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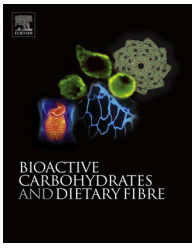

Antibacterial and antioxidant activities of aqueous extracts of eight edible mushrooms

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ABSTRACT

Polysaccharides extracts of eight edible mushroom species, including five species collected from New Zealand forests and parks, were tested for their ability to inhibit the growth of five common bacterial strains. Antibacterial activity was assayed using the disc diffusion and microdilution methods. An aqueous extract from *Cordyceps sinensis* inhibited the growth of *Bacillus subtilis* and *Streptococcus epidermidis* with minimum inhibitory concentration (MIC) values of 938 and 469 μg/mL, respectively. A *Pleurotus australis* extract had the same MIC of 469 μg/mL against *S. epidermidis*. Comparatively, the microdilution method was more efficient and accurate than the disk diffusion method at measuring the antimicrobial activity of high molecular weight polysaccharides. All polysaccharides exhibited DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activities, with *P. australis* having the highest antioxidant activity (EC_{50} of 4.03 mg/mL). Fourier transform infrared (FT-IR) analyses indicated that some extracts contained α or β-conformations. Their relative quantities of OH, evaluated by the ratios of OH group/CH group, did not correlate with their scavenging activity based on EC_{50} values. Several of the mushroom polysaccharide extracts investigated in this study have antibacterial and antioxidant activities that warrant further study as potential dietary supplements to improve health and well-being.

1. Introduction

The growing emergence of drug-resistant bacterial strains is a serious threat to the effective treatment of infections (Klein, Smith, & Laxminarayan, 2007). Drug resistance usually happens after long-term misuse of antibacterial agents (Gao, Zhou, Huang, & Xu, 2003), and is a consequence of the acquisition of mutations in the bacterial genome and genes that assist bacterial survival (Mazodier & Davies, 1991). Whilst it is impossible to prevent bacterial evolution, it is important to choose the most appropriate antibiotics and to use them appropriately to minimise the development of drug-resistant strains (Alves, Ferreira, Martins, & Pintado, 2012). The application of antibiotics in animal feed has been

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reduced as a measure to avoid the emergence of new antibiotic resistant strains. Regulatory restrictions on the subtherapeutic use of antibiotics necessitates alternative means to restrain bacterial infections in poultry (Giannenas et al., 2010). There will forever be a need for novel antimicrobial compounds to outwit bacteria and other pathogens (Zgoda & Porter, 2001).

Stress on the body due to aging, obesity, and detrimentral lifestyle choices is another significant health issue, which often takes the form of oxidative damage to tissues. Superoxide radicals (O_2^-), hydroxyl radicals ($\cdot OH$) and hydrogen peroxide (H_2O_2) damage DNA, impair enzymes and structural proteins, and provoke uncontrolled chain reactions including lipid peroxidation (Halliwell & Cross, 1994). The reactive oxygen species (ROS) and oxygen-derived free radicals are known to play key roles in carcinogenesis and cellular degeneration which promote the development of cancer, cardiovascular and neurological diseases, cataracts, diabetes, and rheumatoid arthritis (Circu & Aw, 2010; Cadenas & Davies, 2000; Jeong et al., 2012). Almost all organisms have defence systems to protect against free radical damage. The activity to scavenge radicals has been associated with a rise in the antioxidant enzyme activities. These enzymes include superoxide dismutase (SOD) which catalyzes the dismutation of superoxide anion to hydrogen peroxide, catalase (CAT) which detoxifies hydrogen peroxides and converts lipid hydroperoxides to non-toxic substances, and glutathione peroxidase (GPx) which maintains the levels of reduced glutathione (GSH) (Guo, Ji, & Ping, 2009). However, these systems are not sufficient to prevent damage, and hence antioxidants are employed to form a cooperative defence system. Antioxidants are defined as compounds that possess an ability to protect biological systems against the potentially harmful effects of processes or reactions that can cause excessive oxidation (Krinsky, 1989). Many synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, have side effects and are thought to be responsible for liver damage and carcinogenesis (Grice, 1988). As a result, natural antioxidants are preferred in food applications. Natural substances, such as vitamins A, C and E, carotenoids, flavonoids and other simple phenolic compounds, are proven to prevent oxidative damage and thus protect the human body (Mackerras, 1995).

Numerous antimicrobial agents, including penicillin and griseofulvin, have been isolated from micro-fungi. The rich diversity of different fungal species offers a potential source of new antibiotics (Yamac & Bilgili, 2006). Mushrooms that possess the macroscopic reproductive structures of a diverse range of basidiomycete fungi have been utilized for curative and medicinal purposes since prehistoric times. Mushrooms are a veritable treasure-house of bioactives that display antimicrobial, antitumorgenic, hypolipidemic, and hypoglycaemic properties (Venturini, Rivera, Gonzalez, & Blanco, 2008). Two major groups of mushroom bioactives are the polysaccharides and triterpenes (Ameri, Vaidya, & Deokule, 2011). Polysaccharides are responsible for the rigidity and morphological properties of the fungal cell wall (Wolff et al., 2008). Many display potent activity against common strains of bacteria (Yamac & Bilgili, 2006; Bala, Aitken, Cusack, & Steadman, 2012; Zhu, Sheng, Yan, Qiao, & Lv, 2012). Fungi produce polysaccharides, phenolics, and various metabolites that represent potential sources of novel natural antioxidants (Huang, Cai, Xing, Corke, & Sun, 2007; Cheung, Cheung, & Ooi, 2003).

The current study sought to investigate antibacterial activities of five New Zealand native mushrooms and three cultivated mushrooms. Paper disc diffusion and microdilution techniques were employed to measure antibacterial activity, and compared in order to determine which method was superior. The antioxidant activity of these mushroom extracts was also assessed by measuring their radical scavenging activity.

2. Materials and methods

2.1. Materials

Eight species of mushroom were selected for evaluation of antibacterial and antioxidant activities. Four mushroom species were collected fresh from native podocarp forests around Auckland (New Zealand), namely *Auricularia cornea* Ehrenb., *Calvatia gigantea* (Batsch) Lloyd, *Hericium coralloides* (Scop.) Pers., and *Pleurotus australis* (Cooke & Massee) Sacc. *Ileodictyon cibarium* Tul. & C.Tul. was collected on wood mulch from urban gardens. With the exception of *P. australis*, the other four New Zealand species were reportedly known to Maori as edible mushrooms (Fuller, Buchanan, & Roberts, 2004). Two cultivated mushroom species, *Hericium erinaceum* (Bull.) Pers. and *Lentinula edodes* (Berk.) Pegler, were purchased as Asian imported dried products from a local supermarket. *Cordyceps sinensis* was obtained as a powdered product purchased from Medimushrooms (Dr. Alla's Cordyceps, New Zealand). The free-radicals 1,1-diphenyl-2-picrylhydrazyl (DPPH) and potassium bromide (KBr) were purchased from Sigma (Darmstadt, Germany).

2.2. Extraction of polysaccharides

Water soluble substances were extracted and purified from mushrooms according to the method described by Wang et al. (1993) with additional modifications as shown in Fig. 1. Mushroom fruiting bodies were frozen at −80 °C, lyophilized in a vacuum freeze dryer, and reduced to a fine dried powder (20 mesh) (Vaz et al., 2011). Powdered samples (100 g) were extracted with 150 mL of 80% ethanol for 1 h to eliminate low molecular components such as mono- and disaccharides, oligosaccharides, amino acids, lipids and some phenols. After filtration with a Whatman No. 4 paper, the residue was subjected to aqueous extraction under agitation (150 rpm; magnetic stirrer) with hot water (1 L, 100 °C, 3 h). The aqueous suspension was centrifuged at $8400 \times g$ for 15 min followed by filtration with Whatman No. 4 paper. The supernatant was concentrated to 10 mL under vacuum at 55 °C and then added to 5 volumes of ethanol to induce precipitation at 4 °C overnight (Xie et al., 2010). The precipitated polysaccharides were collected after centrifugation as above. The obtained precipitate was again dissolved in distilled water (100 mL) and dialyzed through a DEAE cellulose tube (molecular weight cut-off of 12,000 Da) against distilled