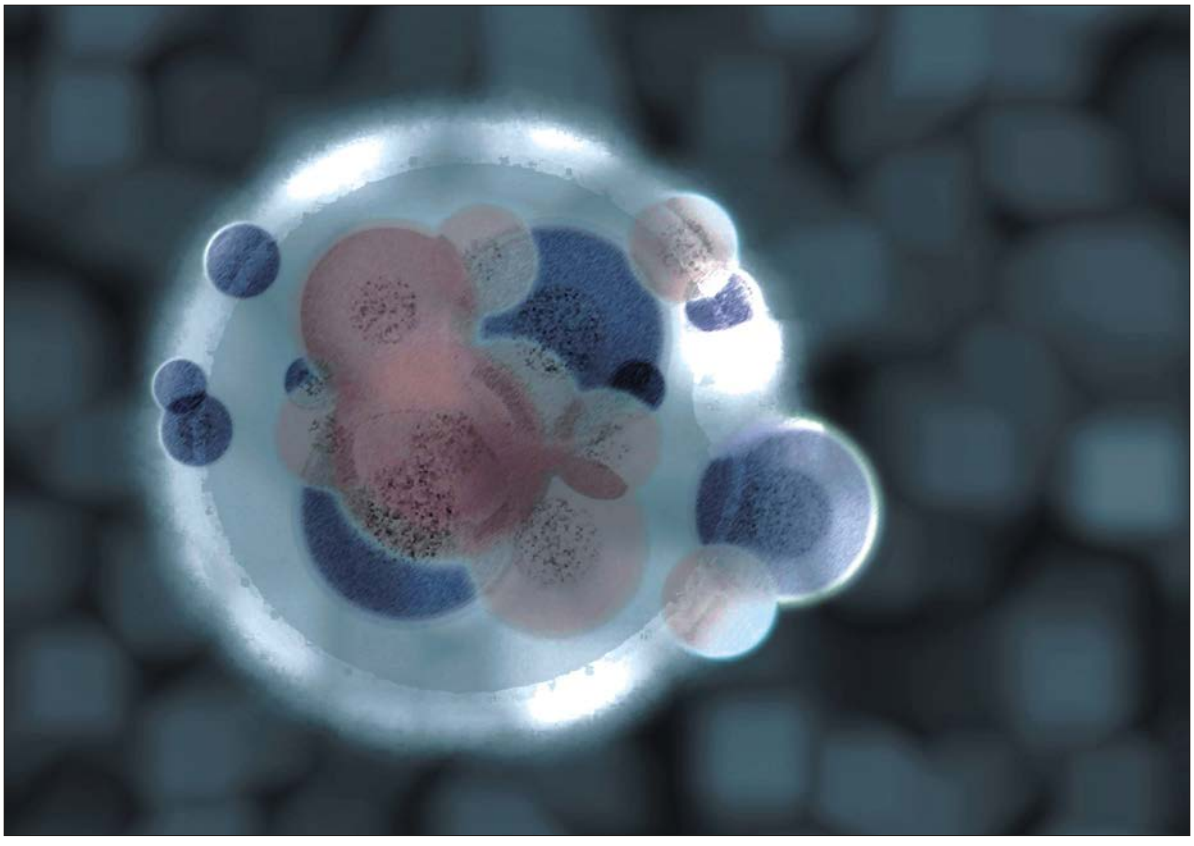

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Antioxidant, Cytotoxic and Antimalarial Activities from Crude Extracts of Mushroom *Phellinus linteus*

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Abstract: Crude extracts of mushroom *Phellinus linteus* were investigated for their antioxidant, cytotoxic, antimalarial and antibacterial activities. The crude EtOAc fraction from large scale extraction showed the strongest antioxidant activity with IC_{50} value of 17.73±0.27 μg mL^{-1} and the highest total phenolic value of 76.39±0.07 EGA. This activity was as good as ($p>0.05$) L(+)ascorbic acid (IC_{50} value of 16.56±0.50 μg mL^{-1}). From small scale extraction, IC_{50} values of crude extracts were in the range of 24.15±0.50 to 207.02±1.95 μg mL^{-1} and the values of total phenolic content ranged from 68.11±0.06 to 5.96±0.18 EGA. Crude MeOH, CH_2Cl_2 and EtOAc extracts from large scale extraction exhibited cytotoxicity against MFC7 and NCI-H187 cancer cells. Crude MeOH and CH_2Cl_2 extracts also displayed antimalarial activity against *Plasmodium falciparum* with IC_{50} values of 3.15 and 3.08 μg mL^{-1}, respectively. None of the extracts were found to have antibacterial activity against five different species of pathogenic bacteria.

Key words: *Phellinus linteus*, antioxidant, antimalaria, cytotoxicity, antibacteria

INTRODUCTION

Mushroom *Phellinus linteus*, commonly referred as Sanghwang in Korea, Meshimakobu in Japan and Songgen in China, is the most well known species of the genus *Phellinus*. This mushroom has long been used as a traditional medicine in oriental countries for the treatment of stomachaches, inflammation, arthritis of the knee, gastroenteric disorders, inflammation, tumors and lymphatic disorders (Cho *et al*., 2002; Kang *et at*., 2004; Kim *et al*., 2004a). The active polysaccharides, especially β-glucan, produced by *P. linteus* have been known to exhibit potent biological activities. There were reports of polysaccharides extracted from *P. linteus* stimulating cell-mediated and humoral immunity and inhibiting tumor growth and metastasis (Han *et al*., 1999; Kim *et al*., 1996). The inhibitory activity of the polysaccharide isolated from *P. lintues* toward melanoma cell metastasis (Han *et al*., 2006) and the role of polysaccharides from *P. linteus* as effective immunomodulator and enhancing the antitumoral activity of peritoneal macrophages (Kim *et al*., 2004b) have also been reported. *P. linteus* was found to act as a natural anticancer agent by preventing the inhibition of gap junctional intercellular communication through the inactivation of ERK1/2 and p38 MAP kinases (Cho *et al*., 2002). Polysaccharides from *P. linteus* have also been reported to exhibit the hypoglycemic effect and they also decreased total cholesterol, triacylglycerol and aspartate aminotransferase activity (Kim *et al*., 2001). Its polysaccharide had an effect on the immunomodulatory activity of the murine splenic lymphocytes (Kim *et al*., 2003a) and also inhibited tumor growth through a mechanism leading to a Th-1 dominant immune state and the activation of $CD11c^+CD8^+$ DC (Kim *et al*., 2004c).

An active polysaccharide from *P. linteus* was reported to have been involved with the prevention and treatment of autoimmune joint inflammation (Kim *et al*., 2003b). The antiinflammatory, antinociceptive and antiangiogenic activities from an *n*-BuOH subfraction from a mycelia culture of *P. linteus* (Kim *et al*., 2004a) and the antiinflammatory activity from its fruiting body via mediation of heme oxygenase-1 (Kim *et al*., 2006) have been investigated. There were reports of *P. linteus* inhibition of inflammatory mediators by suppressing redox-based NF-kB and MAPKs activation in lipopolysaccharide-induced RAW 264.7 macrophage (Kim *et al*., 2007a). The anti-inflammatory activity mediated through the PKCδ/Nrf2/ARE signaling to up-regulation of heme oxygenase-1 (Kim *et al*., 2007b) was documented.

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Crude extracts of *P. linteus* have also been studied for their antioxidant activity. There was a report that the 70% ethanol extract from fruiting body of *P. linteus* showed antiangiogenic and antioxidant activities when compared with standard vitamin C (Song *et al*., 2003). Recently, polysaccharide from hot water extracts from various medicinal mushrooms, including *P. linteus*, were found to be highly active to Reactive Oxygen Species (ROS) which could play an important role in the prevention of infection by destroying potential intracellular pathogens (Wei and Van Griensven, 2008).

In this study, we aim to investigate antioxidant property of crude extracts obtained from various solvent extractions of *P. linteus*, to evaluate these extracts for their cytotoxicity against NCI-H187, MFC7 and KB cancer cells and to characterize antimalarial and antibacterial activities which have never been investigated before.

MATERIALS AND METHODS

Mushroom sample: The fruiting body of mushroom *P. linteus* was kindly provided by Mr. Frankie Chan. The mushroom was identified by Dr. Usa Klinhom based on morphology and deposited at MSUT, Faculty of Science, Mahasarakham University. The specimen was dried at 60°C under a UV lamp, then powdered.

Extraction

Small scale extraction: Ten gram of mushroom powder was extracted with 100 mL (24 h, 3 times) of five different solvents; water, ethanol (EtOH), 50% EtOH, 80% EtOH and ethyl acetate (EtOAc), under two conditions; heated at 60°C and room temperature extractions. The collected EtOH and EtOAc layers (cal. 300 mL each) were each evaporated under reduced pressure at 40°C to give crude EtOH and EtOAc extracts, respectively. The collected water, 50% EtOH, 80% EtOH layers (cal. 300 mL each) were evaporated under reduced pressure and residues from evaporation were subjected to freeze-drying to give crude extracts from water, 50% EtOH and 80% EtOH.

Large scale extraction: Five hundred gram of mushroom powder was extracted with methanol (MeOH) (3 L, 60°C, 3 day, 2 times). The collected MeOH layer (cal. 6 L) was concentrated under reduced pressure to obtain a brownish-black crude MeOH extract. Three hundred milligram of this crude extract was taken for the antioxidant and biological assays. The remaining extract was suspended in water (150 mL) and then partitioned with hexane, dichoromethane (CH_2Cl_2) and EtOAc, respectively. The hexane (cal. 1 L), CH_2Cl_2 (cal. 700 mL) and EtOAc (cal. 1.2 L) layers were evaporated under reduced pressure to give crude hexane (1.67 g), CH_2Cl_2 (1.54 g) and EtOAc (3.39 g) fractions from MeOH, respectively.

Antioxidant assay

Radical scavenging activity toward DPPH radical (DPPH method): Free radical scavenging activity of the crude extracts was measured according to the method of (Chu *et al*., 2000) with the modifications in the concentration and the ratio of mixed solutions. A 2 mL of 0.2 mM DPPH solution in MeOH was added to 1 mL of sample solution (5-100 μg mL^{-1}). The mixture was kept at room temperature for 30 min in the dark. After that, the absorbance values of the solutions were measured at λ_{max} 517. The percentages of radical scavenging activity (%RSA) of each concentration were calculated and then converted to the value of the inhibition concentration at 50% (IC_{50} value). Quercetin, L(+)-ascorbic acid (vitamin C) and butylated hydroxytoluene (BHT) were used as standard antioxidants.

$$RSA\ (\%) = [1 - (A_{sample}/A_{blank}) \times 100]$$

Blank: One milliliter of methanol mixed with 2 mL of 0.2 mM DPPH solution.

Determination of total phenolic content (Folin- Ciocalteu method): Total phenolic content was determined by using the Folin-Ciocalteu method described by Miliauskas *at al*. (2004) with the modifications in the concentration and the ratio of mixed solutions. One milliliter of a 50 μg mL^{-1} of sample solution in MeOH was added with 2 mL of Folin-Ciocalteu's reagent solution (10-fold dilution in H_2O) and 4 mL of 7.5% (w/v) sodium carbonate solution. The absorbance values of the mixed solution were measured at λ_{max} 761 nm after being left to stand for 1 h at room temperature in the dark. The phenolic content was expressed as equivalent of gallic acid (EGA); mg EGA: 100 mg of the extract.

Cytotoxicity and antimalarial assay: Crude MeOH extract and its crude CH_2Cl_2 and EtOAc extracts from large scale extraction were sent to BIOTEC for their cytotoxicity (Skehan *et al*., 1990) and antimalarial (Desjardins *et al*., 1979) activity.

Antibacterial assay: Antibacterial activity was evaluated using paper disc method. Crude MeOH extract its crude hexane, CH_2Cl_2 and EtOAc fractions from large scale extraction were test against five food borne pathogenic bacteria; *Escherichia coli* ATCC25922, *Salmonella typhi* DMST5784, *Shigella flexneri* DMST4423, *Shigella dysenteriae* DMST15111 and *Vibrio cholerae* ATCC14035. Bacterial concentrations of 10^7 cfu mL^{-1} were

incubated before being placed on paper discs containing crude extracts at a concentration of 5,000 μg mL^{-1} on MHA medium. The zone of inhibition was observed and measured at 24 and 48 h.

The extraction, antioxidant and antibacterial activities testing were carried out at the Faculty of Science, Mahasarakham University while the bioassay for cytotoxic and antimalarial activities were carried out at BIOTEC (National Center for Genetic Engineering and Biotechnology), Bangkok, Thailand, during August to December, 2008.

Statistical analysis: The tests for antioxidant activity and total phenolic content were carried out in triplicate. The data was recorded as Mean±SD. The means of all parameters were examined for significance by Analysis of Variance (ANOVA) with Duncan's significant difference post-hoc test (SPSS software). The p-values less than 0.05 were considered significant.

RESULTS

Antioxidant activity

DPPH radical scavenging activity: The strongest DPPH radical scavenging capacity of the extracts was found in crude EtOAc fraction from large scale extraction with IC_{50} value of 17.73±0.27 μg mL^{-1} (Table 1). This activity was as good as ($p>0.05$) L(+) ascorbic acid (vitamin C) (IC_{50} value of 16.56±0.50 μg mL^{-1}), significantly ($p<0.05$) higher than BHT (IC_{50} value of 18.84±0.27 μg mL^{-1}) but lower than quercetin (IC_{50} value of 10.92±0.13 μg mL^{-1}). This activity was three and four times stronger than the scavenging activity of crude EtOAc extracts from heated extraction, IC_{50} value of 52.90±0.30 μg mL^{-1} and room temperature, IC_{50} value of 77.97±1.26 μg mL^{-1}, from small scale extractions. Comparing activity between crude MeOH and EtOH extracts showed that crude MeOH extract from large scale extraction, IC_{50} value of 25.58±0.22 μg mL^{-1}, displayed radical scavenging activity significantly higher ($p<0.05$) than crude EtOH extracts from heated, IC_{50} value 29.18±0.20 μg mL^{-1} and much better than room temp., IC_{50} value 43.06±0.52 μg mL^{-1}, from small scale extractions. Crude CH_2Cl_2 fraction from large scale extraction was found to have low antioxidant property when compared with crude MeOH extract and EtOAc fraction. However, this activity was significant ($p<0.05$) higher than the activity of crude water extracts from small scale extractions. Hexane was used for large scale extraction in order to remove the lipid from crude MeOH extract before fractionation. No significant antioxidant activity (IC_{50} value>1,000 μg mL^{-1}) was observed for crude hexane fraction.

Table 1: Antioxidant activity from crude extracts of mushroom *P. linteus*

		Antioxidant activity	
Extraction condition	Crude extract	DPPH (IC_{50}; μg mL^{-1})	Total phenloic content EGA: 100 g of extract
Large scale	MeOH	25.58±0.22	36.51±0.07
	Hexane	>1,000	-
	CH_2Cl_2	130.78±2.00	32.75±0.30
	EtOAc	17.73±0.27	76.39±0.07
Small scale[a]	H_2O	147.27±2.26	14.19±0.17
	50% EtOH	24.15±0.50	37.13±0.26
	80% EtOH	24.48±0.87	68.11±0.06
	EtOH	29.18±0.20	46.38±0.12
	EtOAc	52.90±0.30	15.87±0.36
Small scale[b]	H_2O	207.02±1.95	5.96±0.18
	50% EtOH	25.54±0.17	35.91±0.52
	80% EtOH	34.42±0.13	51.64±1.47
	EtOH	43.06±0.52	36.85±0.72
	EtOAc	77.97±1.26	25.76±0.76
Standard	Quercetin	10.92±0.13	
	L(+)Ascorbic acid	16.56±0.50	
	BHT	18.84±0.27	

[a]Heated at the temperature of 60°C; [b]Room temperature

For small scale extraction, scavenging activity on DPPH radical from crude extracts from heated extraction exhibited stronger activity than room temperature extraction for all kind of extracting solvents. In this group, crude 50% EtOH extract showed the highest antioxidant activity with IC_{50} values of 24.15±0.50 and 25.54±0.17 μg mL^{-1}, while crude water extracts exhibited the lowest DPPH scavenging ability with IC_{50} values of 147.24±2.26 and 207.02±1.95 μg mL^{-1}.

Total phenolic content: Crude EtOAc fraction from large scale extraction presented the highest amount of phenolic compounds with a value of 76.39±0.07 EGA (Table 1). For small scale extraction, crude extract from 80% EtOH showed the highest total phenolic content for both heated and room temperature extraction conditions with values of 68.11±0.06 (heat) and 51.64±1.47 (room temp.) EGA, respectively. The lowest total phenolic content was found in crude water extracts, 14.19±0.17 (heat) and 5.96±0.18 (room temp.) EGA. High amount of total phenolics relate to good antioxidant property.

Cytotoxic and antimalarial activities: Crude MeOH, crude CH_2Cl_2 and EtOAc fractions from large scale extraction of *P. linteus* showed cytotoxicity against MFC7 (IC_{50} values of 17.36, 25.78 and 27.26 μg mL^{-1}, respectively), NCI-H187 (IC_{50} values of 19.14, 17.88 and 40.15 μg mL^{-1}, respectively) and vero cells (IC_{50} values of 48.42 and 42.58 and non cytotoxic μg mL^{-1}, respectively) while displayed inactive result to KB cell (Table 2). Also, crude extracts from MeOH and CH_2Cl_2 exhibited potent antimalarial activity against the multidrug-resistant strain of *Plasmodium falciparum* with IC_{50} values of 3.15 and 3.08 μg mL^{-1}, respectively.

Table 2: Biological activities from crude extracts of *P. linteus*

	Cytotoxicity (IC_{50}; μg mL^{-1})				
Crude extract	KB	MFC7	NCI-H187	vero	Antimalaria[d] (IC_{50}; μg mL^{-1})
MeOH	>20	17.36	19.14	48.42	3.15
CH_2Cl_2	>20	25.78	17.88	42.58	3.08
EtOAc	>20	27.26	40.15	>50	>20
Standard					
Doxorubicin	0.13	2.15	0.04	-	-
Ellipticine	0.48	-	0.44	0.56	-
Dihydroartemisinin	-	-	-	-	0.005

[d]Against *Plasmodium falciparum* (K1, multidrug-resistant strain), KB: Oral cavity cancer cell, MCF7: Breast cancer call, NCI-H187: Small cell lung cancer cell, vero use African green monkey kidney fibroblasts

Antimicrobial activity: From our results, no significant antibacterial activity was observed from crude extracts of *P. linteus* against five species of pathogenic bacteria tested in this study at maximum concentration of 100 mg mL^{-1}.

DISCUSSION

In our present work, medicinal mushroom *P. linteus* has been investigated for its antioxidant and biological activities. The strongest antioxidant capacity as comparable as ($p>0.05$) vitamin C was observed in crude EtOAc fraction. These results were in good agreement with the study of Kang *et al.* (2004) in the report of the antioxidant activity of L-tyrosine catalyzed by tyrosinase from crude fractions of *P. linteus*. The results indicated that successively fractionation of crude MeOH extract with CH_2Cl_2, EtOAc and *n*-BuOH, the highest active antioxidants came in crude EtOAc fraction. Slightly lower antioxidant property of crude EtOH-water (50% EtOH and 80% EtOH) extracts than the standard BHT, a synthetic antioxidant compound, was investigated. This result were similar to the antioxidant activity of mushroom Chaga extracts from sclerotium (ST) and Fruiting Body (FB) parts reported by Nakajima *et al.* (2007). Crude 80% MeOH extract from both of ST and FB parts showed relatively high activity than 50% MeOH, 20% MeOH and aqueous extracts using DPPH assay.

It has been reported that the antioxidant capacity of edible mushroom extracts was positively correlated with their content of phenolic compounds (Lim *et al*., 2007). Present results showed a good correlation between the amount of total phenolics and antioxidant activity of the *P. linteus* extracts. Yellow pigments that composed of hispidin derivatives and polyphenols which are a major contribution to their antioxidant property are commonly produced by mushrooms *Phellinus* (Lee and Yun, 2007) and pure polyphenol compounds isolated from mushroom *Phellinus* have been reported by Nagatsu *et al.* (2004), Min *et al.* (2006), Mo *et al.* (2004) and Wang *et al.* (2007) The significantly high amount of phenolic compounds in crude EtOAc fraction followed by crude 80% EtOH and EtOH extracts could be explained that the phenolics of *P. linteus* are high in polarity and likely easier soluble in polar organic and alcohol-aqueous solvents.

Crude MeOH extract and its crude hexane, CH_2Cl_2 and EtOAc fractions of *P. linteus* had no antibacterial activity against five species of pathogenic bacteria tested. However, Hur *et al.* (2004) reported antibacterial activity from *n*-BuOH fraction of crude MeOH extract of *P. linteus* against *Staphylococcus aureus* strains at MIC values ranging from 63-125 μg mL^{-1} while antibacterial activity from aqueous extracts of *P. gilvus* and *P. igniarius* against *S. aureus*, *E. coli* and *Klebsiella pneumonia* have been reported by Sittiwet and Puangpronpitag (2008a, b). Interestingly, antibacterial activity from crude water extract of *P. linteus* against pathogenic bacteria should be further studied.

The cytotoxicity against NCI-H187 and antimalarial activity of *P. linteus* are the first time reported. Recently, the activity of *P. linteus* suppressed phosphorylation of AKT at Thr^{308} and Ser^{473} in breast cancer cells has been investigated by Sliva *et al.* (2008).

In conclusion, our research suggested that *P. linteus* collected from Thailand has high potential would be used as natural antioxidants. Due to toxicological concerns associated with the used of synthetic substances in food and increasing awareness about natural foods, there has been increased interest in the use of natural substances as food preservatives and antioxidants (Peschel *et al*., 2006). Its antioxidant property, cytotoxicity and antimalarial activity could also have correlations with other pharmacological actions for its use in folk medicine. Isolation and structure elucidation of pure compound from crude CH_2Cl_2 and EtOAc fractions of *P. linteus* are under the process.

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REFERENCES

Cho, J.H., S.D. Cho, H. Hu, S.H. Kim, S.K. Lee, Y.S. Lee and K.S. Kang, 2002. The roles of ERK1/2 and p38 MAP kinases in the preventive mechanisms of mushroom *Phellinus linteus* against the inhibition of gap junctional intercellular communication by hydrogen peroxide. Carcinogenesis, 23: 1163-1169.

Chu, Y.H., C.L. Chang and H.F. Hsu, 2000. Flavonoid content of several vegetables and their antioxidant activity. J. Sci. Food Agric., 80: 561-566.

Desjardins, R.E., C.J. Canfield and J.D. Chulay, 1979. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. Antimicrob. Agents Chemother., 16: 710-718.

Han, S.B., C .W. Lee, Y.J. Jeon, N.D. Hong, I.D. Yoo, K.H. Yang and H.M. Kim, 1999. The inhibitory effect of polysaccharides isolated from *Phellinus linteus* on tumor growth and metastasis. Immunopharmacology, 41: 157-164.

Han, S.B., C.W. Lee, J.S. Kang, Y.D. Yoon and K.H. Lee *et al*., 2006. Acidic polysaccharide from *Phellinus linteus* inhibits melanoma cell metastasis by blocking cell adhesion and invasion. Int. Immunopharmacol., 6: 697-720.

Hur, J.M., C .H. Yang, S.H. Han, S.H. Lee, Y.O. You, J.H. Parck and K.J. Kim, 2004. Antibacterial effect of *Phellinus linteus* against methicillin-resistant *Staphylococcus aureus*. Fitoterapia, 75: 603-605.

Kang, H.S., J.H. Choi, W.K. Cho, J.C. Park and J.S. Choi, 2004. A sphingolipid and tyrosinase inhibitors from the fruiting body of *Phellinus linteus*. Arch. Pharm. Res., 27: 742-750.

Kim, H.M., S.B. Han, G.T. Oh, Y.H. Kim, D.H. Hong and I.D. Yoo, 1996. Stimulation of humoral and cell mediated immunity by polysaccharide from mushroom *Phellinus linteus*. Int. Immunopharmacol., 18: 295-303.

Kim, D.H., B.K. Yang, S.C. Jeong, J.B. Park and S.P. Cho *et al*., 2001. Production of a hypoglycemic, extracellular polyssacharide from submerged culture of the mushroom *Phellinus linteus*. Biotechnol. Lett., 23: 513-517.

Kim, G.Y., S.K. Park, M.K. Lee, S.H. Lee and Y.H. Oh *et al*., 2003a. Proteoglycan isolated from *Phellinus linteus* activates murine B lymphocytes via protein kinase C and protein tyrosine kinase. Int. Immunopharmacol., 3: 1281-1292.

Kim, G.Y., S.H. Kim, S.Y. Hwang, H. Y. Kim and Y.M. Park *et al*., 2003b. Oral administration of proteoglycan isolated from *Phellinus linteus* in the prevention and treatment of collagen-induced arthritis in mice. Biol. Pharm. Bull., 26: 823-831.

Kim, S.H., Y.S. Song, S.K. Kim, B.C. Kim, C.J. Lim and E.H. Park, 2004a. Anti-inflammatory and related pharmacological activities of the *n*-BuOH subfraction of mushroom *Phellinus linteus*. J. Ethonopharmacol., 93: 141-146.

Kim, G.Y., G.S. Choi, S.H. Lee and Y.M. Park, 2004b. Acidic polysaccharides isolated from *Phellinus linteus* enhances through the up-regulation of nitric oxide and tumor necrosis factor-a from peritoneal macrophages. J. Ethnopharmacol., 95: 69-76.

Kim, G.Y., W.K. Oh, B.C. Shin, Y.I. Shin and Y.C. Park *et al*., 2004c. Proteoglycan isolated from *Phellinus linteus* inhibits tumor growth through mechanisms leading to an activation of $CD11c^{+}CD8^{+}$ DC and type I helper T cell-dominant immune state. FEBS Lett., 576: 391-400.

Kim, B.C., J.W. Choi, H.Y. Hong, S.A. Lee and S. Hong *et al*., 2006. Heme oxygenase-1 mediates the anti-inflammatory effect of mushroom *Phellinus linteus* in LPS-stimulated PAW264.7 macrophages. J. Ethnopharmacol., 10: 364-371.

Kim, H.G., D.H. Yoon, W.H. Lee, S.K. Hun and B. Shrestha *et al*., 2007a. *Phellinus linteus* inhibits inflammatory mediators by suppressing redox-based NF-kB and MAPKs activation in lipopolysaccharide-induced RAW 264.7 macrophage. J. Ethnopharmacol., 114: 307-315.

Kim, B.C., W.K. Jeon, H.Y. Hong, K.B. Jeon and J.H. Hahn *et al*., 2007b. The anti-inflammatory activity of *Phellinus linteus* (Berk, and M.A. Curt.) is mediated through the PKC?/Nrf2/ARE signaling to up-regulation of heme oxygenase-1. J. Ethnopharmacol., 113: 240-247.

Lee, I.K. and B.S. Yun, 2007. Highly oxygenated and unsaturated metabolites providing a diversity of hispidin class antioxidants in the medical mushrooms *Inonotus* and *Phellinus*. Bioorg. Med. Chem., 15: 3309-3314.

Lim, H.W., J.H. Yoon, Y.S. Kim, M.W. Lee, S.Y. Park and H.K. Choi, 2007. Free radical scavenging and inhibition of nitric oxide production by four grades of pine mushroom (*Tricholoma matsutake* Sing.). Food Chem., 103: 1337-1342.

Miliauskas, G., P.R. Venskutonis and T.A. Van Beek, 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem., 85: 231-237.

Min, B.S., B.S. Yun, H.K. Lee, H.J. Jung, H.A. Jung and J.S. Choi, 2006. Two novel furan derivatives from *Phellinus linteus* with anti-complement activity. Bioorg. Med. Chem. Lett., 16: 3255-3257.

Mo, S., S. Wang, G. Zhou, Y. Yang, Y. Li, X. Chen and J. Shi, 2004. Phelligridin C-F: Cytotoxic pyrano[3,2-c]pran-4-one derivatives from the fungus *Phellinus linteus*. J. Nat. Prod., 67: 823-828.

Nagatsu, A., S. Itoh, R. Tanaka, S. Kato and M. Haruna *et al*., 2004. Identification of novel substituted fused aromatic compounds, meshimakobnol A and B, from natural *Phellinus linteus* fruit body. Tetrahedron Lett., 45: 5931-5933.

Nakajima, Y., Y. Sata and T. Konishi, 2007. Antioxidant small phenolic ingredients in *Inonotus obliquus* (persoon) Pilat (Chaga). Chem. Pharm. Bull., 55: 1222-1226.

Peschel, W., F. Sanchez-Rabaneda, W. Dieckmann, A. Plescher and I. Gartzia *et al*., 2006. An industria approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chem., 97: 137-150.

Sittiwet, C. and D. Puangpronpitag, 2008a. Anti-*Staphylococcus aureus* activity of *Phellinus igniarius* aqueous extract. Int. J. Pharmacol., 4: 503-505.

Sittiwet, C. and D. Puangpronpitag, 2008b. Antibacterial activity of *Phellinus gilvus* aqueous extract. Int. J. Pharmacol., 4: 500-502.

Skehan, P., R. Storeng, D. Sgudiero, A. Monks and J. McMahon *et al*., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Nat. Cancer Inst., 82: 1107-1112.

Sliva, D., A. Jedinak, J. Kawasaki, K. Harvey and V. Slivova, 2008. *Phellinus linteus* suppresses growth, angiogenesis and invasive behaviour of breast cancer calls through the inhibition of AKT signaling. Br. J. Cancer, 98: 1348-1356.

Song, Y.S., S.H. Kim, J.H. Sa, C. Jin, C.J. Lim and E.H. Park, 2003. Anti-angiogenic, antioxidant and xanthine oxidase inhibition activities of the mushroom *Phellinus linteus*. J. Ethnopharm., 88: 113-116.

Wang, Y., X.Y. Shang, S.J. Wang, S.Y. Mo and S. Li *et al*., 2007. Structures, biogenesis and biological activities of pyrano[4,3-c]isochromen-4-one derivatives from the fungus *Phellinus igniarius*. J. Nat. Prod., 70: 296-299.

Wei, S. and L.J.L.D. Van Griensven, 2008. Pro-and antioxidant properties of medicinal mushroom extract. Int. J. Med. Mushr., 10: 315-324.