Mycobiology

Mini-review

Health Effects of Small Volatile Compounds from East Asian Medicinal Mushrooms

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Abstract Medicinal fungi, taken whole or as various forms of extracts, have been used to alleviate, cure or prevent human ailments since pre-historic times. In particular, Asian cultures have incorporated a variety of mushrooms into their medical practices. Chemically pure, bioactive metabolites from fungi have been a mainstay of modern pharmacological research and in addition to antibiotics, include anticancer agents, immunosuppressants, enzyme inhibitors, antagonist and agonists of hormones, and a variety of psychotropic substances. However, to date not many studies have focused on the possible health benefits of odorant volatile organic compounds (i.e., gas phase compounds). An analysis of these compounds for their health related effects will expand the range of compounds available for the treatment of chronic and acute diseases. This review highlights phenolic acids and monoterpenes from Asian medicinal mushrooms (AMMs), which not only produce pleasant odors but also have antioxidant and antibacterial effects. Odorant bioactive volatile phase compounds from medicinal mushrooms remain an essentially untapped source for future medicines, and AMMs remain a promising resource for future pharmacological research.

Keywords Antimicrobial, Antioxidant, Monoterpene, Odor, Phenolic acid

Scientists who study traditional healing practices are in pursuit of a unified concept of medicine in which the use of complex mixtures of natural compounds from mushrooms and plants can be integrated with contemporary Western approaches that use purified single crystalline compounds as drugs. In particular, natural products with antimicrobial properties (antibiotics) transformed therapeutic medicine and changed the character of the modern pharmaceutical industry. While useful drugs have been developed from many different bacterial, fungal and plant sources, it is interesting to note that of the approximately 200,000 known natural products, approximately one quarter are fungal metabolites [1].

Most pharmaceutical research has focused on the discovery of new antibiotics and other therapeutics such as antihypertensives, anticancer agents, antiparasitics and immunosuppressants. Botanists, microbiologists and mycologists categorize such compounds together as "secondary metabolites", i.e., members of a chemically heterogeneous group of low molecular weight natural products that are produced by specialized biosynthetic pathways and that serve apparently "dispensable" functions [2-4]. Specific secondary metabolites are usually restricted to narrow taxonomic groups where they are biosynthesized by gene clusters [5]. Bioinformatics features of putative alkaloid, non-ribosomal polypeptide, polyketide and terpenoid pathways can be detected by their distinctive genomic signatures in fungal genome data, and many cryptic pathways have been discovered in recent years [5]. It is now believed that many organisms encode the potential to produce many more secondary metabolites than originally expected [6].

In this review, we focus on a group of even lower weight natural products that are easily volatilized. These gas phase metabolites are responsible for the distinctive aroma properties of different organisms and have received relatively little attention by pharmacologists in their search for new bioactive metabolites. In particular, our review will target phenolic and monoterpene compounds from Asian medicinal mushrooms (AMMs).

VOLATILE ORGANIC COMPOUNDS

Volatile organic compounds (VOCs) are low molecular mass, carbon-containing compounds that readily evaporate

Mycobiology 2015 March, **43**(1): 9-13
http://dx.doi.org/10.5941/MYCO.2015.43.1.9
pISSN 1229-8093 • eISSN 2092-9323

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Received January 6, 2015
Revised March 3, 2015
Accepted March 3, 2015

at normal atmospheric temperatures and pressures [7]. Fungi produce VOCs of many molecular sizes and include acids, alcohols, aldehydes, aromatics, esters, heterocyclics, ketones, phenols, terpenes, thiols and so forth. Over 300 different VOCs have been identified from fungi where they are usually produced as complex mixtures in individually low concentrations [8]. The shortage of investigations into the health effects of these compounds in fungi is related in part to the difficulties in sample preparation, extraction and concentration of odorant molecules that are often found in the parts-per-billion range. It can be difficult to isolate sufficient amounts of them for physiological studies and characterization [9]. Furthermore, some VOCs have negative health effects. Perhaps the best-studied volatiles are industrial compounds such as chemical solvents (benzene, carbon tetrachloride, chloroform, formaldehyde, glycol ethers, methylene chloride, toluene, and so forth), many of them have widespread commercial application and are known to have both short and long-term negative effects on human health [10]. Far less is known about the possible health effects of biogenic VOCs, either beneficial or deleterious.

The many functionalities of fungal VOCs and their roles in interorganismal interactions have been reviewed by Bitas *et al.* [11]. The single most abundant volatile produced by mushrooms is 1-octen-3-ol, also known as "mushroom alcohol," which gives many commercially available edible mushrooms their characteristic smell and which has an extremely low (0.01 ppm) odor threshold in humans [1]. Mushroom alcohol has been isolated from almost every fungal species studied. It is widely used as a food and flavoring agent as well as a component of perfumes. Moreover, the compound is suspected to contribute to "sick building syndrome", causing headaches, dizziness and nausea in those exposed to it [12]. Our lab also has demonstrated that mushroom alcohol induces neurotoxic effects in a *Drosophila melanogaster* model for Parkinson's disease [13, 14] and found that it is 80 times more toxic than toluene in human embryonic stem cells [15].

MEDICINAL MUSHROOMS

For centuries, cultures across the globe have used mushrooms for nourishment, medication and creative inspiration. As natural remedies, mushrooms and mushrooms extracts are utilized in the treatment and prevention of acute and chronic ailments including asthma, cancer, heart disease, infections, insomnia and ulcers. The medical traditions of China, Japan and Korea, in particular, have incorporated some of the most ancient, distinctive and widely used fungal remedies. The major fungi used in traditional East Asian medical practices include *Ophiocordyceps sinensis* (Chinese: dōng chóng xià cǎo; English: caterpillar mushroom), *Ganoderma lucidum* (Chinese: líng zhī; Japanese: reishi), *Grifola frondosa* (Chinese: huī shù huā; Japanese: maitake; English: chicken-of-the-woods), *Hericium erinaceus* (Chinese: hóu tóu; Korean: norugongdengi beoseot; English: lion's mane mushroom), *Antrodia camphorata* (Chinese: niu chang), *Flammulina velutipes* (Chinese: jīn zhēn gū; Korean: paengi beoseot; Japanese: enokidake) and *Lentinula edodes* (Chinese: xiāng gū; Japanese: shiitake). Traditionally, these mushrooms are ingested whole or extracts containing the curative compounds are prepared by boiling the fruiting bodies in water; many modern commercial products are also available consisting of powdered or pill forms of the mycelia, fruiting bodies and/or spores [16].

Numerous studies have been published on the therapeutic compounds found within medicinal fungi. Scientific research on beneficial fungal compounds primarily focus on macromolecules (polysaccharides, proteins and lipids) or non-volatile secondary metabolites with molecular masses of approximately 300~900 g/mol. For example, the antitumor properties of *Pleurotus* spp. have been attributed to (1 → 3)-β-D-glucans while complex branched polysaccharides, and triterpenes are considered the major pharmacological components of *G. lucidum* [16]. However, medicinal mushrooms also produce a considerable quantity of smaller less-studied, fragrant compounds with putative health benefits and detriments.

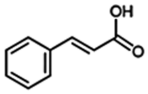
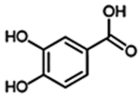
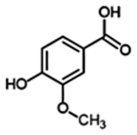
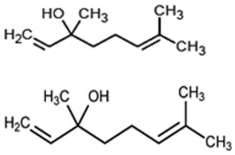
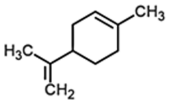
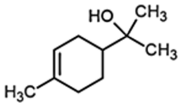
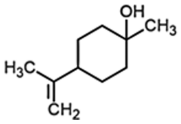
AMM PHENOLICS ACIDS AND MONOTERPENES

For the sake of this review, we emphasize two groups of active compounds which have been isolated from AMMs: simple phenolic acids and monoterpenes (Table 1) [17-27]. Phenolic acids are a class of organic compounds containing an aromatic benzene ring directly bonded to a hydroxyl group and a carboxylic acid substituent. Generally known and marketed for their antioxidant prowess and other health promotion activities in plant foods such as berries and teas, studies have turned to determining the absorption and circulation of phenolic compounds within consumers, as recently reviewed by Velderrain-Rodríguez *et al.* [28]. Terpenes, which are secondary metabolites biosynthetically derived from isoprenes, are the largest known group of natural products and have been largely characterized from plants. As many of them have pleasant odors, they have been intensively researched by the food and flavor industries [29]. Less is known about their health effects.

Similarly, not much is known about the biosynthesis of either of these groups of compounds in fungi. In plants, phenols are produced mostly through shikimate and acetate pathways [28]. Bacteria and fungi are known to biotransform or biodegrade camphor, *p*-cymene and citronellol/geraniol [30]. Using bioinformatics predictions, more pathways and the involved enzymes can be annotated [5, 30]. It is also possible that some of these compounds may be obtained from the surrounding environment as in the case of α-terpineol, which seems to act as an extracellular signal for the production of triterpenes in *A. camphorata* [26].

Total phenol content within *G. lucidum*, *F. velutipes*, *Panus tigrinus* and other AMM extracts range from 0.2 to

Table 1. Six odorous phenolic acid and monoterpene compounds found in Asian medicinal mushrooms

IUPAC name and synonym	Structure	Odor	Associated AMM
Phenolic acids			
(E)-3-Phenylprop-2-enoic acid Cinnamic acid		Honey-like	*Ganoderma lucidum* [17] *Lentinula edodes* [18] *Pleurotus eryngii* [18] *Pleurotus ostreatus* [18]
3,4-Dihydroxybenzoic acid Protocatechuic acid		Phenolic	*Agaricus blazei* [19] *Flammulina velutipes* [19, 20] *G. lucidum* [19, 21] *Inonotus obliquus* [19] *L. edodes* [19] *Ophiocordyceps sinensis* [21] *Phellinus linteus* [19] *P. eryngi* [19] *P. ostreatus* [19, 20] *Trametes versicolor* [20] *Sparassis crispa* [19]
4-Hydroxy-3-methoxybenzoic acid Vanillic acid		Vanilla-like	*S. crispa* [19]
Monoterpenes			
3,7-dimethylocta-1,6-dien-3-ol Linalool		Floral	*G. lucidum* [22] *Hericium erinaceus* [23] *Piptoporus betulinus* [24]
1-Methyl-4-(1-methylethenyl)-cyclohexene Limonene		Citrusy	*G. lucidum* [22] *H. erinaceus* [23] *Lentinula edodes* [25]
2-(4-Methyl-1-cyclohex-3-enyl)propan-2-ol α-Terpineol		Fruity-spicy	*Antrodia camphorata* [26] *G. lucidum* [22] *P. betulinus* [24] *P. eryngi* [27]
1-Methyl-4-(prop-1-en-2-yl)-cyclohexan-1-ol β-Terpineol		Woody	*A. camphorata* [26] *H. erinaceus* [23]

Note: Chemical structures were generated using ChemDoodle 2D sketcher (http://web.chemdoodle.com/demos/sketcher).

10 mg/g (in terms of chlorogenic acid equivalents; measured using Folin-Ciocalteu reagent), while individual concentrations of some phenolic acid compounds are up to 3 mg/g [20]. Protocatechuic acid is one of the highest accumulating simple phenolic acids in some AMMs [18-20]. Mushroom extracts also have been found to have 25~50% terpene content by gas chromatography-mass spectrometry with some monoterpenes present at up to nearly a fifth of the extract [26, 27]. However, the phenolic acid and monoterpene compounds presented in Table 1 accumulate at lower concentrations. For example, when detected, limonene is only 0.1% to 0.9% of an extract [23, 24, 27]. The highest-accumulating monoterpene found to date in an AMM is eucalyptol in *A. camphorata* at 18% [26].

As with other VOCs, the concentrations of phenolic acids and monoterpenes vary among parts of the mushrooms and with growth conditions. In *G. lucidum*, the fruiting body has a higher phenol content (12.3 μg/g dry mushroom mass) than does the mycelia harvested from liquid cultures (2.5 μg/g dry hyphal mass) [17]. In *A. camphorata*, only the fruiting bodies contain α-terpineol [26]. Conversely, in *Pleurotus ostreatus*, cinnamic acid is present at a much higher concentration in the mycelia from liquid cultures than in whole mushroom fruiting bodies [18]. Moreover, the concentrations and identities of these compounds may fluctuate due to differences in the origin of the fungus and the substrate on which it grows. Karaman *et al.* [20] found much higher concentrations of phenolic acids in wild Serbian mushrooms than Reis *et al.* [18] did in mushrooms bought at Portuguese supermarkets. Within the same study,

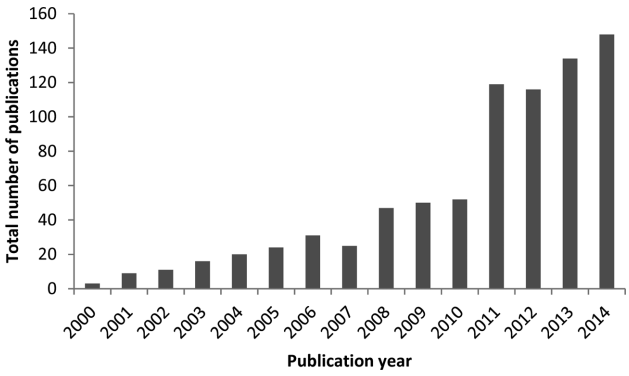


Fig. 1. Relevant publications in PubMed. As of this writing, an NCBI PubMed search of "medicinal mushroom" yields 860 relevant publications of which 851 are research journal articles. While there is a definite increase in publications, it is miniscule compared to the nearly exponential surge seen in other fields.

Rösecke *et al.* [24] detected noticeably different terpene concentrations in *Piptoporus betulinus* from the same source. Finally, the kinds of detected VOCs are also a reflection of the extraction and analytical procedures that are applied [31]. While Karaman *et al.* [20] and Reis *et al.* [18] used different dilutions of methanol, chloroform and ethanol to extract phenolic compounds from powdered fruiting bodies, Kim *et al.* [19] utilized a mixture of acetonitrile and hydrochloric acid. Even methods to generate and store powdered mushrooms differ among these studies. Karaman *et al.* [20] air-dried mushrooms at 50°C before pulverization and storing the powder at room temperature in the dark. Kim *et al.* [19] freeze-dried the mushroom powder before use.

Both phenolic acids and monoterpenes have antioxidant and antimicrobial properties. In fact, most of the antioxidant compounds in commercial AMMs are phenols, especially phenolic acids [20]. The phenol content in mushroom extracts positively correlates with antioxidant and antibacterial activity [20]. Crude AAM extracts generally exhibit effective concentration and inhibitory concentration (IC_{50}) ratios for antioxidant activity at 0.15~0.85 mg/mL [20]. The monoterpenes α-terpineol and linalool have comparable IC_{50}'s around 0.3% (v/v) [32]. Cinnamic acid and one of its metabolites have minimum IC_{50}'s of 0.7~30 μg/mL against certain bacteria and fungi [33], while those of α-terpineol and linalool against several bacteria range from 0.5% to 2% [32]. For cinnamic acid and its metabolic derivative, the IC_{50}'s were generally lower than those of commercial antibiotics streptomycin, ampicillin, bifonazole and ketoconazole. Combinations of monoterpenes may lead to additive or synergistic antibacterial effects [32].

PERSPECTIVES

Purified fungal secondary metabolites such as the β-lactams and statins helped revolutionize twentieth century medicine and have become significant part of humanity's success in treating disease. Consequently, the economic value of products derived from these natural sources is in the billions of dollars [29, 34]. Similarly, even in the absence of rigorous scientific evidence of their efficacies, used both as remedies and as functional foods, medicinal mushrooms are globally popular and lucrative products. For example, the market value of *G. lucidum* products was estimated at US $1,628 million in 1995; global mushroom production was in terms of thousands of metric tons the following year [16]. In Western countries alone, the annual market value of *G. lucidum* products was estimated at over US $1.5 billion in 2006 [16]. It is likely that the market value of traditional fungal medicines have since increased. Unfortunately, rigorous scientific research on the effectiveness of AMMs has not kept pace with the interest; the number of publications relating to AMMs is still small compared to the huge overall volume of pharmacological research (Fig. 1).

While *in vitro* studies using cell cultures have yielded promising results, scientific evidence of *in vivo* effects is still largely limited to anecdotal consumer feedback. Along with the practical difficulties in isolating and identifying VOC compounds, this dearth of overall clinical research into AMMs limits our knowledge of the possible health benefits and economic values of small fungal odorous compounds. Given the ever-increasing preference for natural products and alternative medicine, it is hoped that well-controlled clinical trials will be conducted in the future to determine the benefits of small volatiles after ingestion, inhalation or dermal application. In summary, despite some progress, the scientific study of the health effects of small molecules made by medicinal fungi, especially volatile odiferous compounds, is still in its infancy. We hope this review will stimulate increased interest in their study and change the paradigms of both toxicology and drug discovery research so that they pay more attention to the underexplored medicinal impact of gas phase molecules from fungi.

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