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Total phenolic content and antioxidant activity of crude methanolic extract from five *Pleurotus* species

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Pleurotus species are a popular edible mushroom and easily artificially cultivated. This research was investigated total phenolic content (TPC) and antioxidant activity of the crude methanolic extract of five *Pleurotus* species: *Pleurotus ostreatus*, *P. sajor-caju*, *P. eryngii*, *P. cystidiosus* and *P. eous*. The TPC in the extracts was determined with the Folin–Ciocalteau method. Methanolic extract of *P. ostreatus* showed highest phenolic content (17.63±1.30 mg GAE/g extract). The antioxidant activity was estimated using 2,2 -azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The result showed that the crude methanolic extract of five *Pleurotus* was separated into two groups. *P. ostreatus*, *P. sajor-caju* and *P. eous* showed relatively high antioxidant activity, while *P. eryngii* and *P. cystidiosus* showed low antioxidant activity. Thus, the present study establishes some *Pleurotus* species are used both in food supplements and in medicinal products. Including, further studies of those mushroom should be directed isolation of pure chemical constituents and biological activities evaluation in order to prepare high value food and pharmaceutical products.

Keywords: *Pleurotus* species, total phenolic content, antioxidant activity

Introduction

A free radical is an atoms with one or more unpaired eletrons which have extremely high chemical reactivity. Therefore, free radicals are able to damage numerous biological substances, including DNA, protein and lipid membranes (Tsai *et al*., 2007). These conditions may be a cause or symptom of illness in humans such as cancer, cardiovascular diseases, impaired immune function, atherosclerosis and aging (Wu and Hanson, 2008; Xu *et al*., 2009). Prevention from free radical damage, the body has a defense system called antioxidants. These antioxidants are actually delay or inhibit cellular damage mainly through

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their free radical scavenging property. In vivo, the antioxidants are generated from either endogenous or exogenous sources. The exogenous antioxidants are externally supplied through food that commonly called dietary antioxidants. Besides mushrooms have a great nutritional value, the chemical and nutraceutical composition that used directly in human diet to promote health. Mushrooms also accumulate a great variety of secondary metabolites exspecilly phenolics compounds. The phenolic compounds in mushrooms show excellent antioxidant capacity (Hung and Nhi, 2012; Finimundy *et al*., 2013; Valverde *et al*., 2015).

The genus *Pleurotus* (Basidiomycota, Agaricales), also known as oyster mushrooms, is one of the most diverse groups of cultivated mushroom that have important economic and medicinal properties (Cohen *et al*., 2002). The genus *Pleurotus* have been reports on biological activity. However, there are only data for some species such as *P. ostreatus* (Lavi *et al*., 2006; Tong *et al*., 2009; Jedinak *et al*., 2011), *P. eryngii* (Mishra *et al*., 2013) and *P. sajor-caju* (Ngai and Ng, 2004; Kanagasabapathy *et al*., 2011; Finimundy *et al*., 2013). Therefore, the aim of the present study was to investigate total phenolic content (TPC) and antioxidant activity of the crude methanolic extract of five *Pleurotus* species: *P. ostreatus*, *P. sajor-caju*, *P. eryngii*, *P. cystidiosus* and *P. eous*.

Materials and methods

Collection and preparation of samples

Pleurotus species: *P. ostreatus*, *P. sajor-caju*, *P. eryngii*, *P. cystidiosus* and *P. eous* were collected from Taladthai, Pathum Thani in March 2015. The fresh mushrooms were washed, sliced and dried in an oven 45 ℃ until a constant weight. The dried mushrooms were milled and kept in a plastic bag until analysis. The dried mushroom powder was extracted by maceration with methanol at room temperature at 150 rpm for 1-3 days and then filtered through Whatman No. 1 paper. The methanolic extracts were evaporated at 40 ℃ to dryness using rotary evaporator and stored at 4 ℃ for further use.

Determination of total phenol content

The total phenol content of the crude methanolic mushroom was estimated by the Folin-Ciocalteu colorimetric method (Armania *et al*., 2013) with some modifications. Briefly, a volume of 0.5 ml of the extract (1-2 mg/ml) was mixed with 2.5 ml of 10% Folin-Ciocalteu reagent. Five minutes later, add 2 ml of 7.5% Na_2CO_2 to the mixture and mixed thoroughly. The mixture was

kept in the dark for 90 min at 40 ℃. The absorbance of the mixture was read at 765 nm. The calibration line was constructed using a standard curve range from 20-100 µg/ml of gallic acid. The total amount of phenol content was expressed as mg of gallic acid equivalents per gram extract (mgGAE/g of extract).

DPPH radical scavenging activity

The DPPH method was used to determine for radical scavenging activity of extracts. The analysis was carried out according to the method of Armania *et al*. (2013) with some slight modifications. Briefly, the 100 µl of various concentrations (0.625-10 mg/ml) of test samples were added to 100 µl of 0.2 mM methanolic DPPH solution and mixed thoroughly. The mixture was incubated for 30 min in the dark at room temperature and the absorbance was measured at 492 nm. The percentage inhibition was calculated according to the formula: $[(A_0 - A_1)/A_0] \times 100$, where A_0 was the absorbance of the control and A_1 the absorbance of the sample. Including, expressed as milligrams trolox equivalent antioxidant capacity (mgTEAC)/g extract.

ABTS radical cation scavenging activity

The ABTS radical cation scavenging activity was described by Armania *et al*. (2013) with some slight modifications. The $ABTS^{\cdot+}$ cation radicals were produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate. The solution was incubated in the dark at room temperature for 12 h or until the reaction was turned to blue-green colors. Prior to use, the solution was diluted with methanol to get an absorbance of 0.700 ± 0.025 at 734 nm. Free radical scavenging activity was assessed by mixing 100 µl of a test sample with 1000 µl of ABTS working solution in a microcuvette. The decrease in absorbance was measured exactly after 6 min. The percentage inhibition was calculated according to the formula: $[(A_0 - A_1)/A_0] \times 100$, where A_0 the absorbance of the control without the samples, and A_1 the absorbance of the samples as well as expressed as milligrams trolox equivalent antioxidant capacity (mgTEAC)/g extract.

Statistical analysis

All experimental measurements were carried out in three parallel measurement and expressed as mean±standard deviation (SD). The data were analyzed by SPSS version 17.0. One-way analysis of variance (ANOVA) were used to show the mean differences between all samples. Duncan's multiple

range tests (DMRT) were used to determine the significant differences between groups. $P \leq 0.05$ was considered statistically significant.

Results and Discussion

Total Phenolic Content

Phenolic compounds are particularly potent natural products with wide range of biological activities consisted of antioxidant, anti-inflammatory, antimicrobial, chemopreventive and anticancer properties (Halliwell and Gutteridge, 2006). The main characteristic of this group of compounds has been related to its antioxidant activity (Valverde et al., 2015). *P. ostreatus* has the highest phenolic content (17.63 mg GAE/g extract) followed by *P. sajor-caju*, *P. eous*, *P. eryngii* and *P. cystidiosus* (Table 1). The result showed that the total phenolic content was separated into two groups. *P. ostreatus*, *P. sajor-caju* and *P. eous* showed high content of total phenolic content, while *P. eryngii* and *P. cystidiosus* showed low content of total phenolic content. In contrast, the evaluation of total phenolic and flavonoid contents in eight types of edible mushrooms (*Agaricus bisporus*, *Boletus edulis*, *Calocybe gambosa*, *Cantharellus cibarius*, *Craterellus cornucopioides*, *Hygrophorus marzuolus*, *Lactarius deliciosus* and *Pleurotus ostreatus*). These authors concluded that mushrooms contain 1-6 mg of phenolics/g of dried mushroom that *P. ostreatus* presented low content of phenolic compounds (Palacios *et al*., 2011). Including, *P. eryngii* had the highest contents of phenolics, followed by *P. djamor* when compaired with *P. citrinopileatus*, *P. flabellatus*, *P. florida*, *P. ostreatus* and *P. sajor-caju* (Mishra et al., 2013).

DPPH radical scavenging activity

The radical scavenging of the mushroom extract was tested using a methanolic solution exhibits a deep purple colour with absorption at 492 nm. The purple colour generally disappears when an antioxidant is present in medium (Gursoy *et al*., 2010). The scavenging effect of the methanolic extract increased with concentration which the 50 % effective concentration (EC_{50}) values are given in Table 1. Generally, EC_{50} values of lower than 10 mg/ml indicated that the extracts were effective in antioxidant properties. Crude methanolic extract of *Pleurotus* showed a wide range of antioxidant capacities, 1.59-2.18 mg Trolox/g extract. *P. ostreatus* has the highest antioxidant capacities (2.18 mg GAE/g extract) followed by *P. sajor-caju*, *P. eous*, *P. eryngii* and *P. cystidiosus* that have 2.09, 1.99, 1.71 and 1.59 mg GAE/g

extract, respectively. Including, the antioxidant of *Pleurotus* spp. was separated into two groups. *P. ostreatus*, *P. sajor-caju* and *P. eous* showed high antioxidant activity, while *P. erynii* and *P. cystidiosus* showed low activity (Figure 1).

Table 1 Total phenolic content and 50% effective concentration of antioxidant activity in crude methanolic extract from five *Pleurotus* species

Mushrooms	Total Phenolic Content (mg GAE/g)**	EC_{50} values (mg/ml)*	
		DPPH	ABTS
P. ostreatus	17.63 ±1.30[a]	5.00 ±0.06[a]	5.10 ±0.15[b]
P. sajor-caju	15.75 ±2.60[ab]	5.22 ±0.07[a]	4.51 ±0.12[ab]
P. eous	11.02 ±2.21[bc]	5.43 ±0.06[a]	3.92 ±0.01[a]
P. eryngii	08.77 ±1.08[c]	8.48 ±0.87[b]	6.98 ±0.33[c]
P. cystidiosus	07.17 ±2.24[c]	9.13 ±0.17[b]	7.18 ±0.57[c]

*Values expressed are mean ± SD of triplicate measurements
**mgGAE/g of extract: milligrams of gallic acid equivalents per gram of extract
EC_{50} (mg/ml) = 50 % effective concentration
a-c means with the different letters in the same column were significant at $p \leq 0.05$

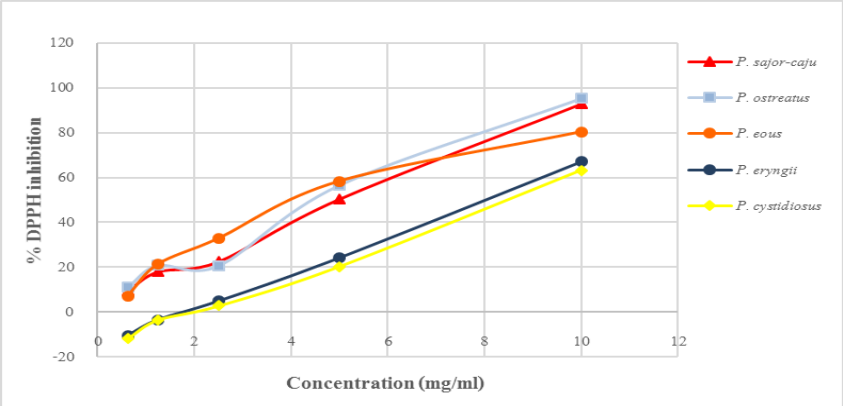

Figure 1 DPPH radical scavenging activity of the crude methanolic extracts of five *Pleurotus* species

ABTS radical cation scavenging activity

ABTS assay is often used in evaluating total antioxidant power of single compounds and complex mixtures of various plants (Katalinic *et al*., 2006). In this assay, ABTS radical mono cation was generated directly in stable from potassium persulfate. The 50 % effective concentration values are given in Table 1. Furthermore, crude methanolic extract of *Pleurotus* showed a wide

range of antioxidant capacities, 26.82-49.13 mg Trolox/g extract. *P. eous* has the highest antioxidant capacities (49.13 mg GAE/g extract) followed by *P. sajor-caju*, *P. ostreatus*, *P. eryngii* and *P. cystidiosus* that have 42.62, 37.69, 27.59 and 26.82 mg GAE/g extract, respectively. However, the ABTS radical scavenging activity of the crude methanolic extract of *Pleurotus* can be separated to two groups similar with DPPH method. Generally, *P. eryngii* and *P. cystidiosus* grow well at low temperature. Therefore, antioxidant capacities as well as phenolic content of *Pleurotus* may be depend on grow temperature.

From this data, the extracts containing high levels of phenolic content generally exhibit high antioxidant activities. It demonstrated that phenolic compounds are very important constituents in controlling antioxidants and scavenging ability. Our result was supported with findings of Kanagasabapathy *et al*. (2011) who reported the *P. sajor-caju* showed antitumor effects and antioxidant properties by the aqueous and butanol extracts exhibited the highest antioxidant activity and corresponded to the total phenolic content. As well as, *P. sajor-caju* have a potential source of antioxidant and anticancer compounds (Finimundy et al.,2013).

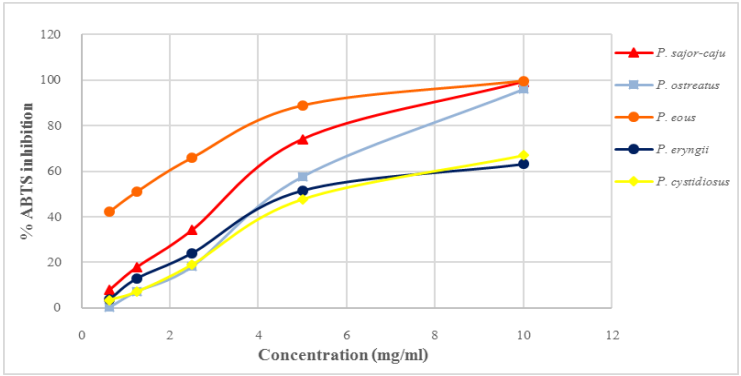

Figure 2 ABTS radical scavenging activity of the crude methanolic extracts of five *Pleurotus* species

Conclusion

Our finding indicate that the crude methanolic extract of five *Pleurotus* was separated into two groups. *P. ostreatus*, *P. sajor-caju* and *P. eous* showed relatively high antioxidant activity, while *P. eryngii* and *P. cystidiosus* showed low antioxidant activity both measured by DPPH and ABTS assays. This research supports positive relationship between antioxidant activity and total phenol content indicated that these compounds contribute to the antioxidant activity. Thus, the present study establishes some *Pleurotus* species are used

both in food supplements cosmatic and in medicinal products. However should be study in other topics such as cytotoxicity, chemical constituents and biological activities evaluation.

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References

Armania, N., Yazan, L.S., Musa, S.N., Ismail, I.S., Foo, J.B., Chan, K.W., Noreen, H., Hisyam, A.H., Zulfahmi, S. and Ismail, M. (2013). *Dillenia suffruticosa* exhibited antioxidant and cytotoxic activity through induction of apoptosis and G_2/M cell cycle arrest. Journal of Ethnopharmacology 146: 525-535.

Cohen, R., Persky, L. and Hadar, Y. (2002). Biotechnological applications and potential of wood-degrading mushroom of genus *Pleurotus*. Applied Microbiology Biotechnology 58: 582-594.

Gursoy, N., Sarikurkcu, C., Tepe, B. and Solak, H. (2010). Evaluation of antioxidant activities of 3 edible mushrooms: *Ramaria flava* (Schaef: Fr.) Qu ĕl., *Rhizopogon roseolus* (Corda) T.M.Fries., and *Russula delica* Fr. Food Science and Biotechnology 19: 691-696.

Finimundy, T.C., Gambato, G., Fontana, R., Camassola, M., Salvador, M., Moura, S., Hess, J., Henriques, J.A., Dillon, A.J. and Roesch-Ely, M. (2013). Aqueous extracts of *Lentinula edodes* and *Pleurotus sajor-caju* exhibit high antioxidant capability and promising in vitro antitumor activity. Nutrition Research 33(1): 76-84.

Halliwell, B. and Gutteridge, C. (2006). Free radicals in biology and medicine. Oxford: Clarendon Press.

Hung, P.V. and Nhi, N.N.Y. (2012). Nutritional composition and antioxidant capacity of several edible mushrooms grown in the Southern Vietnam. International Food Research Journal 19(2): 611-615.

Jedinak, A., Dudhgaonkar, S., Wu, Q.L., Simon, J. and Sliva, D. (2011). Anti-inflammatory activity of edible oyster mushroom is mediated through the inhibition of NF-κB and AP-1 signaling. Nutrition Journal 10: 52.

Kanagasabapathy, G., Malek, S.N.A., Kuppusamy, U.R. and Vikineswary, S. (2011). Chemical composition and antioxidant properties of extracts of fresh fruiting bodies of *Pleurotus sajor-caju* (Fr.) singer. Journal of Agricultural and Food Chemistry 59(6): 2618-2626.

Katalinic, V., Milos, M., Kulisic T. and Jukic M. (2006). Sreening of 70 medicinal plant extract for antioxidant capacity and total phenol. Food Chemistry 94: 550-557.

Lavi, I., Friesem, D., Geresh, S., Hadar, Y. and Schwartz, B. (2006). An aqueous polysaccharide extract from the edible mushroom *Pleurotus ostreatus* induces anti-proliferative and pro-apoptotic effects on HT-29 colon cancer cells. Cancer Letters 244(1): 61-70.

Mishra, K.K., Pal, R.S., Arunkumar, R., Chandrashekara, C., Jain, S.K. and Bhatt, J.C. (2013). Antioxidant properties of different edible mushroom species and increased bioconversion efficiency of *Pleurotus eryngii* using locally available casing materials. Food Chemistry 138(2-3): 1557-1563.

Ngai, P.H.K. and Ng, T.B. (2004). A ribonuclease with antimicrobial, antimitogenic and antiproliferative activities from the edible mushroom *Pleurotus sajor-caju*. Peptides 25(1): 11-17.

Palacios, I., Lozano, M., Moro, C., Arrigo, M.D., Rostagno, M.A., Martínez, J.A., García-Lafuente, A., Guillamón, E. and Villares, A. (2011). Antioxidant properties of phenolic compounds occurring in edible mushrooms. Food Chemistry 128(3): 674-678.

Tong, H., Xia, F., Feng, K. (2009). Structural characterization and in vitro antitumor activity of a novel polysaccharide isolated from the fruiting bodies of *Pleurotus ostreatus*. Bioresource Technology 100(4): 1682-1686.

Tsai, M., Song, T., Shih, P. and Yen, G. (2007). Antioxidant properties of water-soluble polysaccharides from *Antrodia cinnamomea* in submerged culture. Food Chemistry 104: 1115-1122.

Valverde, M.E., Hernández-Pérez, T. and Paredes-López, O. (2015). Edible mushrooms: improving human health and promoting quality life. International Journal of Microbiology: 1-14.

Wu, X.J. and Hansen, C. (2008). Antioxidant capacity, phenol content, and polysaccharide content of *Lentinus edodes* grown in whey permeate-based submerged culture. Journal of Food Science 73: 1-8.

Xu, W., Zhang, F., Luo, Y., Ma, L., Kou, X and Huang, K. (2009). Antioxidant activity of a water-soluble polysaccharide purified from *Pteridium aquilinum*. Carbohydrate Research 344: 217-222.

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