

The Antioxidant in Local Edible Mushrooms : Upper Northeastern Thailand

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ABSTRACT

The antioxidant was studied in ten local edible mushrooms in Kalasin Province : Hed Koh (*Russula* sp.), Hed Phuang (*Boletus* sp.), Hed Pluak Khao Door (*Termitomyces* sp.), Hed Ra Ngoke Khai (*Amanita* sp.), Hed Khlai (*Russula delica* Fr.), Hed Teen Plok (*Lentinus sajor-caju*), Hed Mun Phor Leaug (*Cantharellus* sp.), Hed Kradang (*Lentinus polychrous* Lev.), Hed Taan (*Russula* sp.) and Hed Had (*Lactarius* sp.). The samples were oven dried at 40°C and extracted with methanol. The crude extract was examined antioxidant by DPPH assay method and using BHT (2,2'-diphenyl-1-picrylhydrazyl) as the standard substance, then measure the absorbance at 517 nm with spectrophotometer. It was found that Hed Koh, Hed Phuang, Hed Ra Ngoke Khai, Hed Teen Plok, Hed Kradang and Hed Taan had $EC_{50} = 10.73 \mu\text{g/ml}$, $8.89 \mu\text{g/ml}$, $6.23 \mu\text{g/ml}$, $9.65 \mu\text{g/ml}$, $5.93 \mu\text{g/ml}$ and $3.08 \mu\text{g/ml}$, respectively lower than BHT, $EC_{50} = 13.57 \mu\text{g/ml}$. It was showed that they had higher antioxidants. The others had lower antioxidant : Hed Had, Hed Pluak Khao Door, Hed Khlai and Hed Mun Phor Leaug had $EC_{50} = 15.41 \mu\text{g/ml}$, $18.31 \mu\text{g/ml}$, $20.17 \mu\text{g/ml}$ and $16.48 \mu\text{g/ml}$, respectively.

Keywords: DPPH assay method , antioxidants , Edible mushroom

INTRODUCTION

Mushroom is classified as low-class life in a fungus family. Mushrooms have only a cell wall, but no pipes to convey water and food (Vascular Bundle). They are breed by spore. They have no Chlorophyll so they can not photosynthesize to create food for themselves. Most are living as a Saprophytic (Mushroom, 2009), degradation from remains of living things. There are 2 kinds of mushrooms: Edible Mushroom and Poisonous Mushroom. Poisonous Mushrooms will severe physical distress-including vomiting, diarrhea, cramps and loss of coordination (Edible Mushroom and Poisonous Mushroom, 2009). Although the symptoms of poisoning from these mushrooms may be alarming, they usually pass in 24 hours or less with no lasting effects. You should, however, notify your doctor immediately if you suspect mushroom poisoning of any kind. Edible mushrooms are found in every region of Thailand. Thai people use them as food and herb for long time. There are many edible mushrooms were found in Thailand, especially in upper northeastern

region which people often use them as their favorite dishes almost every meal. Some mushrooms are from forests, rain forest mountain range such as Phupan Mountain Range, they are very delicious and called “Hed-Pa”. The mushrooms are such as Hed Khon Khao (*Clitocybe sp.*), Hed Poa (*Astreaus hygrometricus*) and Hed Taptao (*Boletus edulis Bull.*) etc., They have many nutritious and necessary elements such as protein, vitamin, calcium and antioxidant etc. for our bodies.

An antioxidant is a molecule capable of decreasing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals (Superoxide anion radical (O_2^{\bullet}), Hydroxyl radical (HO^{\bullet}), Peroxy radical (ROO^{\bullet}), Oxy radical (RO^{\bullet}), Hydrogen peroxide (H_2O_2), Hydrogen radical (H^{\bullet}), Methyl radical (CH_3^{\bullet})) (<http://www.thaiclinic.com/antioxidant.html>, 2009.), which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves.

Simpler assay on antioxidative activity with DPPH Radical Scavenging Assay. DPPH (2,2'-diphenyl-1-picrylhydrazyl) (Bondet *et al.*, 1997). are free and stable radical (Figure 1), can receive electron or hydrogen atom from other molecule, the element has changed to be non-radical (DPPH:H, Figure 2)

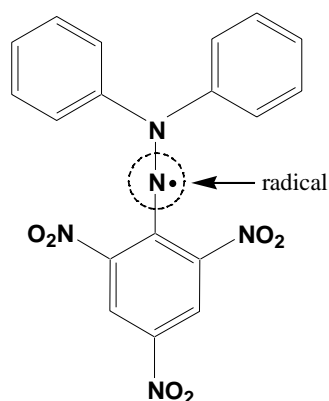


Figure 1 DPPH

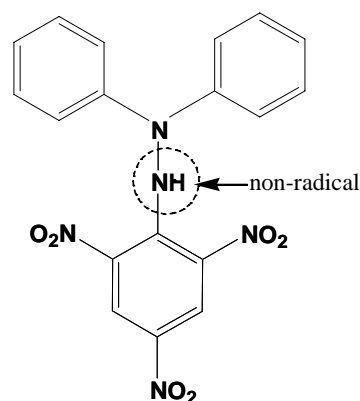


Figure 2 DPPH:H

Free radicals are atomic or molecular substances contain one or more unpaired electrons in an outer valence shell. Molecules of free radicals are written with a point raised (\bullet) on the front or back of an atom or molecule. These unpaired electrons are usually highly reactive (figure 3). Radical scavengers are antioxidants : a substance that is not inhibited or restrained reaction to free radicals occurs (Radical Scavengers, 2009). Inhibit chain reaction of free radicals (figure 4). Passenger resistance to free radicals trap free radicals that damage cells and stop reaction and reduce cancer, cardiac ischemia etc.

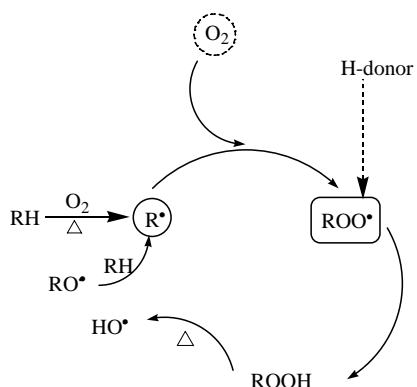


Figure 3 Free radicals

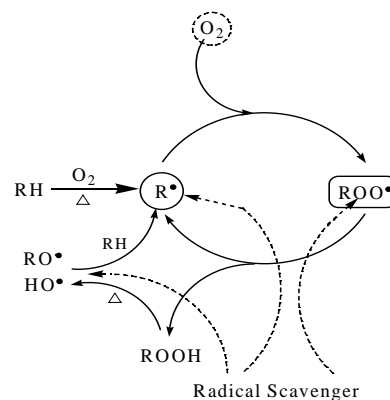


Figure 4 Radical scavenger

Not only the worth of antioxidant in local edible mushrooms including nutritious meals and medicine. As people living in upper northeastern region of Thailand, the mushrooms are not only our foods, but our valuable ancestor’s knowledge. So, this research had paid attention to study the local edible mushrooms which are found in our area.

METHODOLOGY

Materials

Ten samples of edible local mushrooms were collected from Kalasin Province, Thailand during May – August 2008. These are Hed Had, Hed Koh, Hed Teen Plok, Hed Pluak Khao Door, Hed Kradang, Hed Khlai, Hed Ra Ngoke Khai, Hed Taan, Hed Phuang and Hed Mun Poo Lueng. The samples were oven dried at 40 °C in 24 hour and crush in the blender. Powder samples were extracted with methanol (MeOH) 1:20 (w/v) at room temperature for 3 days. After that filter them by vacuum filtration for getting crude extract, then take the extract to determinate antioxidant by DPPH assay method.

DPPH assay method

The sample solution 1.562, 3.125, 6.25, 12.5, 25, and 50 and µg/ml, 1.0 ml in methanol added 0.2 mM of DPPH (2,2'-diphenyl-1-picrylhydrazyl). After reaction in darkness for 30 minutes measured the absorbance at 517 nm with spectrophotometer. Percentage of radical scavenging was calculated from equation (1) and determined effective concentration at 50 percent (EC₅₀) from the graph between Percentage of radical scavenging and concentration of sample solution

$$\% \text{Radical Scavenging} = [(1 - A_{\text{Sample}}) / A_{\text{Control}}] \times 100 \dots \dots \dots (1)$$

Control : 1 mL of methanol mixed with 1 mL of 0.2 mM DPPH solution.

RESULTS AND DISCUSSION

1. Percentage of crude extract

From the study of antioxidant potential of methanol crude extract from ten edible local mushrooms found that Hed Khlai had the highest percentage in Crude MeOH = 17.89 % and Hed Pluak Khao Door, Hed Koh, Hed Phuang, Hed Had, Hed Teen Plok, Hed Mun Poo Lueng, Hed Ra Ngoke Khai, Hed Taan and Hed Kradang, 17.01, 15.31, 14.59, 14.44, 13.75, 12.47, 10.73, 8.08 and 7.29 % respectively (Table 1).

Table 1 Percentage of Crude MeOH on sample

Local Name	Science name	%Crude MeOH
Hed Had	<i>Lactarius</i> sp.	14.44
Hed Koh	<i>Russula</i> sp.	15.31
Hed Teen Plok	<i>Lentinus sajor-caju</i>	13.75
Hed Pluak Khao Door	<i>Termitomyces</i> sp.	17.01
Hed Kradang	<i>Lentinus polychrous</i> Lev.	7.29
Hed Khlai	<i>Russula delica</i> Fr.	17.89
Hed Ra Ngoke Khai	<i>Amanita</i> sp.	10.73
Hed Taan	<i>Russula</i> sp.	8.08
Hed Phuang	<i>Boletus</i> sp.	14.59
Hed Mun Poo Lueng	<i>Cantharellus</i> sp.	12.47

Organic solvents are used to extract various kinds, such as methanol, ethanol, water, etc. The Crude Extracts of Mushroom *Phellinus linteus* comparing activity between crude methanol and ethanol extracts showed that crude methanol extract displayed radical scavenging activity significantly higher than crude ethanol extracts (Samchai. *et al*, 2009). While the crude chloroform extracts had the highest inhibitory effect which completely inhibited seed germination of the *P. setosum* (Swartz.) L.C. Rich than hexane and methanol (Wirat *et al*, 2001).

2. Percentage of Radical Scavenging

When combining antioxidant with DPPH, dark purple DPPH had reacted with antioxidant for 30 minutes, the color of DPPH solution changed into yellow, which could measure an absorbance at 517 nm by spectrophotometer after calculated percentage of radical scavenging from equation (1) are shown in Figure 5 and Figure 6. Effective concentration at 50 percent (EC_{50}) values of crude extract were determined from the graph between percentage of radical scavenging and concentration of sample solution are shown in Figure 7. Six mushrooms which were Hed Koh, Hed Teen Plok, Hed Kradang, Hed Ra Ngoko Khai, Hed Taan and Hed Phuang had EC_{50} = 10.73 $\mu\text{g/ml}$, 9.65 $\mu\text{g/ml}$, 5.93 $\mu\text{g/ml}$, 6.23 $\mu\text{g/ml}$, 3.08 $\mu\text{g/ml}$ and 8.89 $\mu\text{g/ml}$, respectively lower than BHT, it means the higher antioxidant. The others : Hed Had, Hed Pluak Khao Door, Hed Khlai and Hed Mun Phor Leung had EC_{50} = 15.41 $\mu\text{g/ml}$, 18.31 $\mu\text{g/ml}$, 20.17 $\mu\text{g/ml}$ and 16.48 $\mu\text{g/ml}$, respectively, higher than BHT it means the lower antioxidant.

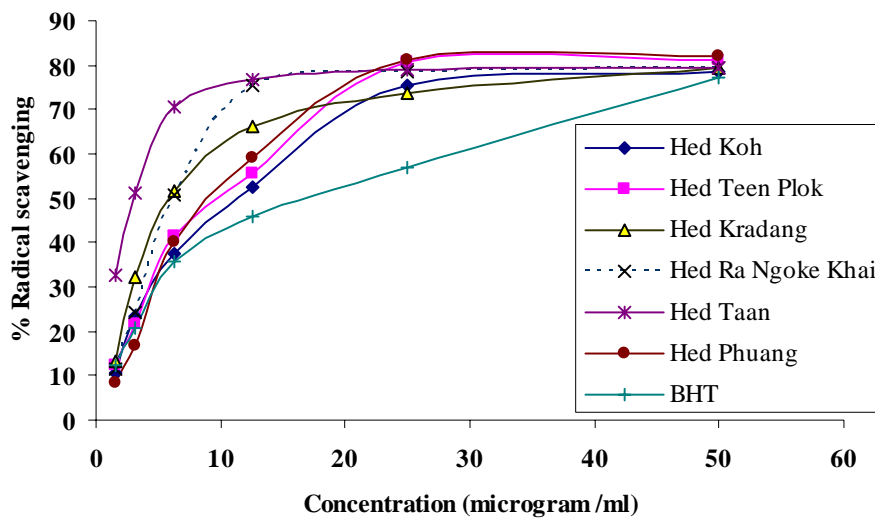


Figure 5 Percentage of radical scavenging of six higher antioxidant compared with BHT

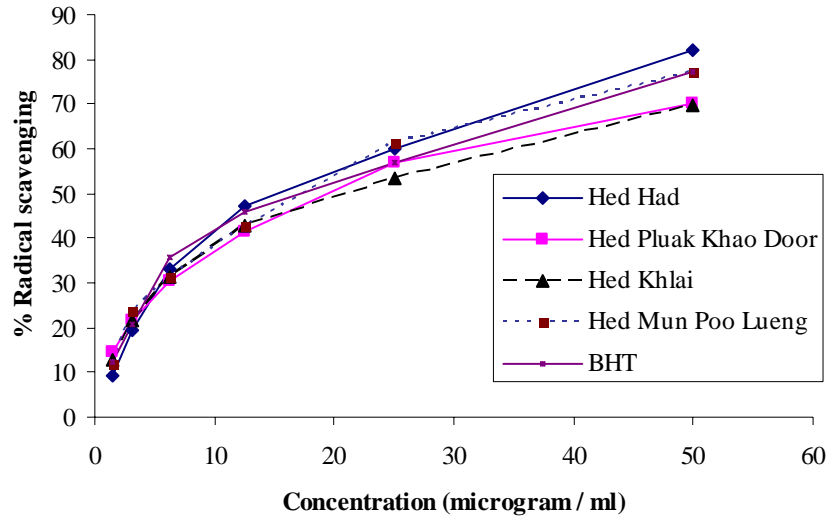


Figure 6 Percentage of Radical Scavenging of four lower antioxidant compared with BHT

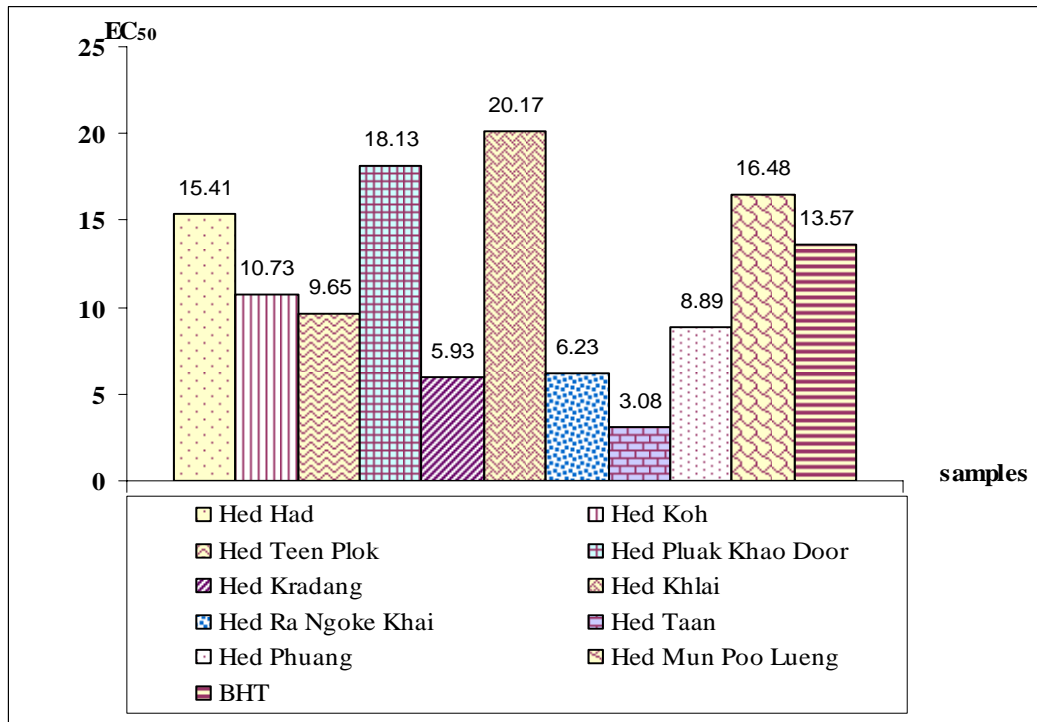


Figure 7 Effective concentration at 50 percent (EC₅₀, μg/ml) of samples compared with BHT

There are several mushroom that are antioxidant: Hed Tabtao (*Boletus edulis* Bull), Hed Poe (*Astreaus hygrometricus* (Pers.) Morg), Hed Lom (*Lintinus praerigidus* Brek), Hed Khon Khao (*Clitocybe sp.*) Hed Krang (*Commune* Fr.) (Trakoontivakorn and Saksitpitak, 2000). Not only the worth of antioxidant in local edible mushrooms including nutritious meals and medicine such as Hed Lom had higher calcium, vitamin B1 and Iron, and Hed Tabtao had higher Iron (Jiramongkholkarn, 2004). In Japan, Hed Krang (local name is “suehirtake”) found polysaccharide and can be inhibited the progressess of the tumor (Mizuno, 1995). Also several indigenous vegetables that are antioxidant; Ya Nang (*Tilacora triandra* (Colebr.) Diels), Phak Kratung Maba (*Dregea volubilis* (L.f) Hook.f.), Phak Mun Pla (*Fagraea fragrans* Roxb.), Phak Mad (*Zanthoxylum limonella* (Dennst.) Alston), Phak Som Moung (*Garcinia cowa* Roxb. ex DC.), Phak Seang (*Xanthophyllum lanceatum* (Miq.) J.J. Sm.) (Sirival, 2008). The potential of Thai indigenous vegetables could heal, protect diseases and keep healthy along the report of antitumor promoting activities research in 112 Thai eatable plants found that 34% had higher antitumor potential (Trakoontivakorn and Saksitpitak, 2000).

CONCLUSIONS

The local edible mushroom in the upper Northeastern Thailand were extracted with methanol and examined antioxidant by DPPH assay method. Six mushrooms had higher antioxidant : Hed Taan, Hed Kradang, Hed Ra Ngoke Khai, Hed Phuang, Hed Teen Plok and Hed Koh, had $EC_{50} = 3.08, 5.93, 6.25, 8.89, 9.65$ and $10.73 \mu\text{g/ml}$, respectively. Four mushrooms had lower antioxidant : Hed Had, Hed Pluak Khao Door, Hed Khlai and Hed Mun Phor Leaung had $EC_{50} = 15.41 \mu\text{g/ml}, 18.31 \mu\text{g/ml}, 20.17 \mu\text{g/ml}$ and $16.48 \mu\text{g/ml}$, respectively. So, it is useful for Consumption mushroom as food due to higher antioxidant, Calcium, Vitamin B1 and Iron etc. Also, Thai edible mushrooms and local vegetables has the antioxidants that help to prevent degenerative diseases such as heart disease, cancer, arthritis and stroke etc. The study can be another eating alternative for good health and can apply for more products and values in industries such as drug production. If we can prove and acknowledgement about the value of these Thai edible mushrooms for good health, the demand of the mushrooms will be increase and the producers are confident to promote their selling so many related researchs will come.

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