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# Antioxidant and Antihyperglycemic Activities of Four Edible Lentinus Mushrooms

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## Abstract

Four Lentinus mushrooms: *Lentinus edodes*, *Lentinus polychrous, Lentinus sajor-caju,* and *Lentinus squarrosulus* were investigated for their chemical compositions as well as their antioxidants and hyperglycemic activities. The chemical composition of Lentinus mushrooms contained total phenolic and total flavonoid contents, normally used as an indirect measurement of antioxidant capacity. Scavenging activity on DPPH radicals and FRAP assays were used for confirming antioxidant activity while assays of α-glucosidase inhibitory activity were used for evaluating antihyperglycemic activity in these mushroom extracts. The highest amount of total phenolic content (TPC) displayed in the extract of *L. squarrosulus* which correlated well with antioxidant properties of mushroom extracts indicated by DPPP and FRAP assays. It is firstly reported herein on α-glucosidase inhibitory activity in Lentinus mushrooms, in which *L. polychrous* revealed the highest inhibitory activity ($IC_{50}$ value 39.59±0.01 μg/mL) and correlated well with total flavonoid compound.

**Keywords:** α-glucosidase inhibitor, *Lentinus edodes*, *Lentinus polychrous, Lentinus sajor-caju L. squarrosulus*, antihyperglycemic, antioxidation

## 1. Introduction

Nowadays, mushrooms are not only intended to satisfy consumers and provide essential nutrients, but also to prevent disease and improve their physical and mental well-being. Edible mushrooms characteristically contain many different bioactive compounds such as polysaccharide, amino acid, glycolipid, terpenoid, and so on with highly diverse biological activities like anticancer, antibacterial, antifungal and immunomodulatory potential [1, 2]. Some advantages of using edible mushrooms over plants as sources of bioactive compounds are that mushrooms can be produced in much less time, and can be manipulated to produce optimal quantities of active compounds [3-5]. Apart from the research of potent α- glucosidase inhibitors from alternative sources such as fungi and others microorganisms, Lentinus mushrooms which were identified as rich sources of nutrients and popular consumption in Thailand were selected for this study. Four Lentinus mushrooms: *L. edodes*, *L. polychrous, L. sajor-caju* and *L. squarrosulus* belong to Family Polyporaceae, are recognized as many as 40 species over the world [6,7]. Chemical composition and the potential of antioxidant activity of the four Lentinus mushrooms and the effect of extract on the activity of α–glucosidase were investigated in this study.

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## 2. Materials and Methods

### 2.1 Materials

All four Lentinus mushrooms which have fully cap of fruiting body or were in harvest stage were cultivated in local mushroom farm in Amphor U-thai, Phranakhon Si Ayutthaya, Thailand and preserved in cellophane bags with air tight conditions. Taxonomic identification was done following the description of Pegler [8]. Mushroom specimens were deposited at Herbarium of Science and Technology Center, Phranakhon Si Ayutthaya Rajabhat University, Thailand.

### 2.2 Extraction

The fruiting bodies of these four edible Lentinus mushrooms (500 g) were collected, cleaned and air-dried before grinding and soaking in the extraction solvent for 24 h. Each mushroom was stirred with 80% ethanol (250 mLx4) at 25°C for 24 h, and then filtered through Whatman filter paper No.4. The ethanol extract was then evaporated under reduced pressure to yield ethanolic extracts. The dried extracts were resuspended in ethanol and stored at 4 °C for analyses of total flavonoid contents and α-glucosidase.

### 2.3 Total phenolic compound analysis

The total phenolic contents of the extract were determined using a modified version of the Folin-Ciocalteu method [9]. The extract (0.1 ml) was mixed with 50 µl of 2 N Folin Ciocalteu reagent, and allowed to stand for 3-5 min at room temperature before adding 0.3 ml of 20% $Na_2CO_3$ to the mixture. After leaving for 15 min at room temperature, 1 ml of distilled water was added. The absorbance was measured at 725 nm using a UV-spectrometer. Total phenolic was quantified by calibration curve obtained from measuring the absorbance of gallic acid standard. The concentration was expressed as mg of gallic acid equivalents per gram of extract [10].

### 2.4 Total flavonoid contents analysis

The total flavonoid content of the extract was determined according to the colorimetric method as described by Moreno *et al* [11]. Each extract (0.5 ml) was added to test tubes containing 0.1 ml of 10% $Al(NO_3)_3$ (w/v), 1 M of $CH_3COOK$ and 4.3 ml of 80% ethanol. After incubation for 40 min at room temperature, the absorbance was determined at 415 nm. The total flavonoid content was expressed in milligrams of quercetin equivalents (QE) per gram of extract.

### 2.5 DPPH assay

The DPPH (2, 2-diphenylpicrylhydrazyl) assay [12] was used to determine the free radical scavenging activity of mushroom extracts. Each mushroom extract in ethanol was mixed with 1 ml of methanolic solution containing DPPH radicals, resulting in a concentration of 0.2 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in dark condition, and the absorbance was then measured at 517 nm. The DPPH free radical scavenging activity was expressed in the percentage inhibition which was calculated by $[(A_0-A_1)/A_0] \times 100$, where $A_0$ is the absorbance without the sample, and $A_1$ is the absorbance with the sample.

### 2.6 FRAP assay

The reducing power was recorded while FRAP (Ferric reducing antioxidant power assay) was determined according to the method described by Benzie and Strain [13] with some modifications by Jeong *et al*. [14]. The stock solutions included 300 mM acetate buffer, pH 3,6,10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution included 40 mM HCl, 20 mM TPTZ solution, and 2.5 ml $FeCl_3.6H_2O$ solution and was warmed at 37 °C. The mushroom tested sample was then mixed with 0.5 ml extracts and allowed to react with FRAP solution for 30 min in dark condition. The colored product (Ferrous

tripyridyltriazine complex) was undertaken at 539 nm absorbance. The standard curve was linear between 25 and 800 nM Trolox (6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid). The results were expressed in mM TE (Trolox equivalent antioxidant capacity) perform of dried mushrooms.

### 2.7 α-Glucosidase assay

An inhibitory effect against α-glucosidase (from baker's yeast) was performed using the modified protocol previously reported [15-17]. Briefly, the α-glucosidase (0.1 U/mL) and substrate (1 mM *p*-nitrophenyl-α-D-glucopyranoside) were dissolved in 0.1 M phosphate buffer, pH 6.9. A 10 μL of test compound (1 mg/mL in DMSO) was incubated with 40 μL of α-glucosidase at 37 ºC for 10 min. A 50 μL substrate solution was then added to the reaction mixture and incubated at 37 ºC for additional 20 min. The reaction was terminated by adding 100 μL of 1 M $Na_2$ $CO_3$. Enzymatic activity was quantified by measuring the absorbance at 405 nm (Bio-Red microplate reader model 3550 UV). The percentage inhibition was calculated by $[(A_0-A_1)/A_0] \times 100$, where $A_0$ is the absorbance without the sample, and $A_1$ is the absorbance with the sample. The $IC_{50}$ value was determined from a plot of percentage inhibition versus sample concentration. Acarbose was used as a standard control and the experiment was performed in triplicate.

### 2.8 Data Analysis

The concentrate of tested compounds required to inhibit 50% of the α-glucosidase activity under the assay conditions was determined from dose-response curves and defined as $IC_{50}$ value. Each value means SD of triplicate determinations. Mean separation within columns was done by Duncan's multiple range test at 0.1% level and significant differences of these data were calculated using analysis of variance (ANOVA).

## 3. Results and Discussion

The ethanolic extract yields from four Lentinus mushrooms were 3.11±0.001, 4.45±0.003, 3.20±0.004 and 1.99±0.006 mg/g dried weight mushroom in *L. edodes*, *L. polychrous, L. sajor-caju* and *L.squarrosulus*, respectively, as shown in Figure 1. The extract yields were in agreement with the finding of previous report by Attarat and Phermthai [4].

The ethanolic extracts of Lentinus mushrooms were analyzed for the total phenolic content (TPC) and total flavonoid content (TFC). Phenolic and flavonoid exhibited a wide range of biological effects such as antioxidation, antibacterial, antiinflammatory and antihyperglycemic. In this study, TPC used as indirect testing of antioxidant capacity in Lentinus mushrooms extracts. Total phenolic and flavonoid compounds in Lentinus mushrooms extracts are presented in Table 1.

The extract of *L. squarrosulus* contained the highest amount of TPC at 300.5±0.6 mg GAE/g. The finding was very similar to that of previous report [4] and for TFC, it was firstly reported in Lentinus mushrooms with *L. Polychrous* presenting the highest amount of TFC at 120.9±0.2mg QE/g. In addition, TPC of mushroom extract was also used as an indirect measure of antioxidant capacity that was further revised by different assays as DPPH and FRAP assays. Thus, the antioxidant properties as percentage inhibition of DPPH of Lentinus mushroom extracts were showed in Figure 2.

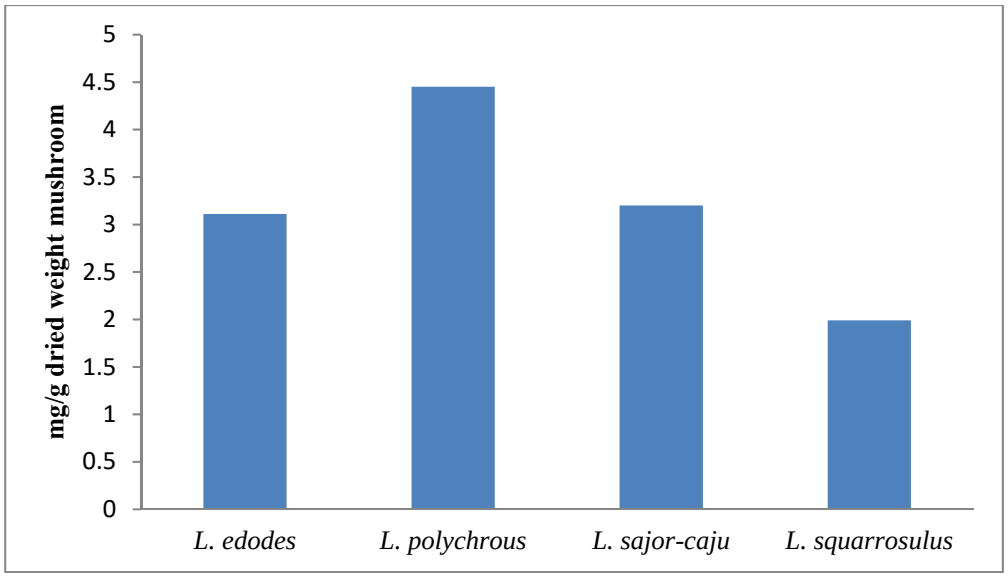


**Figure 1**. The ethanolic extract yields from four Lentinus mushrooms

**Table 1**. Total phenolic and total flavonoid contents in extracts of Lentinus mushrooms

| Species | TPC* (mg GAE/g) | TFC** (mg QE/g) |
|---|---|---|
| *L. edodes* | 140.1±0.2c | 62.8±0.1c |
| *L. polychrous* | 120.1±0.1d | 120.9±0.2a |
| *L.sajor-caju* | 196.0±0.3b | 112.2±0.4b |
| *L. squarrosulus* | 300.5±0.6a | 19.18±0.6d |

*Total phenol content analysed as gallic acid equivalent mg/g of extract, values are averages of triplicates.
**Total flavonoid content analysed as quaercetin equivalent mg/g of extract, values are averages of triplicates.
Note Different letters within the same column means significantly different ($P \leq 0.1$)

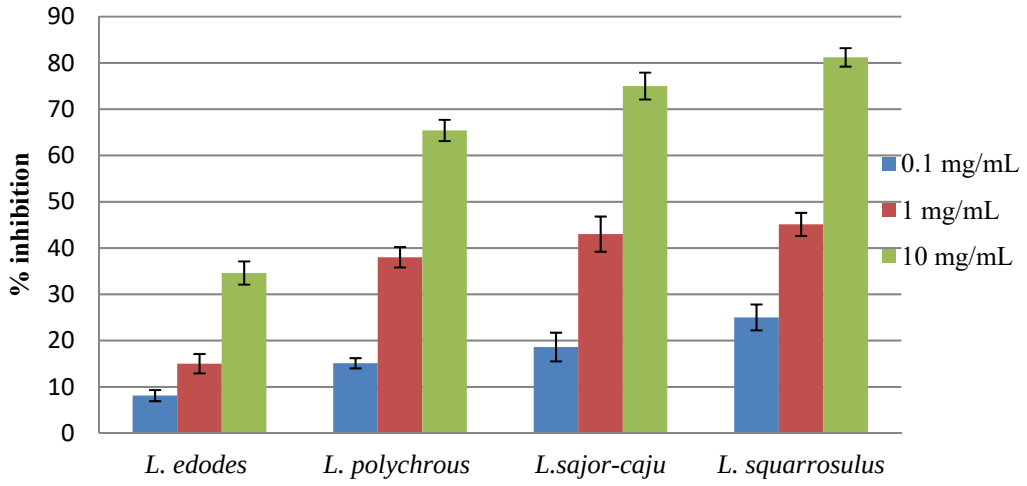


**Figure 2**. Percentage inhibition of DPPH radical of Lentinus mushrooms

Among the Lentinus mushroom extracts, *L. squarrosulus* showed the highest percentage inhibition of DPPH in all concentrations which were dose-dependent inhibition. At a concentration of 1.0 mg/mL the percentage inhibition of DPPH of *L. polychrous, L. sajor-caju and L. squarrosulus* was about 38, 43 and 45.1%, respectively.

The FRAP assay was expressed by comparison of micromolar of trolox equivalent per gram dried mushroom as shown in Figure 3. The extract of *L. squarrosulus* showed 94.1 μM TE/g dried mushroom suggesting that it was more potent antioxidant than other extracts. This data was correlated with TPC and DPPH assays.

Additionally, the antihyperglycemic properties were evaluated in Lentinus mushroom extracts which were carried out by α-glucosidase inhibitory activity as shown in Table 2. Acarbose was used as the positive control in the treatment of diabates millitus as α-glucosidase inhibitor. The extracts of *L. polychrous and L. squarrosulus* revealed α- glucosidase inhibitory activities with $IC_{50}$ 39.59±0.01 and 46.24±0.02 at 10 mg/mL while the extracts of *L. edodes* and *L. sajor-caju* revealed α- glucosidase inhibitory activities less than 30%.

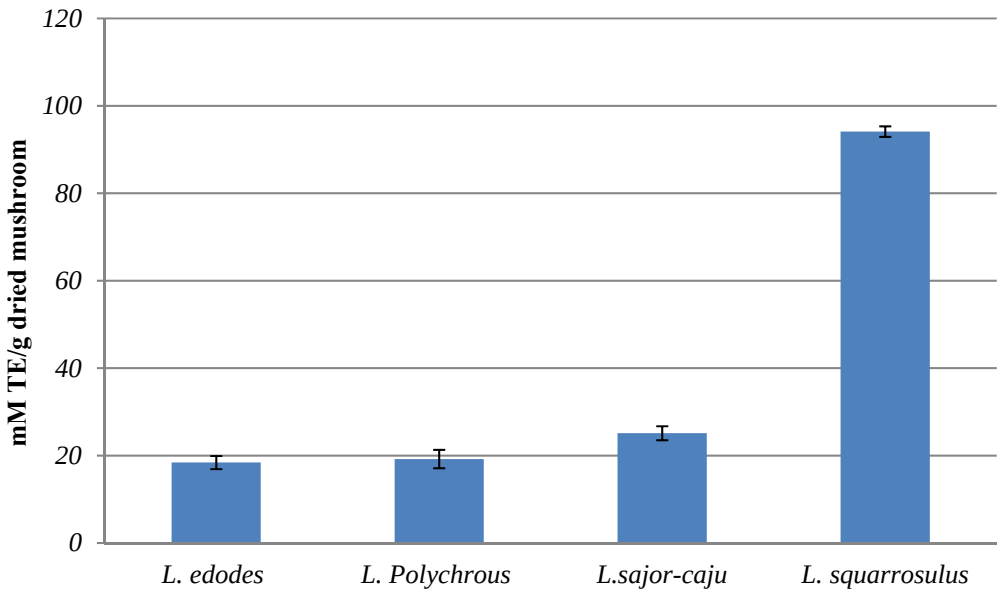


**Figure 3**. FRAP assay, expressed in μM Trolox equivalent antioxidant capacity

**Table 2**. Inhibitory activity of α- Glucosidase in Lentinus mushrooms

| Extract | $IC_{50}$ (μg/mL)* |
|---|---|
| *L. edodes* | NI |
| *L. polychrous* | 39.59±0.01 |
| *L. sajor-caju* | NI |
| *L. squarrosulus* | 46.24±0.02 |
| Acarbose | 52.29 |

*NI= No inhibition, inhibitory effect less than 30% at 10 mg/mL.

## 4. Conclusions

Four Lentinus mushrooms: *L. edodes*, *L. polychrous L.* sajor-caju and *L. squarrosulus* contained high amount phenolic compounds which were antioxidants that correlated well with DPPH and FRAP assays. Both assays used a similar mechanistic basis, the electrons were transferred from antioxidant in order to reduce an oxidation reaction. However, the evaluation of these antioxidants has to be confirmed by other assays. This research firstly reported on α- glucosidase inhibitory activity in Lentinus mushrooms, in which *L. polychrous* gave the highest inhibitory activity with $IC_{50}$ value of 39.59±0.01 μg/mL. Two mushrooms, *L. polychrous* and *L. squarrosulus* were highly effective in antioxidation and antihyperglycemia. In addition, the highest total flavonoid contents of *L. Polychrous* showed highest α-glucosidse inhibitory activity, indicating the correlation of flavonoids to α-glucosidase activity. Therefore, the richness of bioactive components in these mushrooms make them appropriate for functional food and nutritional supplements.

## 5. Acknowledgements

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