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Determination of Antioxidant Activity of *Cantharellus cibarius* Fr.

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Oxidative stress is a general term used to describe the steady state level of oxidative damage in a cell. It can affect a specific molecule or the entire organism. Reactive oxygen species (ROS), such as free radicals and peroxides exist inherently in all aerobic organisms. Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods by free radicals. These antioxidants bind and inactivate the free radicals plant and mushrooms are good antioxidant materials. In present study, antioxidant activitiy of *Cantharellus cibarius* Fr. determined. Total phenolic concentration of the extracts was estimated with Folin-Ciocalteu reagent using gallic acid as standard. Free radical scavenging activities were determined based on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). Results were compared with standard butylated hydroxytoluene and butylated hydroxyanisole.

Key Words: Oxidative stress, *Cantharellus cibarius*, 1,1-Diphenyl-2-picrylhydrazyl radical, Folin-Ciocalteu.

INTRODUCTION

Antioxidants are of great importance in terms of preventing oxidative stress that may cause several degenerative diseases[1]. Many plants are potentially useful for decreasing the risks of several chronic diseases, such as coronary heart disease and some cancers[2-4]. These protective effects have been particularly attributed to various antioxidant compounds, such as vitamin C and E, β-carotene and flavonoids[5]. Mushrooms have long been appreciated for their flavour and texture. They are now recognized as a nutriatious food as well as an impotent source of biologically active compounds of medicinal value[6]. Medicinal mushrooms have an established history of use in traditional oriental therapies. Modern clinical practice in Japan, China, Korea and other Asian countries continue to rely on mushroom-derived preparations. Mushrooms have been used for many years in oriental culture as tea and nutritional food and because of their special fragrance and texture[7]. *Cantharellus cibarius* Fr. is a well-known and

extraordinary mushroom species found in Turkey. It grows on soil in hardwood and conifer forests from the Black Sea region. It has been known by the collector as "Cuce kiz" and sold from the open market during the collection season.

EXPERIMENTAL

The mushroom samples were collected from Ordu region of Turkey in 2006. The identification and classification of macrofungus were carried out by mycologist Dr. Hasan Huseyin Dogan and all specimens were deposited at the laboratory of Department of Biology, Selcuk University, Konya, Turkey. 1,1-Diphenyl-2-picrylhydrazine, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), methanol, Folin reagent, (Sigma-Aldrich), Na_2CO_3, gallic acid, (Merck). Rotary evaporator (Heidolph), Shimadzu 1700 spectrophotometer.

Preparation of the methanol extracts: The air-dried and finely ground sample was extracted by using the method described elsewhere[8]. The sample (100 g), was extracted in a soxhlet apparatus with methanol at 60 ºC for 6 h. The extract was then filtered and concentrated in vacuo at 45 ºC Finally, the extracts were then lyophilized and kept in the dark at +4 ºC until tested.

Free radical-scavenging method: The antioxidant activity of plant extract was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, ($DPPH^{\bullet}$)[9]. A methanolic solution of sample of various concentrations was placed in a cuvette and 4 mL of 6×10^{-5} mol/L methanolic solution of DPPH was added. After a 0.5 h incubation period at room temperature, the absorbance was read against a blank at 515 nm. The same procedure was repeated with synthetic antioxidant, BHT and BHA, as positive control and a blank. Inhibition of the free radical $DPPH^{\bullet}$ in per cent (I %) was calculated as follows:

$$I\ \% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Extract concentration providing 50 % inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate and BHT and BHA were used as positive controls.

Total phenolic compuond assay: The amounts of phenolics in the plant extract was determined with Folin-Ciocalteu reagent[10] using the method of to 50 mL of each sample, 2.5 mL of 10 % dilution of Folin-Ciocalteu reagent and 2 mL of Na_2CO_3 (7.5 %, w/v) were added and the resulting mixture was incubated at 45 ºC for 15 min. The absorbance of all samples was measured at 765 nm. Gallic acid was used as a standard for the calibration curve. The total amount of phenolic compounds was calculated and expressed as milligrams of gallic acid equivalent.

Statistical analysis: All results were obtained in triplicate and data were presented as mean ± standard deviation of three determinations (data were not shown). Statistical analyses were performed using a one-way analysis of variance[11].

RESULTS AND DISCUSSION

Antioxidant activity of mushroom extract according to the DPPH• radical scavenging method. The result (Fig. 1) showed that the decrease in absorbance of the DPPH• radical was due to its reduction by different antioxidants. Absorbance decreases as a result of a colour change from purple to yellow as the radical was scavenged by antioxidant through donation of hydrogen to form the stable DPPH-H. Solution was bleached with all the samples tested. However, differences could be observed through different antioxidants used and their concentrations.

Total phenolic compound assay was measured by Folin-Ciocalteu method[10]. The Folin-Ciocalteu reagent assay was used to determine the total phenolics content. Phenolic contents of mushroom and standart antioxidant compounds are correlated with the antioxidant activity, as shown Fig. 2.

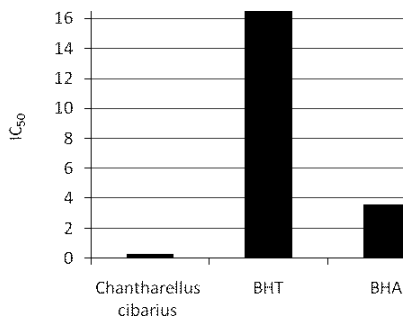

Fig. 1. IC_{50} value of *C. cibarus*, BHA and BHT

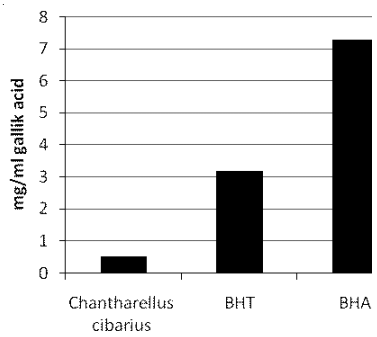

Fig. 2. Total phenolic compounds of *C. cibarus*, BHA and BHT (eq. mg/mL GAE)

All the material in this research exhibited different extent of antioxidant activity. Even though total phenolic concentration of *C. cibarus* is lower than BHA and BHT, IC_{50} value is higher than them and lower IC_{50} value indicates higher antioxidant activity[12]. Therefore, *C. cibarius* extract showed a higher potency than BHT and BHA in scavenging of DPPH free radical. Because mushrooms are a good source of the B vitamin complex, vitamin C and some minerals (potassium, selenium, copper and phosphorus)[13-16]. Selenium and copper have antioxidant properties, rendering harmful free radicals safe and is therefore implicated as an anticancer agent[17,18].

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