Conclusion and Discussion

As similar with previous results showed that reactive oxygen species(ROS), from both endogenous and exogenous sources causes in diverse human diseases such as arteriosclerosis, ischemic injury, cancer and neurodegenerative disease, as well as in processes like inflammation and aging. There is increasing verification that indigenous antioxidants may be useful in preventing the harmful penalty of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs and medicinal plants. Mushrooms were also used as functional and medicinal foods for a long time. Edible mushrooms in many species are high content in fiber, some vitamins and some minerals such as ascorbic acid, vitamin D, potassium, selenium and etc. The compositions of minerals and vitamins have potential to inhibit tumor growth antioxidant and enhance the immune system. Antioxidant activity of methanolic extracts is measured to inhibit initial stage of lipid peroxidation by FTC. The Lom and Kornkaow mushrooms in Phitsanulok showed strong antioxidant activity which is indicated by their low absorbance values. At the same concentration, the relatively upper activity was shown in the extracts of Lom followed by Kornkaow, Horm and got minimum in Kradang mushrooms when they were compared with BHA. However we may confirm lipid peroxidation inhibition with TBA method which measure the amount of lipid peroxidation in second stage. Another mechanism of antioxidants perform is scavenging of reactive oxygen and nitrogen free radicals. In this study we used DPPH assay to scavenge total free radical. This method is based on the reduction of DPPH, a stable free redical and any molecule that can donate an electron or hydrogen to DPPH can react with it and therrby bleach the DPPH absorption. As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, that is, a free radical scavenging is decreased and the resulting decolorization is stoichiometric with respect to the number of electrons captured. After mushrooms extracts were tested for the DPPH free radical scavenging ability. The methanoic extract of natural Lom mushrooms showed strongest radical scavenging activity with minimum IC_{50} at 0.5 mg/ml whereas Horm and Kornkaow mushrooms presented at higher IC_{50}. Kradang showed the poorest free radical scavenging activity. Those IC_{50} are comparable with commercial α-tocopherol and BHA as shown in Fig. 3. Previous studies about other mushroom species of DPPH assay were reported. Mau et al., 2002 reported that scavenging effects of methanolic extracts from medicinal mushrooms on DPPH radical increased with the increased concentrations. At 0.64 mg/ml, scavenging effects were 67.6-74.4% for *Ganoderma lucidum* (Ling-chih), *Ganoderma tsugae*(Sung-shan-chih) and 24.6% for *Coriolus versicolor*(Yun chih). Huang, L.C.2002 also found that excellent scavenging effects(96.3-99.1 and 97.1%) were observed with methanolic extracts from *A.camphorata* and *Brazillian*

mushrooms at 2.5 mg/ml, respectively. Lin, H.-C. reported that at 0.64 mg/ml, the methanolic extract from stinkhorn scavenged DPPH radical by 92.1% whereas scavenging effects of methanolic extracts from other specialty mushrooms were 63-67.8%. At 0.64 mg/ml, the methanolic extract from three oyster mushrooms scavenged DPPH radical by 81.8%, whereas scavenging effects of extracts from other commercial mushrooms were 42.9-69.9%. Our antioxidant activity of Lom mushrooms were rather well compared with other mushrooms. This should conclude that Lom mushrooms contain polyphenolics that can donate electron/hydrogen easily. We verified this hypothesis with measurement of total phenolics concentration equivalents of gallic acid. Gallic acid was the most important polyphenol in natural products in the range of 60-109 mg/g. From our result in Fig.6 high content of total phenolic compound was detected in Lom and Kornkaow mushroom, this result correlate with their potency in antioxidant activity. Total phenolic contents were rather low in Kradang and Horm mushroom and those mushrooms also showed rather poor in antioxidant activity. We should concern about a large number of phytocompound groups are implicated for antoxidant activity. Hot water Lom mushroom extracts were also exhibited strong antioxidant activity at 80% of potency free radical scavenging. The order of scavenging activity showed maximum in Lom followed by Kownkaow, Horm and Kradang mushrooms, respectively. This result correlated with methanolic mushrooms extract. The antioxidant activity of those mushroom extracts is probable from some soluble vitamins such as vitamin C or water soluble of some phenolic compound.

A good quality 2D gel is not only important for composition of protein profiles between samples. Most of the protein spots in Lentinus mushrooms were concentrated in pI range 5-7 and between molecular mass 20-100 kDa, but also essential for reliable protein identification by MS or N-terminal amino acid sequencing. This mainly depends on what extraction protocol is engaged and optimized for a particular sample tissue. In this study we have modified tris buffered phenol method which is performed by Kiyotaka, H. and *et al* (2008). From Fig. 9-16, the spots of 2D gel were rather well however in future we should use longer strip 18 cm to work for MS or N-terminal amino acid sequencing to confirm our interesting proteins. We detected spot L-Seryl tRNA selenium transferase which was identified at pI ~ 8.5 and Mw ~ 51 kDa from previous study of *S. crispa* 2D gel (Kiyotaka, H. and *et al* , 2008) in Lom and KornKaow mushrooms. Selenium is an essentially required element for synthesis of selenoproteins including glutathione peroxidase(GPX) that is an important antioxidant enzyme. L-seryl tRNA selenium transferase is involved in the biosynthesis process. We also detected spot protein at pI ~ 6.43 and Mw~19 kDa which was identified as glutathione peroxidase in the same previous study. Both of the protein spots were presented in high peak of Lom and Kornkaow mushrooms by Melanie viewer 7 program supported of Swiss Institue of Bioinformmatics. Another interesting result, Se content of Lom mushrooms

were also rather high in the rate of 0.42 mg/kg dry weight. These correlated data of Se content, L-seryl tRNA selenium transferase and glutathione peroxidase may be another index of antioxidant properties of Lom and Kornkaow mushrooms. However we plan to use MS or N-terminal sequencing to confirm spots of glutathione peroxidase and L-seryl-tRNA(Sec)Selenium transferase. However we should realize that there are having other groups of proteins which play role in antioxidant in table 4 by Kiyotaka, H. and *et al* (2008).

From our previous data (data not shown), we got preliminary data in anticancer property of Lom mushrooms by inhibition of proliferation in cancer cell lines (cholangiocarcinoma (K-100) in KKU and HT-29) by MTT-assay. In this study we also characterized their antioxidant activity and detected some antioxidative proteins (glutathione peroxidase and L-seryl-tRNA(Sec)Selenium transferase). We also detected some selenium and phenolic compound. Their good characterizations will challenge us to study more for application in future.

Table 4. Group of antioxidative proteins

Accession number	Protein name	Analytical Mw	Theoretical pI	Organism	Peptide/Function
gi|2776	Catalse	57848.89	6.42	Fungi	LFSYPDTHR
Q2PCV2	Laccase	54040.94	6.26	Fungi	Sptvxvndvvpsgtfty/Secondary metabolites biosynthesis, transport, and catabolism
Q9B6D8	Cytochrome c oxidase subunit 3	30467.04	6.32	Fungi	Asatehtltvrdgln/Energy production and conversion
Q7VL68	L-séryl-, tRNA(Sec)Seleniu m transferase	50580.15	8.5	Bacteria	Slqvaliayqkndyh/Translation, ribosomal structure and biogenesis
gi|30580366	Catalase 3	79227.71	5.75	Fungi	FEASHVTNEQVKK
gi|38566870	Glutathione peroxidase	18888.76	6.43	Fungi	FLIGKDGKVK
Q9P4T6	Superoxide dismutase [Mn]	22194.25	6.03	Fungi	Vhtlpdlpyaydalepyfsr/Inorganic ion transport and metabolism
Q1EBD9	Glutathione S-transferase	23889.39	6.60	Fungi	Secondary metabolites biosynthesis, transport, and catabolism

Applied from Kiyotaka, H. and *et al* (2008).