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Effects on Tyrosinase Activity by the Extracts of *Ganoderma lucidum* and Related Mushrooms

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Abstract The inhibitory effects on tyrosinase activity by extracts of several mushrooms belonging to Basidiomycetes were evaluated. Among the tested mushrooms (*Ganoderma lucidum*, *Antrodia camphorata*, *Agaricus brasiliensis*, and *Cordyceps militaris*), *G. lucidum* exhibited significant inhibition of tyrosinase activity (IC_{50} value 0.32 mg/ml), compared to those prepared from other Basidiomycetes. Tyrosinase inhibitors are effective components of skin-lightening compounds and other cosmetics; currently many of the facial mask cosmetics in the market contain *Ganoderma* extracts in their ingredients. The finding that mushroom extracts contain tyrosinase activity inhibition will contribute to better understanding of how their 'healing' properties in various Chinese traditional herbal on skin care products.

Keywords *Ganoderma lucidum* · Lingzhi · Medicinal mushroom · Tyrosinase

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Introduction

Ganoderma lucidum (also known as "Lingzhi" in the herbal medicine community) is a Basidiomycete which is highly valued as a traditional Chinese herb. It is believed to contribute to a person's longevity. The extracts of *Ganoderma lucidum* include bioactive compounds like polysaccharides, triterpenoids, alkaloids, enzymes, and proteins (including glycoprotein) [1]. Numerous pharmacological studies have demonstrated that various extracts of *Ganoderma* spp. contain immunomodulatory, antiperoxidative, and anti-tumor activities [2–6]. Additionally, other studies also reported that the extracts from these medicinal mushrooms could facilitate skin wound healing [7, 8]. "Lingzhi" is also highly popular as a health supplement and is often used to combat fatigue [2, 9].

"Lingzhi" is a common ingredient in various cosmetic lines and may contribute to the whitening of skin. This property is highly valued by many oriental (Chinese and Japanese) women [7, 8, 10]. Skin-whitening is believed to be partly due to the

inhibition of tyrosinase activity, and various skin-whitening cosmetics are believed to be effective because of the presence of arbutin or kojic acid which act as tyrosinase inhibitors [11–13]. Tyrosinase is a copper-containing enzyme present in plant and animal tissues that catalyzes the production of melanin and other pigments from tyrosine by oxidation [14].

In this study, we aim to evaluate the effectiveness of the extracts of *Ganoderma lucidum* and related Basidiomycetes in tyrosinase inhibition and thus its usefulness as a skin whitener.

Materials and Methods

Preparation of Mushroom Extracts

The Basidiomycetes *Ganoderma lucidum*, *Antrodia camphorata* (*Antrodia cinnamomea*), *Agaricus brasiliensis* (*Agaricus blazei* Murill), and *Cordyceps militaris* were purchased from Bioresource Collection and Research Center (BCRC), Food Industry Research and Development Institute, Hsinchu, Taiwan. Cultures of the Basidiomycetes were grown in 1,000-ml Erlenmeyer flasks containing 200 ml potato dextrose broth (BD Diagnostics, Spark, MD, USA) and incubated at 25°C with 100 rpm shaking for 7 days. The mycelia were then filtered using Whatman Grade No. 1 filter paper (VWR International, West Chester, PA, USA), lyophilized, and powered before use. Dried mycelium powder (5 g) was extracted first with 70% (v/v) ethanol, 50% (v/v) ethanol, and distilled water at room temperature for 3 days, respectively. Extracts were filtrated, pooled, and the water allowed evaporating completely in a rota vapor at mild temperature (40°C). The dry residues were finally dissolved in distilled water into different concentrations for further experiments.

Determination of Inhibitory Effect on Tyrosinase Activity of the Extracts

Extracts of *G. lucidum*, *A. camphorata*, *A. brasiliensis*, and *C. militaris* were diluted to different concentrations to assay for tyrosinase inhibition. One hundred-microliter tyrosinase (350 units/ml; Sigma, St. Louis, MO, USA) was mixed with 0.9 ml of various concentration of extracts prepared as described above (0.1 and 1 mg/ml) in phosphate buffer (pH 6.8) and 1 ml of L-tyrosine (1 mmol/l). The reaction mixture was incubated at 37°C for 25 min. Absorbance at 280 nm was measured to determine the activity of tyrosinase [15]. Experimental controls were performed with the same reaction mixture in the absence of extracts with or without tyrosinase. The inhibition rate of tyrosinase activity was calculated as the inhibition $(\%) = [1 - (B - B_0)/(A - A_0)] \times 100$, where A and A_0 represent the absorbance of control with and without tyrosinase, respectively; B and B_0 represent the absorbance of the experimental sample with and without tyrosinase, respectively.

Results and Discussion

The inhibitory effects on tyrosinase activity by the extracts of the different mushrooms are shown in Figs. 1 and 2. Among the extracts examined, all extracts prepared from *G. lucidum* showed significant inhibition of tyrosinase activity (IC_{50} value was about 0.32 mg/ml). About 80% of tyrosinase activity was inhibited when the reaction mixture contained 1 mg/ml of the extract (Fig. 1), and still about 40% of inhibition effect was observed when the reaction mixture contained 0.1 mg/ml of the extract (Fig. 2). No difference in inhibitory effects on tyrosinase activity was observed by *G. lucidum* extracts obtained by the three different extraction methods (75%, 50% ethanol, and distilled water extraction). The only other extract that showed some inhibitory

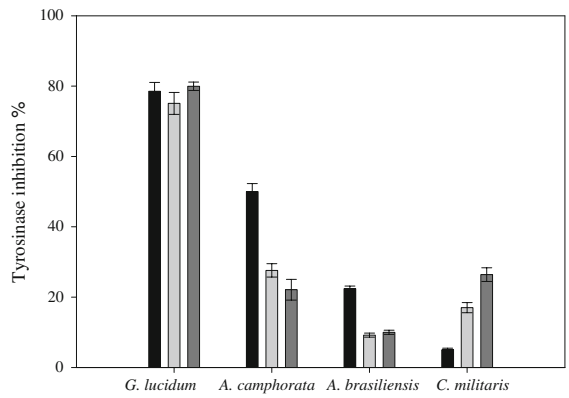

Fig. 1 Inhibitory effects of extracts of mycelia of different mushrooms on tyrosinase activity. 75% ethanol extracts (black bars); 50% ethanol extracts (gray bars); distilled water extracts (dark-gray bars). The concentration of the extracts used in the reaction mixture is 1 mg/ml reaction mixture

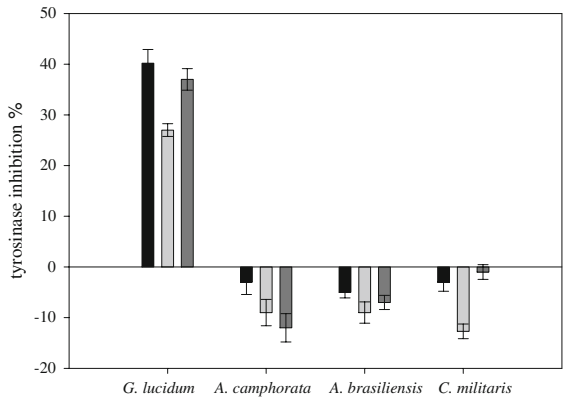

Fig. 2 Inhibitory effects of extracts of mycelia of different mushrooms on tyrosinase activity. 75% ethanol extracts (black bars); 50% ethanol extracts (gray bars); distilled water extracts (dark-gray bars). The concentration of the extracts used in the reaction mixture is 0.1 mg/ml reaction mixture

effect on tyrosinase activity was prepared from *A. camphorata* using 75% ethanol extraction (IC_{50} was about 1 mg/ml) (Fig. 1). Extracts of *A. camphorata* prepared by 50% extraction and distilled water, and all extracts prepared from *A. brasiliensis* and *C. militaris* showed less than 25% inhibition in the reaction mixtures contained 1 mg/ml extracts (Fig. 1). No significant inhibitory effect on tyrosinase activity was observed when 0.1 mg/ml extracts were used in the reaction mixture (Fig. 2).

We subsequently examined four different strains of *G. lucidum* for their ability to inhibit tyrosinase activity. The reaction mixtures contained 1 mg/ml distilled water extraction of mycelia of the four *G. lucidum*. Mycelia from these four strains of *G. lucidum* were washed with distilled water twice before extraction and used for the experiment. Inhibitory effects on tyrosinase activity were still evident (IC_{50} was about 1 mg/ml), although the inhibition rate was lower than extracts prepared from mycelia (This was obtained by removing the mycelia from cultured medium using filtration with a Whatman Grade No. 1 filter paper; Fig. 1) This implies that the active components of the inhibition are water soluble.

Tyrosinase inhibitors are effective components of skin-lightening compounds and cosmetics [13, 16, 17]. Some traditional Chinese herbal medicines have been investigated for their potential use in various lines of cosmetic products, and the extracts of *Pharbitis nil*, *Sophora japonica*, *Spatholobus suberectus*, and *Morus alba* showed potent tyrosinase inhibitory effects [18]. The Basidiomycete *Ganoderma* is often regarded as a panacea in traditional Chinese culture. Recent scientific studies have demonstrated that *Ganoderma* can be a remedy to many diseases such as hepatopathy, chronic hepatitis, nephritis, hypertension, etc. [2]. The extracts of the mycelium of *G. lucidum* have also been demonstrated to have antiperoxidative, anti-inflammatory antimutagenic activities, and antioxidant properties [3, 19]. The residues of fruiting body of *Ganoderma tsugae* exhibit the effect of promoting skin wound healing [7, 8]. *G. lucidum* extracts were also shown to exert immunomodulating and anticancer effects [20]. Laccase activity has been detected from fresh fruiting bodies of *G. lucidum*, and a laccase gene from *G. lucidum* has also been cloned and expressed for studying its antioxidative properties [21, 22]. Whether these enzyme activities are related to the nature of tyrosinase inhibition is to be examined in the future. Currently, many of the facial mask cosmetics in the market contain *Ganoderma* in their ingredients. However, there are no reports presently on *Ganoderma* being the potential source of tyrosinase inhibitors and this could be the direct effect of 'skin-whitening'. In this study, we demonstrate for the first time that extracts of *G. lucidum* contain tyrosinase-inhibiting activities. The extracts also do not show any toxicity to human fibroblast Hs68 (results not shown). This trait may contribute to the effectiveness of this Chinese traditional herbal on skin care and cosmetology.

The composition of fruiting body and/or mycelia of *G. lucidum* were very complicated, and it is still hard at present stage to identify which component(s) attribute to the inhibitory effect [1, 9, 23, 24]. The active constituents of *G. lucidum* on various diseases or health function can be very different such as polysaccharides and triterpenes [24, 25]. *G. lucidum* are widely used as health food additives and now appear in some cosmetic additives. Results of this study suggested another possible mechanism of this magic traditional Chinese medicinal herbal on its effectives of preventing aging and may also be useful as additives in skin care cosmetics.

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