Mal J Nutr 16(2): 281 - 291, 2010

Antioxidant Activities and Total Phenolic Content of Aqueous Extract of *Pleurotus ostreatus* (Cultivated Oyster Mushroom)

Yim HS[1,2*], Chye FY[2], Tan CT[1], Ng YC[1] & Ho CW[1]

[1] *Department of Food Science & Nutrition, Faculty of Applied Sciences, UCSI University 56000 Kuala Lumpur, Malaysia*
[2] *School of Food Science & Nutrition, Universiti Malaysia Sabah Locked Bag 2073, 88999 Kota Kinabalu, Sabah, Malaysia*

ABSTRACT

Pleurotus ostreatus better known as oyster mushroom is widely cultivated and consumed as food in Malaysia. The present study aims to assess the antioxidative potential and total phenolic content of *P. ostreatus* aqueous extract. The antioxidant activities were evaluated against DPPH and ABTS radical-scavenging activity, ferric-reducing antioxidant power (FRAP) and β-carotene-linoleate bleaching assay, and the Folin-Ciocalteu method for total phenolic content (TPC). The DPPH and ABTS radical-scavenging activity was found to be 63.20% and 87.29% respectively; antioxidant activity using FRAP at 1.45 mM FE/100g and β-carotene-linoleate bleaching assay was 83.51%, while the TPC was found to be 798.55 mg GAE/100g. These antioxidant activities were compared to synthetic antioxidant, BHA and ascorbic acid. Ascorbic acid showed highest scavenging effects on DPPH and ABTS radical, followed by *P. ostreatus* and BHA (at maximum safety limit). The ferric reducing power of *P. ostreatus* was significantly higher than BHA and ascorbic acid. The antioxidant activity as assessed in β-carotene-linoleate bleaching assay was found to be higher in BHA compared to *P. ostreatus*. The aqueous extract of *P. ostreatus* was found to respond differently in antioxidant assays. The antioxidative activity of the aqueous extract of *P. ostreatus* correlated with its total phenolic content. Generally, the antioxidant activities of *P. ostreatus'* aqueous extract are comparable to that of BHA and ascorbic acid to a certain extent.

Keywords: Antioxidant activities, aqueous extract, *Pleurotus ostreatus*, total phenolic content

INTRODUCTION

Food industries have long used synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) as preservatives in food products to prevent lipid oxidation which leads to off-odour in lipid-rich food. However, the restricted use of these synthetic antioxidants in food due to their carcinogenic effects has led to increased interest in antioxidant substances from natural resources (Naveena *et al*., 2008). Natural antioxidant substances are generally considered safe because they are found abundantly in vegetables, fruits and many others of plant origin. Numerous naturally occurring substances have been studied and found to possess antioxidant activities, for example, phenolic compounds

*Correspondence author: Hip Seng Yim; Email: *hsyim@ucsi.edu.my*

which have been shown to have the ability to scavenge free radicals.

Mushrooms are good sources of vitamins, minerals, proteins, carbohydrates, unsaturated fatty acids (particularly oleic and linoleic acids), high amounts of fibers and low in energy, fat and sodium, and often regarded as an ideal and healthy food for people with high blood cholesterol and hypertension (Manzi *et al.*, 1999). Mushrooms have been reported to contain a wide variety of free radical scavenging molecules, such as polysaccharides and polyphenols (Cui, Kim & Park, 2005). Besides the phenolics, the relatively large amounts of vitamins A, vitamin C and β-carotene in mushrooms have been shown to be the main contributor to its antioxidant activity (Cheung, Cheung & Ooi, 2003; Barros *et al.*, 2007).

Several species of mushrooms contain a wide variety of free radicals or reactive oxygen species scavengers which have made mushrooms attractive as nutritionally beneficial foods and as a source for drugs development (Guerra-Dore *et al.*, 2007). Cheung *et al.* (2003) found that the presence of phenolic compounds in two mushrooms extract contributed high β-carotene-linoleate bleaching inhibition and radical scavenging activity. Mau *et al.* (2004) reported high chelating effects on ferrous ions from three mushrooms, namely *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia (90.3-94.4%). The probable inhibitory effect on LDL oxidation in relation to phenolic compounds from mushroom has been reported (Cheung & Cheung, 2005). Barros *et al.* (2008) reported that flavonoids from mushroom can act as free radical scavengers to terminate the radical chain reactions that occur during the oxidation of triglycerides in the food system. But to date, there is still relatively scarce information on antioxidant activities of the mushroom *Pleurotus ostreatus*, which is widely cultivated in Malaysia. Thus, the present study aims to evaluate the antioxidant activities of aqueous extract of *P. ostreatus* by free radical scavenging ability, reducing power and β-carotene-linoleate bleaching inhibition system. In addition, the correlations between antioxidant activities and total phenolic content were also evaluated.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and reagents were of analytical grade. Gallic acid, 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-s-triazine (TPTZ), linoleic acid, Tween 40 (polyoxyethylenesorbitan monopalmitate), ascorbic acid, sodium carbonate anhydrous, chloroform, iron (III) chloride anhydrous, and potassium persulfate were purchased from Fisher Scientific (UK). Folin-Ciocalteu's phenol reagent, and 2,2'-azino-di[3-ethyl-benzthiazoline sulfonate] (ABTS) were from Merck (Germany). 2,2-diphenyl-1-picryhydrazyl (DPPH), β-carotene, butylated hydroxy anisole (BHA) and sodium acetate buffer (0.3 M) were purchased from Sigma-Aldrich (St. Louis, MO). Water used was of Millipore quality.

Preparation and extraction of edible wild mushroom extracts

The commercially cultivated edible mushroom, *P. ostreatus* (oyster mushroom) was purchased from a local market in Kuala Lumpur. The mushroom sample was washed, air dried and dried completely in an oven at 45°C for 24 hours. The dried samples were ground to powder using a miller (MF 10 basic; IKA® Werke, Germany) with 0.5mm mesh size and vacuum packaged into a nylon-linear low density polyethylene film by using vacuum packaging machine (DZQ 400/500) prior to analysis.

The powdered mushroom was extracted with water in a water bath shaker (Memmert, Germany) at 50°C for 330 minutes. This extraction time and temperature were

determined previously with total phenolic content, total flavonoid content and condensed tannins as determinant factors as reported elsewhere (Yim *et al.*, 2009). The crude extract was directly used for antioxidant activities and total phenolic analysis. The antioxidant activities were evaluated as relative activities against ascorbic acid and synthetic antioxidant, BHA.

DPPH radical scavenging activity

Radical scavenging activity by antioxidants in the mushroom extract was evaluated using DPPH radicals based on the method by Xu & Chang (2007) with slight modification. The DPPH• solution was prepared by dissolving 5.9 mg of DPPH• in ethanol (100 ml). An accurate amount of 3.8 ml of ethanolic DPPH• solution was added to 0.2 ml of mushroom extract. The mixture was shaken vigorously for 1 minute and left to stand at room temperature in the dark for 30 minutes. Absorbance was measured against the blank reagent at 517 nm (XTD 5, Secomam, UK). All determinations were carried out in triplicate. Radical scavenging activity was calculated according to the equation as follows:

$$Radical\ Scavenging\ Activity\ (\%) = \left[1 - \left(\frac{Abs_{sample}}{Abs_{control}}\right)\right] \times 100$$

ABTS radical cation scavenging activity

Determination of ABTS radical cation scavenging activity of mushroom extract was performed according to the method of Bilgari, AlKarkhi & Easa (2008) with some modifications. The ABTS•$^+$ reagent was prepared by mixing 5 ml of 7 mM ABTS•$^+$ solution with 88 μl of 140 mM potassium persulfate ($K_2S_2O_8$). The mixture was added into an amber bottle and kept in the dark at room temperature for 16 hours to allow the completion of radical generation. After 16 hours, 95% ethanol was used to adjust the absorbance of the ABTS•$^+$ reagent to 0.70 $\pm$ 0.05 at 734 nm (XTD 5, Secomam, UK). Approximately 1 ml of ABTS•$^+$ reagent was added to 10 μl of mushroom extract. The mixture was allowed to stand at room temperature for 6 minutes after the addition. Absorbance was measured against the blank reagent at 734 nm (XTD 5, Secomam, UK). All determinations were performed in triplicate. The radical scavenging activities were calculated according to the equation as follows:

$$Radical\ Scavenging\ Activity\ (\%) = \left[1 - \left(\frac{Abs_{sample}}{Abs_{control}}\right)\right] \times 100$$

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing activity of mushroom extract was estimated based on the method by Xu & Chang (2007) with slight modifications. The FRAP reagent was prepared by adding 2.5 ml of 10 mM TPTZ into 40 mM HCl. After dissolving TPTZ in HCl, 2.5 ml of 20 mM $FeCl_3 \cdot 6H_2O$ (ferric trichloride hexahydrate) was added followed by 25 ml of 0.3 M acetate buffer at pH 3.6.

The freshly prepared FRAP working reagent was warmed to 37°C. Then, approximately 3 ml of the FRAP reagent was added to 100 μl of mushroom extract and 300 μl of deionised water. The absorbance was measured at 593 nm against the blank (XTD 5, Secomam, UK) after 4 minutes. FRAP value was calculated and expressed as mM Fe^{2+} equivalent (FE) per 100 g sample using the calibration curve of Fe^{2+}. Linearity range of the calibration curve was 0.1–1 mM (R^2 = 0.99).

β-carotene-linoleate bleaching assay

The antioxidant activity of mushroom extract was determined by β-carotene-linoleate model system as described in Amarowicz *et al.* (2004) with slight modifications. Firstl, β-carotene solution was prepared by dissolving 0.2 mg of β-

carotene into 1 ml of chloroform. Then, 1 ml of the freshly prepared β-carotene solution was added to a round bottom flask containing 0.02 ml of linoleic acid and 0.2 ml of Tween 40. After evaporation of chloroform at 40°C (Rotary R-200, Buchi, Switzerland), 50 ml of oxygenated deionised water was added and the mixture was shaken vigorously to form an emulsion (β-carotene-linoleate emulsion).

An aliquot of 4.8 ml of the reagent mixture was added to 0.2 ml of mushroom extract. The mixtures were then vortexed vigorously to form a liposome solution, and the test tubes were allowed to incubate in the water bath (Memmert, Germany) at 50°C. The zero time absorbance (Abs_0) was measured at 470 nm (XTD 5, Secomam, UK). Then, the absorbance was taken at time intervals (Abs_t) of 20 minutes for 120 minutes. A blank was prepared with the same chemical as above except the β-carotene in chloroform as background subtraction. Every sample was extracted in triplicate. The bleaching rate (R) of β-carotene was calculated according to the equation as follows:

$$R = \ln\left[\frac{Abs_0}{Abs_t}\right] \div t$$

where, ln = natural log; Abs_0 = absorbance at time 0; Abs_t = absorbance at time t =20, 40, 60, 80, 100 and 120 minutes. The antioxidant activity (ANT) as percent inhibition rate of β-carotene bleaching relative to the control at 120 minutes, was calculated using the equation as follows:

$$ANT(\%) = \left[\frac{R_{control} - R_{sample}}{R_{control}}\right] \times 100$$

Total phenolic content (TPC) analysis

TPC analysis was performed using Folin-Ciocalteu method by Barros *et al.* (2007) with slight modifications. A 1 ml of sample was mixed with 1 ml of Folin-Ciocalteu's solution. After 3 minutes, 1 ml of 7.5% sodium carbonate solution was added to the mixture and adjusted to 10 ml with deionised water. The mixture was allowed to stand at room temperature in a dark environment for 90 minutes. Absorbance was measured against the blank reagent at 725 nm using (XTD 5, Secomam, UK). Gallic acid was used for the calibration curve with a concentration range of 50–1000 μg/ml (R^2 = 0.99) and analysed as above. Results were expressed as mg gallic acid equivalent (GAE)/100g sample. All experiments were performed in triplicate.

Statistical analysis

All analyses were performed in triplicate and averaged. Statistical analyses were conducted using MINITAB (Minitab Inc., State College, PA) version 14. One-way analysis of variance (ANOVA) with Tukey's multiple comparisons and Pearson's correlation coefficient were performed. Significance level was defined using $p < 0.05$.

RESULTS AND DISCUSSION

DPPH free radical compound has been widely used to test the free radical-scavenging ability of various food samples; the antioxidant present neutralises the DPPH• by the transfer of an electron or hydrogen atom. The colour changes from purple to yellow and the reduction capacity of DPPH• is determined by the decrease in its absorbance at 517 nm. The present study evaluated the DPPH• scavenging ability of the aqueous extract of *P. ostreatus* as relative activities against ascorbic acid and synthetic antioxidant, BHA.

The scavenging ability of DPPH• increases with increasing concentrations of ascorbic acid and BHA. Figure 1 shows that the scavenging ability of *P. ostreatus* (63.20 ± 0.24%) was significantly higher than the BHA at a concentration of 1000 μM (58.69 ±

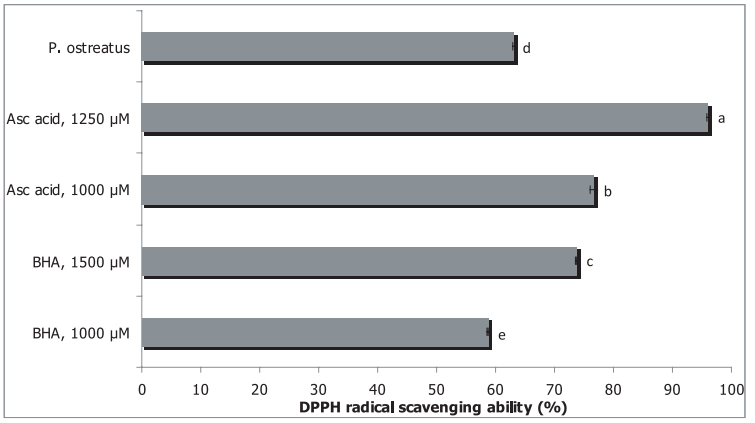

Figure 1. DPPH radical scavenging ability of *P. ostreatus* aqueous extract, BHA & ascorbic acid

Note: Bars with different letters are significantly different (p<0.05).

0.10%), but significantly lower when compared to ascorbic acid at concentrations of 1250 μM (95.98 ± 0.17%), 1000 μM (76.65 ± 0.56%) and BHA at a concentration of 1500 μM (73.74 ± 0.10%) (p<0.05). Such a high DPPH scavenging ability found in ascorbic acid, which is a potent antioxidant was expected and in agreement with Du *et al.* (2009) who found a highly significant linear correlation between vitamin C and DPPH scavenging ability in *Actinidia* fruit. Jayakumar *et al.* (2009) reported 56.2% hydroxyl radical-scavenging effect in *P. ostreatus* extract at a concentration of 10 mg/ml, but this was significantly lower than ascorbic acid (60.2%). However, according to the Malaysian Food Act (1983), the maximum safety limit of BHA in food system is at 200 ppm (equiv. 1100 μM); therefore, *P. ostreatus* could be considered as a good source of natural antioxidant in a food system when compared to the maximum safety limit of BHA.

The blue and green ABTS radical cation was generated through the reaction between ABTS and potassium persulfate. Radical formation prior to adding antioxidant-containing samples prevents interference, which may affect radical formation. When stable absorbance was achieved, the *P. ostreatus* aqueous extract was added and the scavenging ability measured in terms of decolorisation at 734 nm. The ABTS$^{\bullet+}$ scavenging ability of *P. ostreatus* aqueous extract was relatively high compared to ascorbic acid and BHA.

Figure 2 shows that the ABTS$^{\bullet+}$ scavenging ability of *P. ostreatus* (87.29 ± 0.54%) was significantly higher than that of ascorbic acid at concentrations of 1000 and 1500 μM (49.88 ± 0.17% and 73.11 ± 0.22%, respectively) and BHA at concentrations of 1000 and 1500 μM (48.64 ± 0.36% and 65.84 ± 0.17%, respectively) (p<0.05). The aqueous extract of *P. ostreatus* was found to possess better ABTS$^{\bullet+}$ scavenging ability when compared to BHA at a maximum safety limit of 200 ppm (equiv. 1100 μM) according to the Malaysian Food Act (1983). High ability to quench ABTS$^{\bullet+}$ was observed in high molecular weight phenolics such as tannins (Hagerman *et al.*, 1998), as well as catechins, pelargonin, leucopelargonin derivatives and flavonoids found in green tea (Manian *et al.*, 2008). Hence, it is suggested that the mushroom *P. ostreatus* may contain high molecular weight phenolics and can be considered as a good scavenger of radical cation.

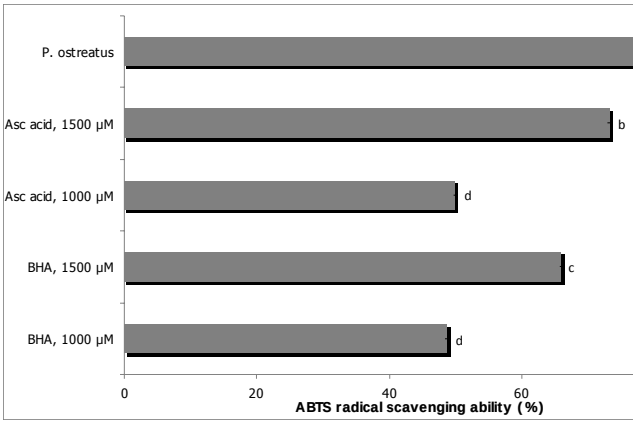

Figure 2. ABTS radical cation-scavenging ability of *P. ostreatus* aqueous extract, BHA & ascorbic acid
Note: Bars with different letters are significantly different (p<0.05).

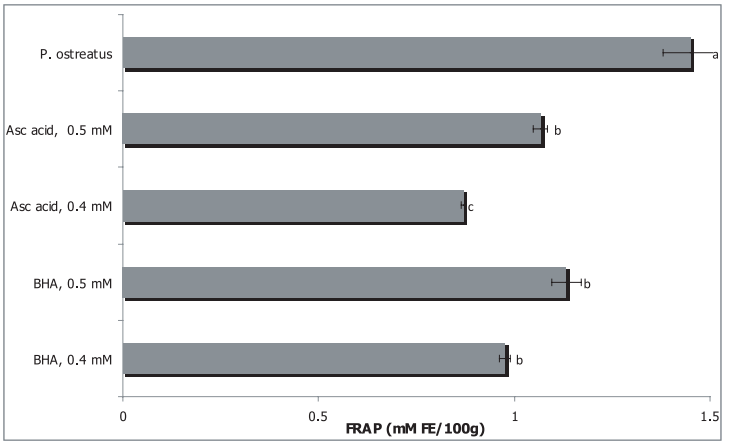

Figure 3. FRAP of *P. ostreatus* aqueous extract, BHA & ascorbic acid
Note: Bars with different letters are significantly different (p<0.05).

The principle of the ferric ion reducing power (FRAP) method is based on the reduction of a ferric-2,4,6-tri(2-pyridyl)-*s*-triazine [Fe (III)-TPTZ] complex to its ferrous 2,4,6-tri (2-pyridyl) -*s*-triazine [Fe(II)-TPTZ] complex coloured form in the presence of antioxidants. This complex has an intense blue colour that can be monitored at 593nm. The reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity. A higher absorbance indicates a higher ferric reducing power.

Figure 3 shows that the reducing power of *P. ostreatus* (1.45 ± 0.07 mM FE/100g) was significantly higher than that of BHA (0.98 ± 0.01 mM FE/100g; 1.13 ± 0.04 mM FE/100g) and ascorbic acid (0.87 ± 0.01 mM FE/100g; 1.07 ± 0.02 mM FE/100g) at 0.4 and 0.5 mM, respectively (p<0.05). Dastmalchi *et al.* (2007) reported that the reducing

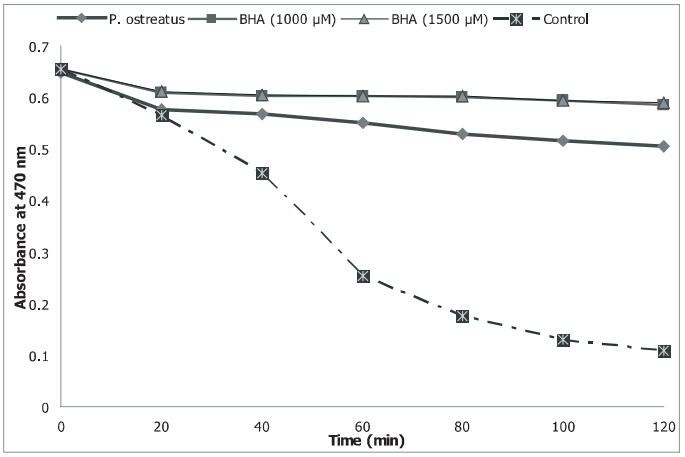

Figure 4. β-carotene bleaching rate of *P. ostreatus* aqueous extract, BHA & control

properties are generally associated with the presence of reductones, which could react with free radicals to stabilise and block radical chain reactions. The reducing power of wild edible mushrooms, namely *Leucopaxillus giganteus*, *Sarcodon imbricatus* and *Agaricus arvensis* reported by Barros *et al.* (2007), and *Russula delica* and *Verpa conica* reported by Elmastas *et al.* (2007) was found to be higher compared to natural and synthetic antioxidants, namely α-tocopherol, BHA and BHT. Jayakumar *et al.* (2009) found that the reducing power of ethanolic extract of *P. ostreatus* at a concentration of 10 mg/ml was better than that of BHT. In the present study, the same trend was observed, which may suggest that such reducing power in *P. ostreatus* might be attributed to the presence of dihydroxy type of benzene derivatives (Siddhuraju, 2007).

In the BCB assay, linoleic acid produced hydroperoxides as free radicals during incubation at 50°C. The loss of the yellow colour of β-carotene was due to its reaction with hydroperoxides. The rate of BCB can be slowed down with the presence of antioxidants. The rate of BCB measured at 470 nm of the *P. ostreatus* aqueous extract was compared with BHA at concentrations of 1000 μM and 1500 μM (Figure 4). The BCB rate of BHA at concentrations of 1000 μM and 1500 μM was stable indicating effectiveness of BHA in preventing degradation of β-carotene, followed by *P. ostreatus* in comparison with control which oxidised most rapidly (observed as steep bleaching rate).

The antioxidant activity (ANT), measured as inhibition of the BCB activity at 120 minutes, is shown in Figure 5. The ANT of *P. ostreatus* was found to be significantly lower than that of BHA at concentrations of 1000 μM and 1500 μM ($p<0.05$), corresponding to the BCB rate as shown in Figure 4. Ascorbic acid was not used as a relative control as it failed to show its strong antioxidant properties, which could be explained by 'polar paradox theory' as reported by Frankel (1996).

According to the 'polar paradox theory', a protective membrane could be formed around the oil droplets by non-polar lipophilic antioxidants (e.g. BHA), hindering the transport of free radicals across the emulsion droplet membrane. This, therefore, prevents oxidation from occurring within the oil droplets. On the other hand, the polar hydrophilic antioxidant (e.g.

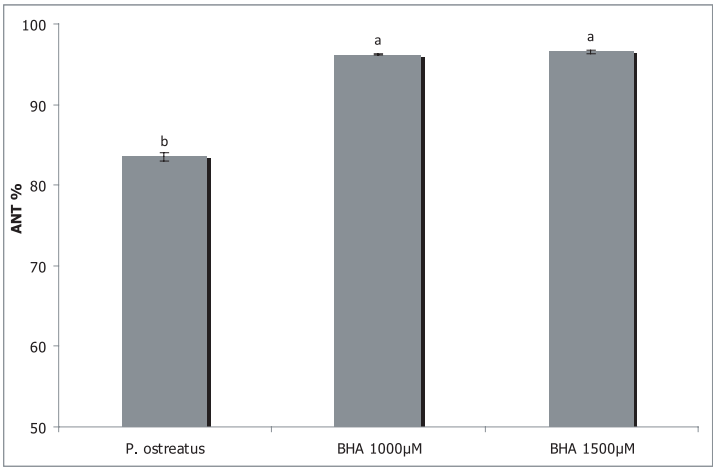

Figure 5. Antioxidant activity (% inhibition of BCB) of *P. ostreatus* aqueous extract & BHA.
Note: Bars with different letters are significantly different (p<0.05)

ascorbic acid) will be markedly diluted by moving to the water phase, as it reduces the protection of linoleic acid. Thus, a hydrophilic antioxidant will be more effective than a lipophilic antioxidant in bulk oil, whereas an lipophilic antioxidant shows greater antioxidant activity in emulsion condition such as the β-carotene-lenoleate emulsion system. A similar result was reported previously by Dastmalchi *et al.* (2007).

The correlation coefficient (r) between antioxidant activities and TPC of *P. ostreatus* aqueous extract was determined (Figure 6). The r between DPPH• scavenging ability (%) and TPC was 0.915 (Figure 6a), the r between ABTS•$^+$ scavenging ability (%) and TPC was 0.767 (Figure 6b), and the r between FRAP and TPC was 0.981 (Figure 6c). This may suggest that the phenolic contents present in the *P. ostreatus* contributed to its antioxidant activity. The result was consistent with other findings that reported such positive correlation between DPPH• scavenging ability (%) and TPC in mushrooms (Cheung *et al.*, 2003) as well as in other plants such as rambutan (Thitilertdecha, Teerawutgulrag & Rakariyatham, 2008) and bitter gourd (Kubola and Siriamornpum, 2008). The positive correlation between ABTS•$^+$ scavenging ability (%) was reported in other natural resources such as medicinal plants (Tawaha *et al.*, 2007), as well as in wine by Villaño *et al.* (2004). Elsewhere, as reported by Wojdyło *et al.* (2007) and Biglari *et al.* (2008), a positive correlation between FRAP and TPC was found on various herbs and date palms.

There was a positive correlation of ANT (as % inhibition of BCB) and TP (r = 0.851) found in the present study (Figure 6d), which might indicate that the extracted antioxidants could be mostly lipophilic antioxidants. This is because the TPC assay gives an indication of the levels of both lipophilic and hydrophilic compounds, whereas BCB assay only gives an indication of the levels of lipophilic compounds (Chew *et al.*, 2008). This suggests that *P. ostreatus* extract may have the potential to be used as antioxidative preservative in emulsion-type systems. Beside mushroom, such positive correlation between ANT and TPC has also been reported in red wines (Alen-Ruiz *et al.*, 2009).

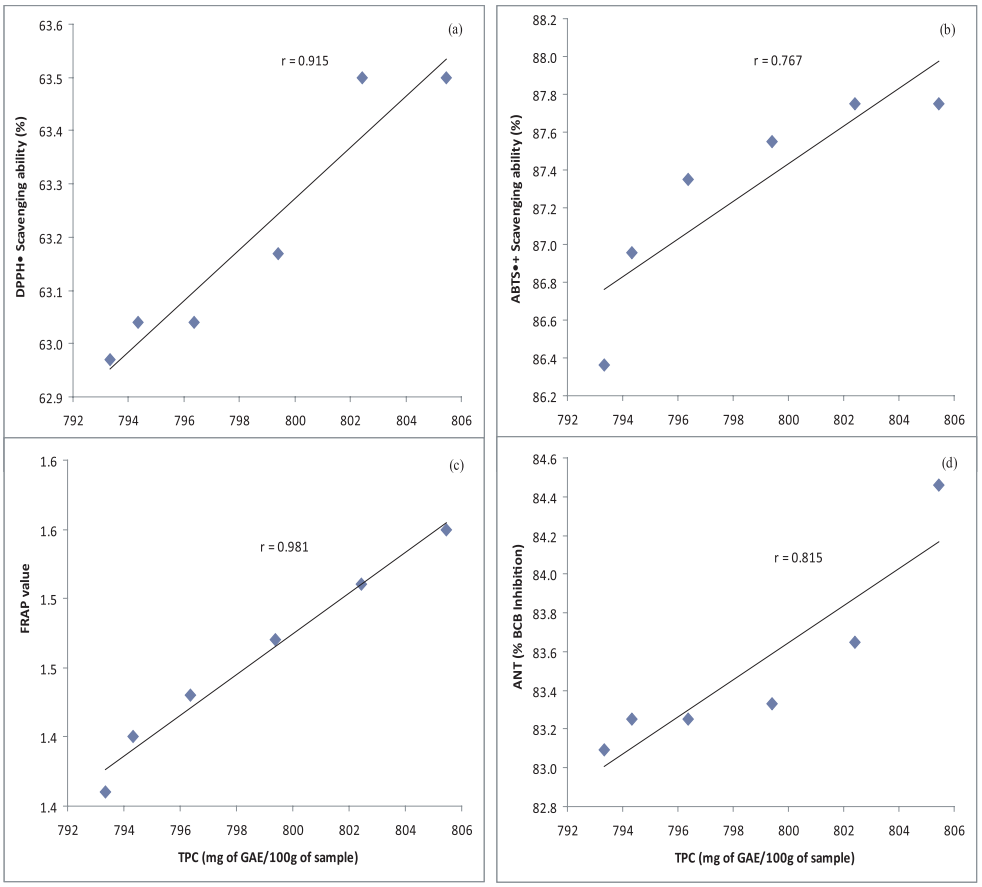

Figure 6. Correlations between TP and (a) DPPH• scavenging ability (%); (b) ABTS•$^{+}$ scavenging ability (%); (c) FRAP value; (d) antioxidant activity (as % inhibition of BCB) of *P. ostreatus* aqueous extract

CONCLUSION

P. ostreatus or oyster mushroom has long been cultivated commercially due to its economic value and its use as a food in Malaysia. *P. ostreatus* has the potential to be a source of antioxidant for the food industry and replace synthetic antioxidants because of its promising antioxidant capacity in comparison with BHA and ascorbic acid to a certain extent. The correlation results indicate that the antioxidant activity could possibly be due to the presence of phenolic compounds. However, other components such as polysaccharides and vitamins C and E present in *P. ostreatus* may also contribute to its antioxidant capacity.

ACKNOWLEDGEMENTS

The authors wish to thank the Malaysian Ministry of Higher Education, Universiti Malaysia Sabah and UCSI University for the financial and laboratory support.

REFERENCES

Alen-Ruiz F, Garcia-Flacon MS, Perez Lamela MC, Martinez-Carballo E & Simal-Gandara J (2009). Influence of major polyphenols on antioxidant activity in Mencia and Brancellao red wines. *Fd Chem* 113: 53-60.

Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B & Weil JA (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Fd Chem* 84(4): 551-562.

Barros L, Cruz T, Baptista P, Estevinho LM & Ferreira ICFR (2008). Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Fd Chem & Toxicol* 46(8): 2742-2747.

Barros L, Ferreira MJ, Queirós B, Ferreira ICFR & Baptista P (2007). Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Fd Chem* 103: 413-419.

Biglari F, AlKarkhi AFM & Easa AM (2008). Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. *Fd Chem* 107(4): 1636-1641.

Cheung LM, Cheung CK & Ooi EC (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Fd Chem* 81: 249-255.

Cheung LM & Cheung PCK (2005). Mushroom extracts with antioxidant activity against lipid peroxidation. *Fd Chem* 89(3): 403-409.

Chew YL, Lim YY, Omar M & Khoo KS (2008). Antioxidant activity of three edible seaweeds from two areas in South East Asia. *LWT-Fd Sc & Tech* 41(6): 1067-1072.

Cui Y, Kim DS & Park KC (2005). Antioxidant effect of *Inonotus obliquus*. *J Ethnopharmacol* 96: 79-85.

Dastmalchi K, Dorman HJD, Laakso I & Hiltunen R (2007). Chemical composition and antioxidative activity of Moldavian balm (*Dracocephalum moldavica* L.) extracts. *LWT-Fd Sc & Tech* 40(9): 1655-1663.

Du G, Li M, Ma F & Liang D (2009). Antioxidant capacity and the relationship with polyphenol and vitamin C in *Actinidia* fruits. *Fd Chem* 113: 557-562.

Elmastas M, Isildak O, Turkekul I & Temur N (2007). Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *J Fd Comp & Anal* 20(3-4): 337-345.

Frankel EN (1996). Antioxidants in lipid foods and their impact on food quality. *Fd Chem* 57(1): 51-55.

Guerra-Dore CMP, Azevedo TCG, de Souza MCR, Rego LA, de Dantas JCM, Silva FRF, Rocha HAO, Baseia IG & Leite EL (2007). Antiinflammatory, antioxidant and cytotoxic actions of β-glucan-rich extract from *Geastrum saccatum* mushroom. *Int Immunopharmacol* 7(9): 1160-1169.

Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW & Riechel TL (1998). High molecular weight plant polyphenolics (Tannins) as biological antioxidants. *J Agric & Fd Chem* 46(5): 1887-1892.

Jayakumar T, Thomas PA & Geraldine P (2009). *In-vitro* antioxidant activities of an ethanolic extract of the oyster mushroom, *Pleurotus ostreatus*. *Innov Fd Sc & Emerg Tech* 10: 228-234.

Kubola J & Siriamornpun S (2008). Phenolic contents and antioxidant activities of bitter gourd (*Momordia charantia* L.) leaf stem and fruit fraction extracts in vitro. *Fd Chem* 110(4): 881-890.

Malaysia Food Act (1983). Food Safety and Quality Division, Ministry of Health Malaysia.

Manian R, Anusuya N, Siddhuraju P & Manian S (2008). The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. *Fd Chem* 107: 1000-1007.

Manzi P, Gambelli L, Marconi S, Vivanti V & Pizzoferrato L (1999). Nutrients in edible mushrooms: an inter-species comparative study. *Fd Chem* 65(4): 477-482.

Mau JK, Chang CN, Huang SJ & Chen CC (2004). Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. *Fd Chem* 87(1): 111-118.

Naveena BM, Sen AR, Vaithiyanathan S, Babji Y & Kondaiah N (2008). Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. *Meat Sc* 80(4): 1304-1308.

Siddhuraju P (2007). Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. *LWT-Fd Sc & Tech* 40(6): 982-990.

Tawaha K, Alali FQ, Gharaibeh M, Mohammad M & El-Elimat T (2007). Antioxidant activity and total phenolic content of selected Jordanian plant species. *Fd Chem* 104(4): 1372-1378.

Thitilertdecha N, Teerawutgulrag A & Rakariyatham N (2008). Antioxidant and antibacterial activities of *Nephelium lappaceum* L. extracts. *LWT-Fd Sc & Tech* 41(10): 2029-2035.

Villaño D, Fernández-Pachón MS, Troncoso AM & García-Parrilla MC (2004). The antioxidant activity of wines determined by the ABTS•+ method: influence of sample dilution and time. *Talanta* 64(2): 501-509.

Wojdyło A, Oszmiañski J & Czemerys R (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Fd Chem* 105(3): 940-949.

Xu BJ & Chang SKC (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *J Fd Sc* 72: S159-S166.

Yim HS, Chye FY, Ho SK & Ho CW (2009). Phenolic profiles of selected edible wild mushrooms as affected by extraction solvent, time and temperature. *As J Fd & Agro-Ind* 2(3): 371-380.