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# Antioxidant analysis of different types of edible mushrooms (*Agaricus bisporous* and *Agaricus brasiliensis*)

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**Abstract**

Consumption of mushroom has increased remarkably because of their desirable aroma, taste and high nutritional content. This study was undertaken to measure and compare the antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC) of Agaricus bisporous (white button mushroom) and Agaricus brasiliensis (Brazilian mushroom) in aqueous and 60% ethanol extract. Results showed that button mushroom (21.47 ± 0.48 mg GAE/g of dry weight) had significant higher TPC in aqueous whereas Brazilian mushroom (12.50 ± 0.22 mg GAE/g of dry weight) had significant higher TPC in 60% ethanol ($p< 0.05$). In terms of TFC, Brazilian mushroom had higher content than button mushroom in both types of solvents. For FRAP assay, Brazilian mushroom had significantly higher total antioxidant activity than the button mushroom in 60% ethanol ($p < 0.05$) but opposite trend with aqueous. For DPPH radical scavenging activity, Brazilian mushroom (60% ethanol) had the lowest EC50 value, followed by button mushroom (60% ethanol), Brazilian mushroom (aqueous) and button mushroom (aqueous). Pearson correlation test ($p < 0.05$) showed strong positive correlation between TPC and FRAP assay in both extracts ($r = 0.969$ for 60% ethanol extract; $r = 0.973$ for aqueous extract). For TFC, there was a strong positive, correlation with FRAP assay ($r = 0.985$) in aqueous extract. In conclusion, high antioxidant activity in ethanol extract of mushrooms due to presence of phenolic content can potentially be used as a source of natural antioxidants.

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## Introduction

Oxidation is essential for the production of energy to fuel biological processes in many living organisms. The recent changes in lifestyle or environmental factors such as pollution, radiation, cigarette smoke and herbicides can generate free radicals among people worldwide. Thus, this can cause further oxidative stress responsible for DNA, protein, membrane damage and contribute to current non-communicable diseases (NCDs). Although antioxidant defense and repair systems are available in humans and other organisms to protect them against oxidative damage, these systems are insufficient tototally prevent the damage (Mau *et al*., 2002).

There are many epidemiological studies suggest that consumption of polyphenol-rich foods and beverages is associated with a reduced risk of cardiovascular diseases, stroke and certain types of cancer in which polyphenol is linked to the antioxidant properties (Barros *et al*., 2007; Jagadish *et al*., 2009). The consumption of dietary antioxidants will help to prevent free radical damage. According to Olajire and Azeez (2011), antioxidants have the ability to scavenge free radicals by inhibiting the initiation step or interrupting the propagation step of oxidation of lipid and as preventive antioxidants which slow the rate of oxidation by several actions.

Mushrooms are widely consumed and have been valued as an edible and medical resource. Many studies have found that some species of mushrooms are having therapeutic properties such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immuno stimulatory effects (Barros *et al*., 2007; Oyetayo, 2009).They accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids (Turkoglu *et al*., 2007).

The Basidiomycete fungus *Agaricus brasiliensis* (*A. blazei*), known as "sun mushroom" is a medicinal mushroom. They are popularly known as 'Cogumelo do Sol' in Brazil and 'Himematsutake' in Japan. They are native to Brazil and have been traditionally used as a health food source in Brazil. In the mid-1960s, spores of *A. blazei* were taken to Japan for commercial cultivation and research (Hetland *et al*.,

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2008). It has been reported that 200,000 to 400,000 kg of the dried body of *A. blazei* is produced every year in Japan (Kimura, 2005). Most research was focused on the therapeutic effects of these medicinal mushrooms, but little information is available about their antioxidant properties (Mau *et al*., 2002).

*Agaricus bisporous* (*A. bisporous*) is commonly known as button or table mushroom. It is an edible Basidiomycete fungus which grown naturally in Europe and North America. It is low in calorie with purine, carbohydrate and sodium content as well as a high content of several vitamins, potassium, phosphorus and some trace elements exist (Savoie *et al*., 2008). To date, most studies have been carried out on the therapeutic effects rather than antioxidant properties of white button mushroom. There is also low availability of Brazilian mushroom in the local hypermarkets. Moreover, consumption of mushrooms such as oyster, shiitake and enoki mushroom have been utilized for a long time in Malaysia compared to white button mushroom and Brazilian mushroom in the public.

According to Sultana, Anwar and Ashraf (2009), the nature of extraction solvent and varying chemical characteristics and polarities of antioxidant compounds can result in different extraction yields and antioxidant activities of the plant. In present study, two different types of extraction solvents (60% ethanol and aqueous) were selected to determine their effects on antioxidant compounds from selected mushrooms.

The findings from the study can be used to disseminate the information regarding antioxidant properties to the public to raise their awareness against non-communicable diseases. Comprehensive studies on the antioxidant properties of mushroom for these two types are not available locally. So, the findings from this study can act as a baseline reference for further research. Thus, this study was to determine the total antioxidant activity, total phenolic and flavonoid content in aqueous and ethanol extract from *A. bisporus* (white button) and *A. brasiliensis* (Brazilian) mushroom.

## Materials and Methods

### *Standards and reagents*

Ethanol (60%, v/v) was purchased from Fisher Scientific (Leicestershire, UK), Deionized distilled water ($ddH_2O$), distilled water was prepared with Favorit W4L water distillation system from Genristo (Nottingham, UK). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), sodium acetate ($C_2H_3NaO_2 \cdot 3H_2O$), acetic acid, 2, 4, 6-tripyridyl-s-triazine (TPTZ), hydrochloric acid (HCl), ferric chloride ($FeCl_3 \cdot 6H_2O$), ferrous sulfate ($FeSO_4 \cdot 7H_2O$), aluminium trichloride ($AlCl_3$), sodium nitrite ($NaNO_2$) were purchased from Sigma Chemical Co. (St. Loius, MO, USA). Folin-Ciocalteu reagent, sodium carbonate, gallic acid, ascorbic acid, sodium hydroxide (NaOH) and quercetin were purchased from Sigma-Aldrich (Steinheim, Germany).

### *Food sampling*

The selection of samples was based on convenience sampling. In this study, white button mushroom (*A. bisporus*) was purchased from a local hypermarket in Selangor, Malaysia while Brazilian mushroom (*A. brasiliensis*) was purchased in a dried form, at a local Chinese medicine shop.

### *Samples preparation*

Every sample comprises complete mushroom fruiting bodies (cap, gills, tubes and stipe) of different sizes. The fruiting bodies of mushroom were cleaned and washed to remove any residual compost by using distilled water. These samples were cut into pieces and stored at -80°C. Then, they were lyophilized using freeze-dryer to remove the moisture content. After freeze-drying, the dried samples were ground into fine powder by using micro-grinder and kept in plastic bag prior to analysis. Due to dried form of Brazilian mushroom may not need a drying step, samples were ground and kept in plastic bag. The samples were kept in airtight container at -20°C prior to analysis.

### *Sample extraction*

Extraction was performed according to the method of Yang *et al*. (2002) with some modifications. Five grams of each powdered sample was extracted with 100 ml of ethanol. The mixture was placed in a conical flask (wrapped with an aluminium foil) and agitated at 200 rpm with orbital shaker for 1 hour at 25°C. The extract was then separated from the residue by filtration through Whatman No. 1 filter paper. The remaining residue was re-extracted twice, and then the two extracts were combined. The residual solvent of ethanol extracts was removed under reduced pressure at 40°C using a rotary evaporator to dryness. For aqueous extraction, the aqueous extract was lyophilized using a freeze dryer. Both of the dried extract was used directly for all analysis.

### *Determination of total phenolic content*

Phenolic compounds in the sample extracts was estimated by using Folin-Ciocalteu assay, based on procedures described by Singleton and Rossi (1965)

and Olajire and Azeez (2011). One milliliter of sample from aqueous or ethanol extract was mixed with 1 ml of Folin and Ciocalteu's phenol reagent (1:9; Folin-Ciocalteu reagent: distilled water). After 5 min, 1 ml of 13% sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min and its absorbance was read at 725 nm. A calibration curve was constructed with different concentrations of gallic acid as standard. The results were expressed as mg of gallic acid equivalents (GAE) per gram of extract.

***Determination of flavonoid compound***

The $AlCl_3$ method was used for determination of the total flavonoid content of the sample extracts according to the method described by Jagadish *et al*. (2009) with some modifications. An aqueous or ethanol extracts (1.5 ml) was mixed with 5 ml of deionized distilled water ($ddH_2O$) and 0.3 ml of 5% $NaNO_2$. After five min incubation at room temperature, 1.5 ml of 2% Aluminium trichloride ($AlCl_3$) solution was added. Two milliliters of 1M NaOH was added after the next 6 min. The mixture was vigorously shaken on orbital shaker for 5 min at 200 rpm and the absorbance was read at 510 nm against a blank. Quercetin with different concentrations was used as a standard.

***Determination of total antioxidant activity***

*Ferric reducing antioxidant power (FRAP) assay*

The FRAP assay was done as described by Benzie and Strain (1996) and Huang *et al*. (2005). The oxidant in the FRAP assay was prepared by mixing TPTZ (2.5 ml, 10 mM in 40 mM HCl), 25 ml of 300 mM acetate buffer, and 2.5 ml of $FeCl_3 \cdot H_2O$ (20 mM). The combination of all these reagents was referred to as "FRAP reagent". An amount of 1.8 ml freshly prepared FRAP reagent was taken in a test tube and incubated at 30°C in water bath for 10 min. Then, absorbance was taken at 0 min ($t_0$). Immediately, 100 µl of sample extract or standard and 100 µl of distilled water was added to the test tube, mixed and incubated at 30°C for 30 min. Then, the absorbance was taken at 593 nm ($t_{30}$). Ferrous sulfate was used as standard. The antioxidant potential of the sample extract was determined against a standard curve of ferrous sulphate and the FRAP value was expressed as µM $Fe^{2+}$ equivalents per gram of extract and calculated using the following equation:

FRAP value = Absorbance (sample + FRAP reagent) – Absorbance (FRAP reagent)

*DPPH radical scavenging activity method*

The scavenging activity of mushrooms was estimated according to the procedure described by Shimada *et al.* (1992) with some modifications. Firstly, an aliquot of 0.5 ml of sample extracts at different concentrations were added to test tubes with 2.9 ml of 200 µM DPPH radical in ethanol. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. The reaction mixture was determined at 515 nm with UV-vis spectrophotometer. Extraction solvent was used as blank while mixture without extract served as control. Ascorbic acid was used as a standard. The scavenging effect was calculated based on the following equation:

Scavenging effect (%) = 1- [(Absorbance sample/ Absorbance control) x 100]

$EC_{50}$ value (mg/ml) was defined as the total antioxidant needed to decrease the initial DPPH free radicals by 50%. It was determined from the plotted graph of scavenging activity against various concentrations of the sample extracts.

***Statistical analysis***

All analyses were performed in triplicate. The data were recorded as means ± standard deviations and analyzed by using Statistical Package for Social Sciences (SPSS version 20.0). The mean values of antioxidant activities between two types of extraction or two types of sample were analyzed by independent-samples t-test. Pearson correlation coefficient was used to determine the relationship between the antioxidant activity, total phenolic and flavonoid content in sample extracts. Differences between means at 5% ($P < 0.05$) level were considered significant.

## Results and Discussion

*Sample preparation and extraction*

Lyophilization was used to give the samples uniform moisture removal and ensure the products for analysis in similar form. Solvent extraction is most frequently used technique for extraction of plant antioxidant compounds. There are no single solvents that are capable to extract all the antioxidant compounds and optimally for all type of samples. In present study, two different types of extraction solvents (60% ethanol and aqueous) were selected to determine their effects on antioxidative compounds from selected mushrooms. 60% of ethanol was used instead of absolute ethanol because extraction yield

will be increased significantly through a mixture of organic solvent with water (Pezente Ferrari *et al.*, 2012).

*Total phenolic content*

When comparison was made between two different types of extraction from Table 1, aqueous extract of both types of mushrooms had a significant higher phenolic content than 60% ethanol extracts. This study was supported by Hip *et al*. (2009) in which their findings had showed that water contributed to the highest yield of total phenolic content in mushrooms under the same extraction condition. On the other hand, contrary results were found on a study done by Mohammedi and Atik (2011).

Through ethanol extraction, Brazilian mushroom has significant higher phenolic content in comparison with button mushroom ($p < 0.05$). The total phenolic content of the samples analyzed in the present study exhibit a higher phenolic content than those reported by Geosel *et al*. (2010) which reported that significantly higher total polyphenolics in all *A. blazei* cultivars than in *A. bisporous* varieties. The higher phenolic concentration observed for Brazilian mushroom can be partially explained by its low water content due to dehydration. The differences in response between different mushroom extracts were due to large variation in the physicochemical properties of polysaccharides, such as sugar composition, molar weights, and structures. Besides, tyrosinase, an enzyme present in mushroom responsible for postharvest browning attribute to the low phenolic content in button mushrooms (Rai and Arumuganathan, 2008).

With regard to aqueous extracts, button mushroom contained higher amount of total phenolic content compared to Brazilian mushroom ($p < 0.05$). Generally, total phenolic content in button mushroom analyzed in the present study was much higher reported in other studies. The phenolic content in a study by Giannenas *et al*. (2011) was 8.85 mg GAE/g of dry weight, while the range in a literature by Dubost *et al*. (2007) was 8.00-10.65 mg GAE/g of dry weight.

*Total flavonoid content*

Table 1 also showed the total flavonoid content of the samples with different extraction solvent. Relating to the ethanolic extraction, flavonoid content in Brazilian mushroom was significantly higher than the button mushroom ($p < 0.05$). The low content of flavonoid content in the button mushrooms studied maybe due to the maturation stages of fruiting bodies. A study reported by Barros *et al.* (2007a) stated that the stages where the fruiting bodiespresented immature spores (stage I and II) revealed a higher content in phenol and flavonoid compounds. For aqueous extract, the result showed the same trend with total flavonoid content in ethanol extract. According to Geosel *et al*. (2010), the low molecular weight antioxidants such as Vitamin A and flavonoids are able to prevent different types of sickness. However, in button mushroom, there was no significant difference of flavonoid content between both solvent extractions as the p value is more than 0.05 in independent t-test analysis.

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenols. A higher content of flavonoid was found in ethanol extract compared to aqueous extract. These results are consistent with previous report. A report by Yeh *et al*. (2011) stated that flavonoid content in ethanol extract of Grifolafrondosa has been found to have significantly higher than cold and hot water extract. This is because the groups such as phenolic acids, lignans, flavonoids with structures containing –OH and –COOH functional groups are easily extracted by the polar solvent in samples. When compared to natural spices and herbs such as rosemary, oregano, and caraway, the flavonoid content in both types of mushrooms were higher than them. The total flavonoid content of the spices varied from 324.08 µg quercetin equivalent (QE)/g for thyme to 3.38µg QE/g for coriander (Kim *et al.*, 2011). Thus, flavonoid compounds in plant are important phytochemical that is present in the human diet.

*Ferric ion reducing antioxidant power assay (FRAP assay)*

Based on table 2, Brazilian mushroom aqueous extract showed significantly higher total antioxidant activity than 60% ethanol extract ($p < 0.05$). Same pattern also occurred in button mushroom. This showed that aqueous extract had better antioxidant capacities than ethanol extract in both types of mushrooms. Both types of solvent extraction were chosen because the antioxidant capacities of plant samples may be influenced by a lot of factors, such as the extraction solvent and test systems used (Fu *et al*., 2010). In addition, it is important to use different types of solvent extraction as natural antioxidants are multifunctional. This was shown through the present result reported that button mushroom exhibited significant higher antioxidant activity in aqueous while Brazilian mushroom showed significant higher antioxidant activity in ethanol solvent.

The total antioxidant activity of the samples

**Table 1.** Summary of all results for mushroom types in different solvent extraction

| Solvents | Samples | Total phenolic content (mg quercetin equivalents/ g of dry weight) | Total flavonoid content (mg gallic acid equivalents/ g of dry weight) |
|---|---|---|---|
| 60% Ethanol | Brazilian | 12.50 ± 0.22b | 5.36 ± 0.20c |
| | Button | 10.25 ± 0.22a | 1.75 ± 0.26a |
| Aqueous | Brazilian | 15.79 ± 0.25c | 3.37 ± 0.17b |
| | Button | 21.47 ± 0.48d | 1.36 ± 0.11a |

Each value is expressed as means ± standard deviation (n = 3). Means with different letters are significantly different within each assay at the level of $p < 0.05$.

**Table 2.** Summary of all results for mushroom types in different solvent extraction

| Solvents | Samples | Ferric Reducing Antioxidant Power Assay (μmol $Fe^{2+}$ equivalents/ g dry weight) | DPPH radical scavenging assay ($EC_{50}$ value, mg/ml) |
|---|---|---|---|
| 60% Ethanol | Brazilian | 107.24 ± 2.19b | 1.67 ± 0.21a |
| | Button | 84.69 ± 3.07a | 2.77 ± 0.25b |
| Aqueous | Brazilian | 158.77 ± 2.8c | 3.80 ± 0.72c |
| | Button | 186.72 ± 2.84d | 4.57 ± 0.40c |

Each value is expressed as means ± standard deviation (n = 3). Means with different letters are significantly different within each assay at the level of $p < 0.05$.

analyzed in the present study were within the range reported by Alvarez-Parrilla *et al*. (2007) except ethanol extract of button mushroom (84.69 μmol $Fe^{2+}$ equivalents/ g dry weight). High antioxidant activities in mushrooms can suppress active oxygen species, which are related to aging and diseases (Yang *et al*., 2002). They also might be developed into functional foods or drugs in the future.

*DPPH free radical scavenging assay*

Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. Scavenging effects of ethanol extracts from mushrooms on DPPH radical increased with the increased concentrations. According to the Table 2, Brazilian mushroom showed higher radical scavenging activity which was 85.44% compared to button mushroom which was 82.39% in ethanol extracts when tested at the highest concentration (25 mg/ml). In general, the scavenging activity of the Brazilian mushroom was higher than that of button mushroom in the concentration range tested. The radical scavenging activity of ethanol extract of Brazilian was better than that of button mushroom probably due to higher content of phenolic compounds and dietary fibers (Abdullah *et al*., 2011).

The scavenging activity of mushroom extracts towards DPPH free radicals was also expressed in terms of $EC_{50}$. A lower $EC_{50}$ value indicates stronger ability of the extracts to act as DPPH radical scavengers. There was no significant difference in $EC_{50}$ value between Brazilian and button mushroom in aqueous extract. The $EC_{50}$ value for Brazilian mushroom was lower compared with button mushroom in both types of solvent extraction. Both samples were reported to show good antioxidant activities as evidenced by $EC_{50}$ value in the range of 1.67 mg/ml to 4.57 mg/ml as compared to other types of mushrooms. It has been reported that the $EC_{50}$ of culinary-medicinal mushrooms such as *Pleurotus flabellatus*, *Lentinula edodes* and *Termitomyces heimii* were 17.857 mg/ml, 19.093 mg/ml and 26.839 mg/ml respectively (Abdullah *et al*., 2011).

Besides, the results also showed that ethanol extract of both mushrooms possessed higher scavenging activity than aqueous extract. This was in agreement with the study done by Govindappa *et al*. (2011). However, the findings were in contrast with the findings found by Alnajar *et al.* (2012). The difference scavenging effect among solvent extraction was due to that free radical scavenging activity is species-dependent. Besides, most the scavenging activities were probably due to light sensitivity of the DPPH radical although with variations of extracts (Masalu *et al*., 2012).

*Correlation between total phenolic content, flavonoid content and antioxidant activity*

From the Pearson correlation tests in table 3, there were a very strong positive and significant ($p < 0.05$) correlation found between total phenolic content and FRAP assay in both extracts ($r = 0.969$ for 60% ethanol extract; $r = 0.973$ for aqueous extract). In $EC_{50}$ of DPPH assay, there was a very strong negative and significant correlation ($p < 0.05$) occur in 60% ethanol extract ($r = -0.939$) but no correlation was found in aqueous extract. It might be feasible to suggest that phenolics do not act as the major antioxidant

**Table 3.** Pearson's correlation coefficient between total phenolic content, FRAP assay and DPPH free radical scavenging assay ($EC_{50}$)

| Total Phenolic content | | Pearson correlation | |
|---|---|---|---|
| | | FRAP | $EC_{50}$ |
| 60% ethanol | r value | 0.969** | -0.939** |
| | p value | 0.001 | 0.005 |
| Aqueous | r value | 0.973** | 0.654 |
| | p value | 0.001 | 0.159 |

**Correlation is significant at the 0.05 level (2-tailed)*

components in the aqueous extracts. There might be possibility of other active constituents, which are nonphenolic in nature that can be extracted in water extracts. According to Chye *et al.* (2008) and Perez-Jimenez and Saura-Calixto (2006), the presence of non-antioxidant compounds especially amino acids and uronic acids in the test solutions may produce higher antioxidant capacity to that produced by the polyphenols alone.

Besides, table 4 displayed that the total flavonoid content in 60% ethanol extract showed a very strong positive and significant ($p < 0.05$) correlation with FRAP assay ($r = 0.985$) but a very strong negative and significant ($p < 0.05$) correlation with FRAP assay in aqueous extract ($r = -0.984$). The positive and highly significant relationship between this effective compound with antioxidant activity indicates that flavonoid plays a major role in the antioxidant activity of mushrooms. The compounds such as flavonoids, which hold hydroxyls groups, are responsible for the radical scavenging activity in the plants (Mohamed Imran *et al*., 2011). There was a negative and significant correlation ($p < 0.05$) found betweenn $EC_{50}$ and total flavonoid content in 60% ethanol extract ($r = -0.933$) but no correlation was found between them in aqueous extract. The negative linear correlations obtained in this study means sample with the highest flavonoid content shows higher antioxidant activity and lower $EC_{50}$ values. This was in accordance to the findings by several authors who reported that total flavonoid content was negatively correlated with the $EC_{50}$ of the edible mushroom Leucopaxillus giganteus (Barros *et al.*, 2007b). The moderate to high scavenging effects of medicinal mushrooms might be associated with some antimutagenic properties (Mau *et al*., 2002).

**Table 4.** Pearson's correlation coefficient between total flavonoid content and FRAP assay and DPPH free radical scavenging assay ($EC_{50}$)

| Total Flavanoid content | | Pearson correlation | |
|---|---|---|---|
| | | FRAP | $EC_{50}$ |
| 60% ethanol | r value | 0.985** | -0.933** |
| | p value | 0.000 | 0.007 |
| Aqueous | r value | 0.984** | -0.592 |
| | p value | 0.000 | 0.216 |

**Correlation is significant at the 0.05 level (2-tailed)*

## Conclusions

A relationship between the $EC_{50}$ of DPPH scavenging activity, FRAP assay, phenolic and flavonoid was established among different types of mushrooms and solvent extraction. In brief, the antioxidant activity was exhibited to a certain extent are probably due to other antioxidant components present in these mushroom extracts besides the phenolic compound and also depends on solvents used. This also indicates that phenolic compounds extracted might cover from moderate polarity to low polarity due to different antioxidant activity. This study suggests that high antioxidant activity in ethanol extract of mushrooms can potentially be used as a source of natural antioxidants due to presence of phenolic compounds since mushrooms are readily available and acceptable to the public. Further investigations on the isolation and purification of the active components from crude extracts of mushrooms can be done by using high performance liquid chromatography (HPLC).

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