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Phenolic profile of various wild edible mushroom extracts from Thailand and their antioxidant properties, anti-tyrosinase and hyperglycaemic inhibitory activities

Khwanta Kaewnarin, Nakarin Suwannarach, Jaturong Kumla, Saisamorn Lumyong *

Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

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ABSTRACT

Four popular wild edible mushroom species (*Rugiboletus extremiorientalis*, *Russula emetica*, *Russula* sp. and *Phlebopus portentosus*) in Thailand were investigated for their total phenolic and flavonoid contents and phenolic profile and were evaluated for their antioxidant properties, as well as their tyrosinase and hyperglycaemic inhibitory activities. Water extraction of *R. extremiorientalis* not only contained high phenolic and flavonoid contents but also exhibited the strongest antioxidant activities as well as significant anti-hyperglycaemic activity. However, the methanolic extract of *R. extremiorientalis* had greater tyrosinase inhibitory activity than that of other mushroom species. The phenolic profile of an effective water extract of *R. extremiorientalis* presented gallic acid, protocatechuic acid, catechin, vanillic acid, syringic acid, sinapic acid, ferulic acid, hydroxycinnamic acid, rutin and apigenin. This is the first report of phytochemical data for selected popular wild edible mushroom species in Thailand, and these findings could be applied to nutraceutical and cosmetic purposes.

1. Introduction

Mushrooms have been widely consumed by humans for centuries, because they are abundant in carbohydrates, proteins and dietary fibre, as well as other important vitamins and minerals. In recent years, interest in mushrooms has significantly increased because of their prevention capabilities against several diseases (Ferreira, Barros, & Abreu, 2009; Kalac, 2012). There is a good deal of scientific evidence that mushrooms not only possess high nutritional properties but also contain numerous bioactive compounds that have proven to be effective antioxidants including phenolic compounds, terpenes, polysaccharides and steroids (Alam, Yoo, & Lee, 2011; Lindequist, Niedermeyer, & Julich, 2005). Among these bioactive compounds, phenolic and flavonoid compounds are particularly noteworthy due to their large array of biological actions, including free radical scavenging, metal chelation (Cheung, Cheung, & Ooi, 2003), enzyme inhibitory activities (Yoon et al., 2011) and inhibition of lipid oxidation.

Wild edible mushrooms are more widely preferred by consumers than cultivated mushrooms in many countries due to

* *Corresponding author.* Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand. Fax: +66 53 892259.
E-mail address: saisamorn.l@cmu.ac.th (S. Lumyong).
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their superior flavour and texture. The most important wild edible mushroom species in the world, with higher price and/or a large market share, are truffle (*Tuber* spp.), porcini (*Boletus edulis*) and matsutake (*Tricholoma matsutake*) including several species of *Boletus*, *Rugiboletus* (syn. *Leccinum*; in Index Fungorum, http://www.indexfungorum.org/Names/Names.asp) and *Russula* (Sitta & Davoli, 2013). *Rugiboletus*, *Russula* and *Boletus* genera are eaten widely in more than 20 countries, such as Bulgaria, China, Japan, Mexico, Russia and Thailand (Boa, 2004; Mortimer et al., 2012; Sanmee, Dell, Lumyong, Izumori, & Lumyong, 2003). Recent studies have reported on the medicinal properties of wild edible mushrooms, such as their anti-tumour properties (Finimundy et al., 2013), antioxidant activities, antimicrobial activities (Ren et al., 2014; Yildiz, Can, Laghari, Sahin, & Malkoc, 2014) and anti-hyperglycaemic activities (Liu et al., 2012; Zavastin et al., 2015). Diplock et al. (1998) indicated that mushrooms are rich sources of antioxidants and that could prevent free radical damage and could reduce the risk of certain chronic diseases.

Oxidative stress conditions are an important cause of the unbalanced generation of reactive oxygen species in cellular organisms. Excessive reactive oxygen species (ROS) production leads to oxidative damage of biological macromolecules (i.e., proteins, lipids and nucleic acids), causing tissue injury (Vaz et al., 2011). Superoxide radicals (O_2^-), hydroxyl radicals (–OH) and hydrogen peroxide (H_2O_2) can induce DNA damage and impair enzyme and protein structures; these types of molecular damage are implicated in the pathogenesis of various chronic diseases, such as cancer, cardiovascular, neurological diseases, cataracts, diabetes and rheumatoid arthritis (Cadenas & Davies, 2000; Circu & Aw, 2010; Jeong et al., 2012).

Diabetes mellitus (DM) is a chronic metabolic disease of carbohydrate, fat and protein metabolisms that lead to an increase in fasting and post-prandial blood glucose levels. Prolonged hyperglycaemia, a significantly high level of glucose in the blood, in diabetic patients contributes to diabetic complications, such as atherosclerosis and cardiovascular disease (Wu et al., 2011). One therapeutic approach for diabetic patients is decreasing the prolonged hyperglycaemia condition by retarding glucose adsorption through the inhibition of α-glucosidase, which is a carbohydrate-hydrolysing enzyme in the small intestine. Diabetes is also associated with ROS generation (Palanisamy, Ling, Manaharan, & Appleton, 2011).

Tyrosinase, a multifunctional copper-containing enzyme, is normally found in fungi, plants and animals (Huang et al., 2006). Tyrosinase is a key enzyme for melanin production, while overmelanin production can be the cause of dermatological disorders leading to skin cancer. Moreover, the melanin synthesis pathway generates many highly reactive intermediate substances, such as hydrogen peroxide and hydroxyl radicals. Therefore, tyrosinase inhibitors may be cosmetically and clinically useful in dealing with skin whitening and skin cancer based on their antioxidative properties and anti-tyrosinase activity (Perluige et al., 2003).

In fact, all free radicals are neutralized by cellular defence systems (enzymes and non-enzymatic molecules) to protect cells against oxidative damage (Ferreira et al., 2009; Valko et al., 2007). However, these systems are not sufficient to completely prevent oxidative stress-induced damage. Therefore, exogenous dietary antioxidants or natural products, which can scavenge free radicals, are very attractive as beneficial substances that can serve as protecting agents for chronic diseases (Hirano et al., 2001; Kanter, 1998).

Presently, an ever widening range of wild mushrooms are being reported from different parts of the world. In Thailand, where there is a great diversity of wild mushroom species, several important wild mushrooms have been found. Wild edible mushroom species are abundant and popular for consumption in Thailand, such as *Rugiboletus* sp., *Russula* sp. and *Phlebopus portentosus*. However, phytochemical analyses of these wild edible mushroom species have not yet been reported. Therefore, the purpose of this study was to evaluate the phenolic profiles, antioxidant properties, anti-pigment and anti-hyperglycaemic activities of four popular wild edible mushroom species (*Rugiboletus extremiorientalis*, *Russula emetica*, *Russula* sp. and *P. portentosus*) in Thailand using different solvent extractions. These phytochemical data have attracted significant interest in wild edible mushrooms as a new source of natural substances that can be applied to nutraceutical and cosmetic properties.

2. Materials and methods

2.1. Chemicals and reagents

All standards (purity >99.0%) for HPLC analysis were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol, ethanol and acetonitrile were obtained from J.T. Baker (Radnor, PA, USA). Sodium carbonate, D-maltose, hydrochloric acid, aluminium chloride and Folin–Ciocalteu's reagent were obtained from Fluka Chemie GmbH (Buchs, Switzerland). Acarbose, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-azinobis(3-ethylbenzothiazoline 6-sulphonic acid) (ABTS), potassium persulphate, Trolox, rat small intestinal α-glucosidase, tyrosinase, PGO enzymes and 3,4-dihydroxy-1-phenylalanine (L-DOPA) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Mushroom species

Information on the four selected wild edible mushrooms is provided in Table 1. The wild edible mushroom species *R. extremiorientalis* and *Russula* sp. were collected from Doi Suthep-Pui National Park, Chiang Mai, Thailand during the rainy season of 2015. *R. emetica* was collected from Mae Taeng District, Chiang Mai, Thailand, while *P. portentosus* was purchased from a local mushroom market in Chiang Mai, Thailand (Fig. 1). These specimens were identified by our teams at the Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. All samples were dried in a hot air oven at 45 °C, reduced to a fine powder and kept at 25 °C for further analysis.

2.3. Extraction of phenolic compounds from wild mushrooms

Approximately 5 g of dried mushroom samples was extracted using various extractants (95% ethanol, 95% methanol,