Antioxidant Polyphenols from the Medicinal Fungi *Inonotus* and *Phellinus*

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Free radicals have been implicated in the pathogenesis of various diseases, such as myocardial and cerebral ischemia, arteriosclerosis, diabetes, rheumatoid arthritis, inflammation and cancer-initiation, and they have also been implicated in the aging process. There is considerable evidence that antioxidants may help prevent illnesses caused by oxidative stress because they have the capacity to quench free radicals, thereby protecting cells and tissues from oxidative damage.

Several mushrooms belonging to the genera *Inonotus* and *Phellinus* have been used as traditional medicines for treatment of gastrointestinal cancer, liver or heart diseases, and stomach ailments. Although they produce a large and diverse variety of secondary metabolites, polysaccharides have been considered to be responsible for their biological effect, and the anticancer and immunomodulating activities of ß-glucans derived from these mushrooms has been well documented. In this study, we focused on the small molecule antioxidants from the cultured broths of the medicinal fungi *I. xeranticus*, *I. obliquus*, *P. baumii*, and *P. linteus* and from their fruiting bodies. To characterize the antioxidative substances present in cultured broths of the medicinal fungi, antioxidant fractions of the cultured broths obtained from *I. xeranticus* and *P. linteus* were analyzed using reversed-phase HPLC, which revealed several antioxidant peaks. To identify these peaks, an *I. xeranticus* strain was mass-cultured, and the cultured broth was separated using antioxidant activity-guided fractionation. As a result, four major active substances were purified and identified as hispidin and its dimers, 3,14-bihispidinyl, hypholomine B, and 1,1-distyrylpyrylethan based on spectroscopic analyses. All compounds exhibited significant scavenging activity against these radical species in a concentration-dependent manner. The fruiting bodies of these medicinal fungi also produce a cluster of yellow pigment with potent antioxidant activity. Our investigation on the chemical constituents of the yellow pigment led the isolation of diverse styrylpyrone analogs with a novel carbon skeleton including inoscavins A-D and interfungins A-C. These compounds exhibited significant free radical scavenging activity against the superoxide radical anion, ABTS radical cation, and DPPH radical.

Natural products that have potent anti-oxidative characteristics are known to be valuable anti-platelets. To determine whether the compounds isolated modulate platelet aggregation, the anti-platelet properties of davallialactone, a major substance, were examined using a platelet aggregation assay. As a result, we found that

davallialactone dose-dependently inhibited platelet aggregation, which was stimulated either by collagen, a potent ligand of integrin α2β1 and glycoprotein VI, or by thrombin, a potent agonist of a protease receptor. To understand the mechanism of anti-platelet activity, we determined whether davallialactone affected the downstream signaling in collagen-activated platelets. Using the Fura-2/AM fluorometric assay, we found that davallialactone dose-dependnetly inhibited intracellular calcium concentration levels. Moreover, davallialactone inhibited the phosphorylation of extracellular signal-regulated protein kinase (ERK)-2 and p38 mitogen-activated protein kinase (MAPK), in a dose-dependent manner. We also explored the anti-inflammatory properties of davallialactone in inflammatory responses mediated by lipopolysaccharide-activated macrophage-like cells. Davallialatone strongly down-regulated LPS-mediated inflammatory responses and up-regulated the expression of anti-inflammatory protein Hemeoxygenase(HO)-1 in activated RAW264.7 cells, without altering their viability and morphology. Taken together, the polyphenols with styrylpyrone moiety from the medicinal fungi might be useful as antioxidants against platelet aggregation and inflammation. Detailed mode of action of davallialactone will be presented.

References

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