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ORIGINAL ARTICLE

Consequence of the antioxidant activities and tyrosinase inhibitory effects of various extracts from the fruiting bodies of *Pleurotus ferulae*

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Abstract This study was initiated to screen the antioxidant activities, tyrosinase inhibitory effects on the fruiting bodies of *Pleurotus ferulae* extracted with acetone, methanol and hot water. The antioxidant activities were performed on β-carotene–linoleic acid, reducing power, DPPH, ferrous ions chelating abilities, and xanthine oxidase. In addition to this, phenolic compounds were also analyzed. The methanolic extract showed the strongest β-carotene–linoleic acid inhibition and high reducing power as compared to other extracts. The scavenging effects on DPPH radicals, the acetonic and methanolic extracts were more effective than hot water extracts. The strongest chelating effect was obtained from the methanolic extract as compared to the tested synthetic antioxidant. Gallic acid, protocatechuic acid, caffeic acid, vanillin, ferulic acid, naringin, resveratrol, naringenin, hesperetin, formononetin and biochanin-A were detected from acetonitrile and hydrochloric acid (5:1) solvent extract. Xanthine oxidase and tyrosinase inhibitory activities of acetonic, methanolic, and hot water extracts of *P. ferulae* increased with increasing concentration. The results suggested that consumption of *P. ferulae* might be beneficial to the antioxidant, xanthine oxidase, and tyrosinase protection system of the human body against oxidative damage and others complications.

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1. Introduction

Pleurotus ferulae is an edible mushroom, belongs to the family Pleurotaceae and order Agaricales. It is mostly pathogenic and grows on the roots of *Ferula communis*, distributed throughout the Mediterranean region (Urbanelli et al., 2002). This mushroom has been known to produce various biologically active molecules and novel enzymes (Choi et al., 2005). Laccase is a ligninolytic enzyme, typically produced as multiple isoenzymes, which have been isolated and characterized in different strains of *P. ferulae* (Soden and Dobson, 2001). Laccase enzymes have also been shown to play an important role in the

development of the disease of some wood-decaying fungi (Schouten et al., 2008).

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centered free radicals and other reactive oxygen species that are continuously produced *in vivo*, result in cell death and tissue damage. Oxidative damage caused by free radicals may be related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis (Halliwell and Gutteridge, 1984). Almost all organisms are well protected against free radical damage by enzymes, such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione. Although almost all organisms possess antioxidant defence and repair systems that have evolved to protect them against oxidative damage, these systems are insufficient to prevent the damage entirely (Simic, 1988). However, antioxidant supplements, or foods containing antioxidants, may be used to help the human body reduce oxidative damage (Yanga et al., 2002). Allopurinol is the clinically used xanthine oxidase inhibitor, which also suffers from many side effects such as hypersensitivity syndrome and renal toxicity (Alam et al., 2011a). Thus, there is a need to develop compounds with xanthine oxidase inhibitor activity which is devoid of the undesirable side effects of allopurinol. A potential source of such a compound can be obtained from mushrooms. Flavonoids and polyphenolic crude extracts have been reported to possess xanthine oxidase inhibitory activity (Zhou et al., 2001; Alam et al., 2011b).

Tyrosinase, also known as polyphenol oxidase, is a copper-containing enzyme, which is widely distributed in mushrooms, animals, and plants. Nowadays mushroom tyrosinase has become popular because it is readily available and useful in a number of applications (Yoon et al., 2011). Despite the clinical importance of *P. ferulae* or the therapeutic potential, there have not been many studies on physiologically beneficial components. However, the antioxidant properties of this mushroom are not available. Accordingly our objective was to evaluate and compare the antioxidant and antityrosinase properties of acetonic, methanolic, and hot water extracts from the fruiting bodies of *P. ferulae*. The profiles of phenolic compounds were also determined.

2. Materials and methods

2.1. Chemicals and reagents

β-Carotene, linoleic acid, chloroform, polyoxyethylene sorbitan monopalmitate (Tween 40), butylated hydroxytoluene (BHT), α-tocopherol (TOC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), L-ascorbic acid, potassium ferricyanide, trichloroacetic acid, ferrous chloride, ferric chloride, ferrozine, Folin–Ciocalteu reagent, gallic acid, methanol, 3,4-dihydroxy-L-phenylalanine (L-DOPA), xanthine, allopurinol, mushroom tyrosinase, and dimethyl sulfoxide (DMSO) were obtained from Sigma–Aldrich (St. Louis, MO, USA). All chemicals and solvents were used as HPLC or analytical grade.

2.2. Mushroom and extraction

Fresh and mature fruiting bodies of *P. ferulae* were obtained from Mushmaru mushroom farm at Cheonan in Korea. A pure culture was deposited in Culture Collection DNA Bank of Mushroom (CCDBM), Division of Life Sciences, University of Incheon, Korea and acquired accession number, IUM-4402. Fruiting bodies were dried with hot air at 40 °C for 48 h and finely pulverized. Five grams of powdered samples was extracted with 100 ml of 60% acetone and 80% methanol with stirring at 150 rpm for 24 h at 25 °C to obtain acetonic and methanolic extracts. The mixture was filtered through two layers of Whatman no. 1 filter paper (Whatman, Maidstone, UK). The same quantity of sample was boiled at 100 °C for 3 h with 100 ml deionized distilled water to obtain a hot water extract. The mixture was cooled to room temperature and filtered through Whatman no. 1 filter paper. The residues were then extracted with two additional 100 ml aliquots of acetone, methanol, and deionized water, as described above. Then the combined extracts were evaporated with a rotary evaporator (Eyela, Saitama, Japan) at 40 °C, and the remaining solvent was removed with a freeze-drier (Optizen, Daejeon, Korea). The yields from the acetonic, methanolic and hot water extracts of *P. ferulae* were 23.18%, 23.80%, and 16.18% (w/w), respectively.

2.3. Antioxidant activity by β-carotene–linoleic acid

Antioxidant activity was determined by measuring the inhibition of volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius et al., 1998). A stock solution of a β-carotene–linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform, and 25 μl of linoleic acid and 200 mg of Tween 40 was added. The chloroform was removed completely using a vacuum evaporator. Then, 100 ml of oxygenated distilled water was added with vigorous shaking; 2.5 ml of this reaction mixture was dispensed to test tubes, 0.5 ml of various concentrations (0.5–20.0 mg/ml) of the extracts in methanol was added, and the reaction mixture was incubated for up to 2 h at 50 °C. The same procedure was repeated with the positive controls BHT and TOC, and a blank. After the incubation, the absorbance of the mixtures was measured at 490 nm using a spectrophotometer (Optizen POP; Mecasys Co. Ltd., Daejeon, Korea). The absorbance was measured until the β-carotene color disappeared. The β-carotene bleaching rate (R) was calculated according to Eq. (1):

$$R = \ln(a/b)/t \quad (1)$$

where, ln = natural log, a = absorbance at time t (0), b = absorbance at time t (120 min). The antioxidant activity (AA) was calculated as the percent inhibition relative to the control using Eq. (2):

$$AA = [(R_{control} - R_{sample})/R_{control}] \times 100 \quad (2)$$

Antioxidant activities of the extracts were compared with those of BHT and TOC at 0.5 mg/ml and a blank consisting of 0.5 ml methanol.

2.4. Reducing power

Reducing power was determined according to the method of Gülçin et al. (2003). Each extract (1–8 mg/ml) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide, and the mixture was incubated at 50 °C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid was added, and the mixture