edible and cultivable. They provide locals with seasonal food, medicine and alternative income (Karunarathna *et al.*, 2011). Many species of *Lentinus* found in Thailand were studies on cultivated and their nutritional value. However, little information is available concerning their bioactivity. Thus,

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we hypothesized that other species of *Lentinus* can show the pharmacological properties as presence in *L. edodes*, especially anticancer properties. In the current study, the biological extracts from *Lentinus* sp. collected in Thailand were studied. The objectives of this work are to evaluate some chemical compounds and the cytotoxic effect on human cancer cell line of polysaccharide extracted from *Lentinus* sp.

MATERIALS AND METHODS

Mushrooms samples

Two mushrooms, *Lentinus* sp. strain EB1001 and *Lentinus* sp. strain EB1101, were collected from the Nakhon Pathom province, Thailand. *Lentinus* sp. strain WCR1104 and *Lentinus* sp. strain WCR1201 were collected from the Ratchaburi and Kanchanaburi province, Thailand, respectively. *Lentinus edodes* AMC#3 was obtained from the Biotechnology Research and Development Office, Department of Agriculture. The cultures were maintained on Potato Dextrose Agar (PDA) at 4 °C.

Morphological characteristic identification

The mushroom specimens were purely isolated and identified at the Department of Microbiology, Faculty of Science, Silpakorn University, Nakhon Pathom, Thailand. The identification of the mushrooms was performed using macro- and micro-characteristics of fresh specimens using taxonomic keys according to Pegler (1983).

Comparison of Internal transcribe spacer (ITS) and phylogenetic tree analysis

The genomic DNA of mushroom samples was extracted from mycelium according to the method described by Chukeatirote *et al.* (2012). The DNA fragment corresponding to ITS region was amplified by the PCR technique using ITS1 (5'-TCCGTAGGTGAACCTTGCCC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers (White *et al.*, 1990), and sequence by First Base Laboratory, Malaysia. The taxa information and Genbank accession numbers in this work are listed in Table 1. Sequences for each strain were aligned using Clustal X (Thompson *et al.*, 1997), and phylogenetic analysis of the aligned sequence was performed with neighbor-joining using the MEGA program version 6.06 (Tamura *et al.*, 2013). Gaps were presented as missing data. The strength of the internal branches of the resulting trees was assessed by bootstrap analysis with 1,000 replicates. Phylogenetic trees were figured in Treeview.

Mushrooms cultivation

Mushroom cultivation was performed in sterilized sawdust bag (90% sawdust, 3% wheat bran, 1% corn meal, 1% cassava powder, 3% gypsum, 1% cane sugar, 0.05% $(NH_4)_2SO_4$ and 0.05% $CaCO_3$ with 60% Rh). The sawdust bags were incubated at 33±2 °C, except for *L. edodes*, which was incubated at 25 °C. After the completion of mycelium running throughout the sawdust bag, the fruiting bodies of *L. sajor-caju* strain EB1001, *L. swartzii* strain EB1101, *L. squarrosulus* strain WCR1201, and *L. velutinus* strain WCR1104 were induced by the removal of plastic bags, and the mushroom substrate was covered with casing soil. The humidity was controlled at 60-70% by watering every day. While the sawdust bags of *L. edodes* were removed from plastic bags and immersed in cold water at 10 °C overnight, after that the substrates were kept at 60-70% Rh at 20 °C.

Mycelial cultivation

The fungal mycelium was cultured on a Potato Dextrose Agar (PDA) plate at 33±2 °C for 7-15 days, except for *L. edodes*, which was incubated at 25 °C for 20 days. Then, plugs of active growing mycelium (diameter 0.5 cm) were inoculated to flat-bottoms containing 25 mL of slightly modified Mushroom Complete Medium (Osman *et al.*, 2009), composed of 1 mg Thiamine-HCl, 10 mg $FeSO_4 \cdot 7H_2O$, 1.6 mg $MnSO_4 \cdot H_2O$, 1 mg $CuSO_4$, 1 g KH_2PO_4, 0.5 g $CaCl_2 \cdot 2H_2O$, 0.5 g $MgSO_4 \cdot 7H_2O$, 2 g $NaNO_3$, 5 g yeast extract, 15 g fructose, 40 g starch soluble and 1 L of distilled water, and cultured at 25-30 °C for 10-30 days.

Crude polysaccharide extraction

The fruiting bodies and mycelium were taken and cut into small pieces and oven-dried at 60 °C. Then, they were blended with blender into fine particles.

For aqueous extraction, the fine particles of mushroom were de-fatted with 95% ethanol at room temperature overnight under shaking condition at 180 revolutions per minute (rpm). Then, the ethanol part was discarded. The mushroom biomass was boiled with 20 volumes of water for 3 h (three times). After centrifugation at 8,000 rpm for 30 min, the supernatants were concentrated with boiling and deproteinized with 1 volume of Sevag's reagent (5:1 $CHCl_3$:n-BuOH) for 3 times. Then the aqueous solution was extracted for crude polysaccharides by adding cold ethanol (final concentration was to 80% EtOH) and kept at 4 °C overnight. The supernatants were discarded after centrifugation at 8,000 rpm for 30 min. The precipitate was washed with cold absolute ethanol, centrifuged, and washed again. The pellet was collected and air-dried to give the crude aqueous extracts. The crude aqueous extracts were stored at -20 °C for further use.

For ethanol extraction, they were soaked in 10 volumes of 95% ethanol solution. The sample was extracted by stirring at 180 rpm at room temperature overnight. The mixture was filtered through Whatman's filter paper no 2. The residue was then extracted with two additional 10 volumes of ethanol as described above. The ethanolic extracts were combined and concentrated in a rotary evaporator at 50 °C. The extract was collected, air-dried and stored at -20 °C for further use.

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Properties of crude extracts

Determination of total carbohydrate content

The carbohydrate content was determined with a phenol–sulphuric acid method (Dubois *et al.*, 1951). Briefly, 0.5 mL of crude extract solution was mixed with 0.5 mL of 5% phenol, followed immediately with 2.5 mL of concentrated sulphuric acid and shake well. After 10 min of shaking the contents in tubes, the reaction mixture was placed at 25-30 °C for 20 min. The absorbance of the mixture was measured at 490 nm. The total carbohydrate content was calculated with D-glucose as standard (0-100 µg/mL).

Determination of reducing sugar

The reducing sugar was determined by the DNS method (Miller, 1959). Briefly, 2 mL of 3,5-dinitrosalicylic acid (DNS) reagent was added to a sample solution (1 mL). Then, the mixture was heated in boiling water for 15 min and cooled immediately. One mL of 40% (w/v) potassium sodium tartrate was added to the mixture and mixed. The volume adjusted to 10 mL with distilled water. The absorbance was measured at 550 nm, and the total reducing sugar was calculated with D-glucose as a standard (0-1,000 µg/mL).

Determination of total phenol content

The total phenol content of the crude extract was measured by the Folin-Ciocalteu colorimetric method, based on the procedure described by Singleton and Rossi (1965) and Thetsrimuang *et al.* (2011) with some modifications. Briefly, a sample (0.5 mL) was mixed with 0.5 mL of 1 N Folin-Ciocalteu reagent. Three minutes later, 0.5 mL of 7% (w/v) Na_2CO_3 was added and kept in the dark for 2 h, the absorbance of the mixture was read at 725 nm. The quantification was based on a standard curve of gallic acid (0-32 µg/mL). The total amount of phenol contents was expressed as gallic acid equivalent (mgGAE/g sample).

Determination of protein content

The total protein content was determined using the procedure described by Lowry *et al.* (1951) with some modification. Briefly, 6 mL of fresh alkaline copper reagent was immediately mixed with an aliquot of sample solution (1.2 mL). After 10 min, 0.3 mL of 1 N Folin-Ciocalteu reagent was added and mixed well. The reaction was incubated for 30 min at room temperature, the absorbance was measured at 500 nm, and the protein content was calculated with bovine serum albumin (BSA) as standard protein (0-1,000 µg/mL).

Determination of cytotoxic effect

The L929 murine aneuploid fibrosarcoma cell line and LLC-MK2 monkey rhesus kidney cell line were used for the cytotoxicity test and the HeLa human epitheloid cervix carcinoma cell line and HepG2 human hepatocellular liver carcinoma cell line were used for *in vitro* anticancer activities test. The cells were grown in RPMI 1640 medium supplemented with 5% fetal bovine serum (FBS) and 50 µg/mL gentamycin. All cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2. *In vitro* anticancer activity and cytotoxicity were evaluated using the MTT assay.

Briefly, 1.2×10^4 cells were seeded in each well of 96-well plates and incubated at 37 °C in a humidified atmosphere of 5% CO_2. After 24 h, fresh medium (100 µL) containing test sample was replaced to each well, followed by further incubation for 24 h. Then, the wells were replaced and incubated with fresh culture media containing MTT (0.5 mg/mL) for 4 h at 37 °C. Finally, the media were removed and DMSO was added to the wells (100 µL/well), and absorbance was measured at 540 nm in a microtiter plate reader. The number of viable cells was determined from the absorbance. Assays were performed in triplet wells. Data were expressed as percent viability compared with control (mean±SD).

RESULTS AND DISCUSSIONS

Mushroom identification

The morphology of four *Lentinus* sp. studied and then they were identified according to Pegler (1983) as *L. sajor-caju* strain EB1001 (Figure 1A), *L. swartzii* strain EB1101 (Figure 1B), *L. squarrosulus* strain WCR1201 (Figure 1C), and *L. velutinus* strain WCR1104 (Figure 1D).

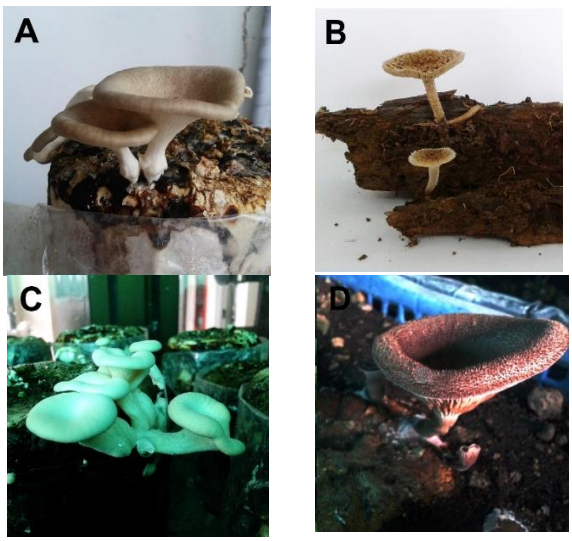

Figure 1: Fruiting body of (A) *L. sajor-caju* strain EB1001, (B) *L. swartzii* strain EB1101, (C) *L. squarrosulus* strain WCR1201, and (D) *L. velutinus* strain WCR1104.

To confirm morphology-based identification, the genomic DNA of mushroom samples was extracted, and the DNA fragment of the ITS region was amplified,

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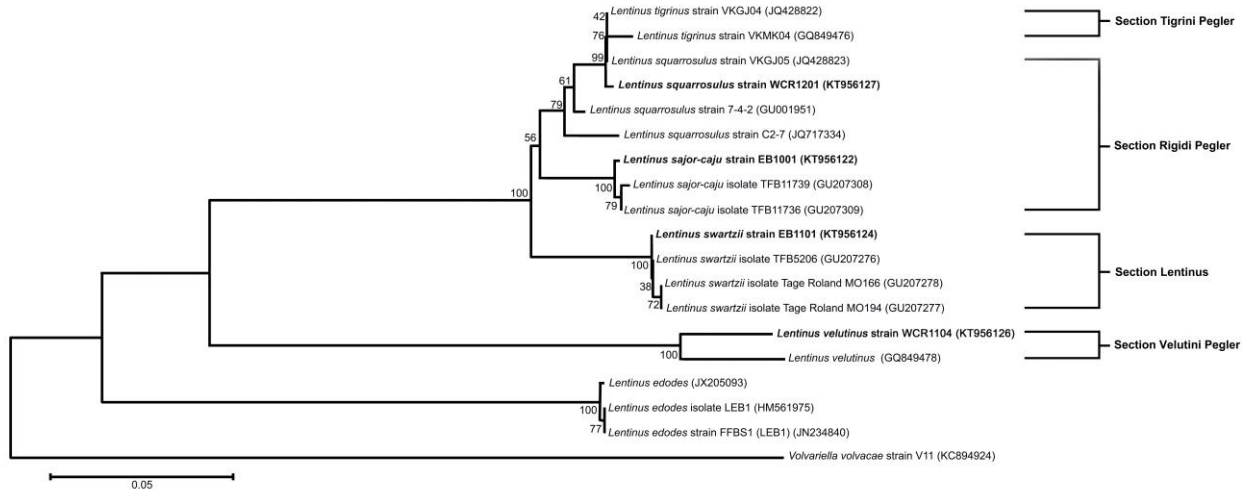

Figure 2: Phylogenetic relationships among four *Lentinus* species, *L. sajor-caju* strain EB1001, *L. swartzii* strain EB1101, *L. squarrosulus* strain WCR1201 and *L. velutinus* strain WCR1104 with some selected *Lentinus* based on ITS sequences. Data were aligned with ClustalW and gaps were treated as missing data. Values above the branches are parsimony bootstrap (≥ 50%). The tree is rooted with *V. volvacae* (KC894924) as outgroup.

sequenced and compared to database. A total of 14 ITS sequences of *Lentinus* spp., classified in section Tigrini, Rigidi, Lentinus, and Velutini, were obtained from GenBank. The ITS sequence of *Volvariella volvacae* used for sequence analysis was referred to as an outgroup. The results indicated that phylogenetic study of the ITS sequences supported the morphological identification (Figure 2).

Crude polysaccharide yields

A number of studies revealed the polysaccharide from hot water and alcohol extracts of mushrooms exhibited cytotoxicity against cancer cell line and anticancer activity. Hence, hot water and ethanol were chosen as the solvents for extraction of the polysaccharide from mushrooms fruiting bodies and mycelia. The yield of crude aqueous polysaccharides from mycelia (13.15-18.16%) was higher than that of fruit bodies (3.99-6.86%). While, the yield of crude ethanolic extracts varied from 3.36% to 52.58% (Table 1). The yield of crude ethanolic extracts was higher than that of crude aqueous extracts because of their textures. The crude ethanolic extracts were light to dark brown viscous liquid with a pungent odor, while the aqueous extraction yielded dark brown crystals. The difference in yields might depend on the strains, species and method of extraction (Thetsrimuang *et al.*, 2011).

Properties of crude polysaccharides

Crude aqueous polysaccharides showed a higher content of total carbohydrates, which ranged from 200.44 mg/g to 433.17 mg/g dwt (20.04-43.32%), than crude ethanolic polysaccharides (8.30-25.17%). The percentage of reducing sugar from aqueous and ethanol extracts ranged from 0% to 12.61% (Table 1). The total protein contents of crude aqueous and crude ethanolic extracts were between 17.7-45.3% and 7.2-16.2%, respectively. The results showed carbohydrates are a major component in crude aqueous extracts of LEFB-Aq, LEM-Aq, LSwM-Aq, LSqM-Aq, and LVM-Aq. While, crude aqueous extract from fruiting bodies of *L. swartzii* contained the proteins as the main content (Table 1). In addition to, the amount of carbohydrates and proteins of crude aqueous extract and crude ethanolic extract was 50-76% and 16-38%, respectively. This indicated that carbohydrate and protein are major component in aqueous extract while, ethanolic extract composed of other substances rather than carbohydrate and protein. The most polysaccharides are generally water-soluble but they could not dissolve in alcohol due to the associated polar character with numerous hydroxyl groups. On the other hands, the low molecular weight sugars e.g. monosaccharide, disaccharide, and oligosaccharide, could soluble in water and alcohol (Balto *et. al.*, 2016). The ratio of polysaccharide to protein from crude aqueous and ethanolic extracts was 1.35 and 1.84 on average, respectively. Our results agree with Dong and Yao (2008), who reported the ratios of polysaccharide to proteins of crude polysaccharides from fruiting bodies (1.0 ratio) and cultured mycelia (1.9 ratio) of *Cordyceps sinensis*. During extraction, free proteins were eliminated by using Sevag's reagent. However, some protein was presented in crude extracts. Thus, it is possible that the obtained crude extracts may be polysaccharide-protein complex substances. Some mushroom extracts were reported as polysaccharide-protein complexes, such as PPC-P11 from *Phaeogyroporus portentosus* (Karnchanatat *et al*., 2013). Nevertheless, this speculation should be examined with respect to the purified fractions. The total phenol contents of crude aqueous extracts and

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Table 1: The yield of crude polysaccharides, amount of proteins, total polysaccharides, reducing sugar, and total phenol content of crude aqueous and ethanolic extracts (n=3).

Crude Polysaccharides	Yields[a] (%)	Total carbohydrates[b] (mg/g)	Reducing sugar[b] (mg/g)	Total proteins[b] (mg/g)	Total phenol contents[b] (mgGAE/g)
LEFB-Aq[c]	6.86	411.98 ± 35.95hi[d]	59.16 ± 4.74d	275.42 ± 9.67h	16.77 ± 0.15fgh
LEM-Aq	14.78	368.91 ± 23.41gh	113.83 ± 3.48f	285.65 ± 6.29hi	20.87 ± 0.68ij
LSaFB-Aq	5.91	246.34 ± 12.78de	49.16 ± 3.13bc	284.28 ± 4.11hi	15.56 ± 0.35f
LSaM-Aq	18.16	326.95 ± 34.87fg	114.79 ± 2.37f	305.04 ± 9.60ij	18.39 ± 0.43ghi
LSwFB-Aq	5.47	207.15 ± 34.13cd	40.85 ± 3.10b	453.81 ± 14.57k	23.43 ± 0.65j
LSwM-Aq	16.16	392.01 ± 47.25hi	58.25 ± 2.17cd	241.20 ± 10.97fg	16.37 ± 0.66fg
LSqFB-Aq	6.16	271.06 ± 6.39ef	44.07 ± 2.09b	216.91 ± 4.91f	14.57 ± 0.09f
LSqM-Aq	13.15	405.71 ± 15.86hi	70.49 ± 1.11e	177.26 ± 4.76e	14.13 ± 0.29f
LVFB-Aq	3.99	200.44 ± 9.09cd	69.89 ± 4.03e	260.52 ± 5.81gh	15.58 ±3.33f
LVM-Aq	14.20	443.17 ± 10.40i	73.82 ± 1.21e	312.73 ± 8.49j	18.94 ± 0.48ghi
LEFB-Et	21.73	83.02 ± 4.37a	20.98 ± 3.61a	77.14 ± 9.05ab	3.29 ± 0.04a
LEM-Et	17.58	191.72 ± 14.29cd	111.46 ± 5.68f	99.26 ± 5.76b	6.42 ± 0.48bc
LSaFB-Et	16.74	172.90 ± 12.85bc	UD[e]	97.31 ± 6.23ab	9.11 ± 0.21de
LSaM-Et	9.84	196.75 ± 8.48cd	56.25 ± 6.57cd	87.90 ± 1.79ab	6.52 ± 0.35cde
LSwFB-Et	3.36	251.70 ± 23.21de	UD	129.12 ± 2.25c	9.74 ± 0.90e
LSwM-Et	20.530	221.72 ± 8.30cde	125.40 ± 1.46g	72.73 ± 1.99a	5.60 ± 0.39abc
LSqFB-Et	32.58	246.14 ± 5.22de	23.76 ± 1.19a	87.31 ± 4.71ab	4.45 ± 0.29ab
LSqM-Et	52.58	123.20 ± 6.44ab	UD	151.28 ± 6.17cd	9.96 ± 0.31e
LVFB-Et	9.69	171.88 ± 18.08bc	126.13 ± 4.66g	162.65 ± 18.69de	19.08 ± 0.21hi
LVM-Et	9.22	175.02 ± 3.78bc	24.31 ± 1.82a	95.78 ± 4.47ab	7.37 ± 0.41cde

[a] Yield per hundred grams dry weight of sample.
[b] Milligram per gram dry weight of crude extract.
[c] Extracts name: the letter referring to each *Lentinus* sp. (LE: *L. edodes*, LSa: *L. sajor-caju*, LSw: *L. swartzii*, LSq: *L. squarrosulus* and LV: *L. velutinus*), FB and M referring to fruit bodies and mycelium, and Aq and Et referring to aqueous and ethanolic extract.
[d] Means with different letters within a column are significantly different ($P < 0.05$).
[e] UD stand for undetected.

crude ethanolic extracts were 14.13-23.43 and 3.29-19.08 mgGAE/g crude polysaccharides, respectively (Table 1).

Determination of cytotoxic effect

A number of studies indicated that crude polysaccharide extract form agarics were able to inhibit the proliferation of cancer cell line. For instance, LEP, an ethanol precipitate of a dried powder extracted from *Lentinus edodes* mycelium, showed direct cytotoxicity to HepG2. The morphology of HepG2 cells treated with LEP were shrunk, rounded, and floated. The viability of HepG2 treated with LEP at 200 µg/mL for 24 h was 59.9% (Yukawa *et al.*, 2012). Li *et al.* (2012) evaluated the effect of acid, water, and alkaline extract of crude polysaccharide from eight Chinese mushrooms on HeLa and HepG2 cell proliferation. The viability of the cancer cell lines treated with extracts at 600 µg/mL for 48 h was 0-67.9%.

The cytotoxicity to the human cancer cell lines, HeLa and HepG2, and the normal cell lines, LLC-MK2 and L929 of crude aqueous extracts was evaluated at concentration 5, 10 and 20 mg/mL for 24 h. The proliferation of cancer cells were not inhibited by any extracts at 5 mg/mL, while all cell lines were completely killed when exposed to the extracts at 20 mg/mL. At 10 mg/mL, the aqueous extracts could not inhibit the growth of normal cell lines, LLC-MK2 and L929. The aqueous extract from fruiting body of *L. velutinus* (LVFB-Aq) inhibited proliferation of both the HeLa and the HepG2 cell lines with 49.83% and 48.59% inhibition, respectively (Figure 3A). The aqueous extract from mycelium of *L. velutinus* (LVM-Aq), fruiting body of *L. edodes* (LEFB-Aq) and mycelium of *L. edodes* (LEM-Aq) showed the cytotoxic effects against only the HepG2. The cell viability after treatment with these crude extracts declined to 55.71%, 36.58% and 62.53% respectively.

In this study, the ethanol extracts were not completely dissolved at concentration higher than 1 mg/mL. Therefore, the cytotoxicity of crude ethanolic extracts to the human cancer cell lines was tested at 1 mg/mL for 24 h due to the limitation of solubility. All ethanol extracts could not inhibit the growth of normal cell lines, LLC-MK2 and L929. The ethanol extract of fruiting body of *L. edodes* (LEFB-Et), *L. sajor-caju* (LSaFB-Et), *L. squarrosulus* (LSqFB-Et) and *L. velutinus* (LVFB-Et) displayed a toxicity effect against only the HeLa cells line by 26.33%, 15.88%, 31.17%, and 35.95% inhibition, respectively. While, the rest showed no effect toward both HepG2 and HeLa cell line.

The differences of cytotoxic effect were probably due to the concentration, the extraction solvents, or the type of obtained substances. Ethanol can extract both polar and nonpolar compounds such as low molecular weight

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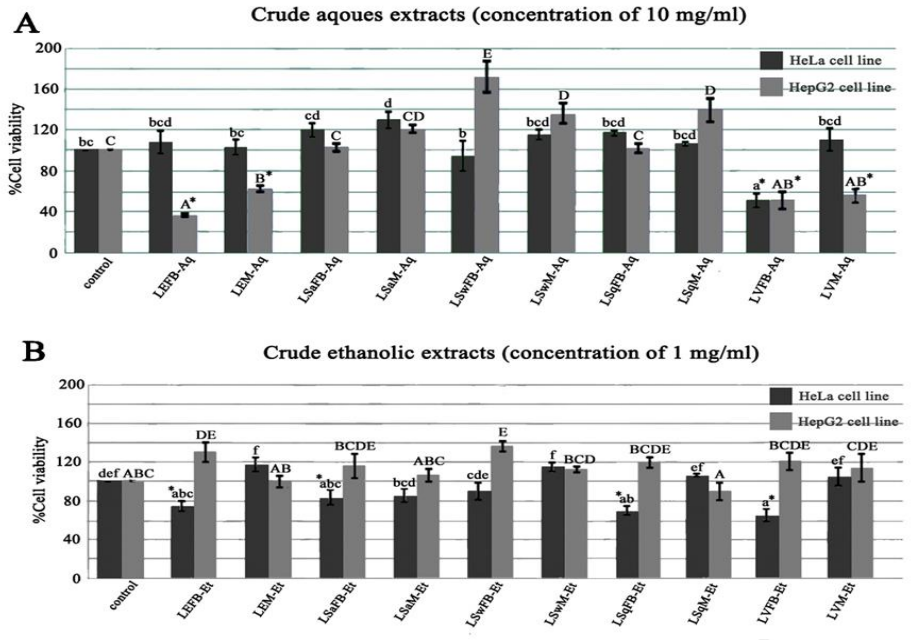

Figure 3: Cell viability (%) of human epitheloid cervix carcinoma cell line (HeLa) and human hepatocellular liver carcinoma cell line (HepG2) after treatment with crude aqueous extracts (A) and crude ethanolic extracts (B) of *Lentinus* sp. for 24 h. Error bars indicate means ± standard deviation (n = 3).

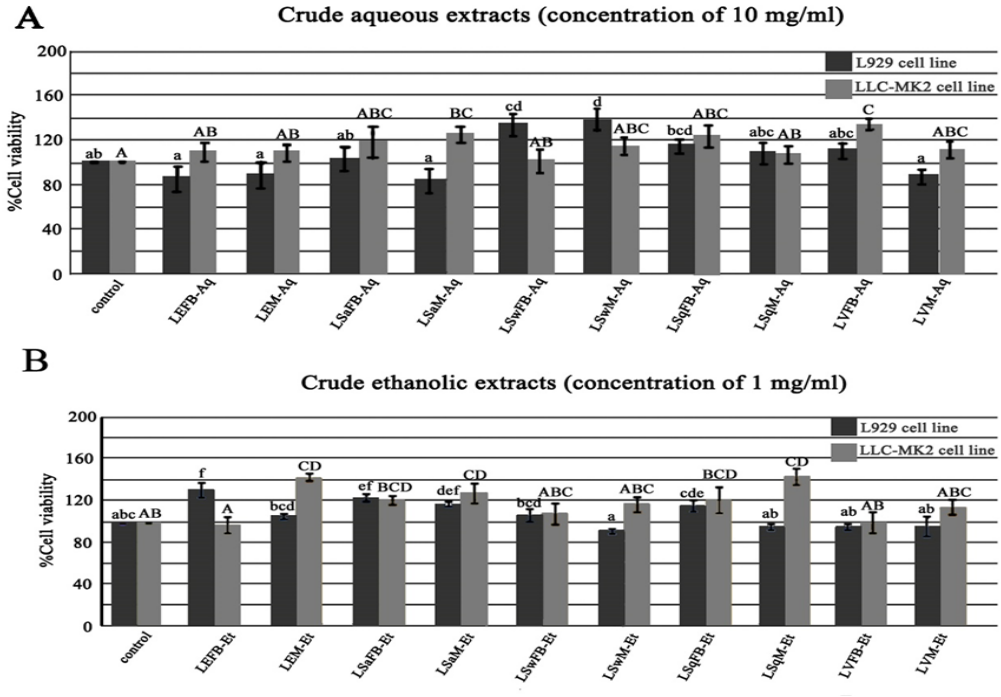

Figure 4: Cell viability (%) of rhesus monkey kidney epithelial cell line (LLC-MK2) and mouse fibroblast cell line (L929) after treatment with crude aqueous extracts (A) and crude ethanolic extracts (B) of *Lentinus* spp. for 24 h. Error bars indicate means ± standard deviation (n = 3).

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sugars, fatty acids, sterols, terpenoids, polypeptides, and amino acids, while carbohydrates and some proteins can be soluble in water (Beattie *et al.*, 2011). Most anticancer substances extracted from mushrooms have been reported as polysaccharides, proteins, and polysaccharide-protein complexes, which was exhibiting both direct and indirect cytotoxic effect to cancer cell line by inducing apoptosis and influencing the cancer cells through the activation of the immune response via several receptor such as dectin-1, complement receptor-3 (CR-3), lactosylceramide, and toll-like receptor (TLR; Zhang *et. al.*, 2007; Silva *et. al.*, 2012; Zong *et. al.*, 2012). The low molecular weight compounds showed direct effect on cancer development by modulate the cellular signal transduction pathways such as NF-κB and MAPK pathway, and support the inhibitory effects on cell differentiation, angiogenesis, and metastasis (Silva *et. al.*, 2012).

The results in this study revealed that the chemical contents of the extracts and their cytotoxic effects against human cancer cell lines were not correlated. For instance, the main component of crude aqueous extracts of LEFB-Aq, LEM-Aq, LSwM-Aq, LSqM-Aq, and LVM-Aq was carbohydrates but, only the crude aqueous extracts of LEFB-Aq, LEM-Aq and LVM-Aq, showed the cytotoxic effect against the HepG2 cell line. This is probably due to the difference of chemical component of the extracts. However, this speculation needs to be clarified.

The crude extracts of *L. sajor-caju* (LSaFB-Et), *L. squarrosulus* (LSqFB-Et) and *L. velutinus* (LVFB-Aq, LVM-Aq and LVFB-Et) showed cytotoxic effect on human cancer cell line but not on normal cell lines (Figure 3 and 4). This indicated that the extracts may be safe and should be further studied for anticancer activity.

CONCLUSION

Four *Lentinus* spp. were collected from central Thailand and identified according to morphology and genetics as *L. sajor-caju*, *L. swartzii*. *L. squarrosulus* and *L. velutinus*. Aqueous and ethanolic extractsn of fruit bodies and mycelia of four *Lentinus* species and *L. edodes* showed amounts of crude polysaccharides ranging from 33.6 to 205.3 mg/g dry weight of sample. The aqueous extract from fruiting body and mycelium of *L. edodes* (LEFB-Aq and LEM-Aq), *L. velutinus* (LVFB-Aq and LVM-Aq) and the ethanol extract of fruiting body of *L. edodes* (LEFB-Et), *L. sajor-caju* (LSaFB-Et), *L. squarrosulus* (LSqFB-Et) and *L. velutinus* (LVFB-Et) displayed a toxicity effect against the human cancer cell lines. However, all crude extracts showed no cytotoxicity to normal cell lines. Hence, the extracts with cytotoxic activity might be a potential source of bioactive compounds for cancer treatment.

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