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Review

The cultivation, bioactive components and pharmacological effects of *Armillaria mellea*

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***Armillaria mellea*, a symbiotic fungus in the underground tubers of Chinese medicinal orchid *Gastrodia elata*, is one of the main biological active components and pharmaceutical effects of its host gastrodia. The purpose of this review is to bring attention to the biological properties of this unique *A. mellea* mushroom and its constituents, as well as to suggest the potential for the development of new drugs related to this fungus. It contains various known and untapped bioactive metabolites such as polysaccharides, sesquiterpene aryl esters, steroids and fibrinolytic enzymes. It could be exploited as an important source of new biological natural products with anticonvulsant, immunomodulatory and antimicrobial functions. The batch culture is preferred as an alternative means of getting bioactive components from *Armellaria* fermentation. Challenges in investigations on *A. mellea* include the optimization of culture parameters, the further elucidation of the molecular pharmacological mechanism and relationship between structure and function of their secondary metabolites.**

Key words: *Armillaria mellea*, cultivation, chemical constituents, pharmacological effect.

INTRODUCTION

Gastrodia (Tian ma), the tuber of the orchid, *Gastrodia elata* Blume (Figure 1A), has been used since ancient times in China for the treatment of convulsions with tetanus or epilepsy, stroke and headaches (Tang and Eisenbrand, 1992). Attempts to isolate the active compounds have focused on the rhizome (tuber, Figure 1B) of *G. elata* and the primary active ingredient isolated was gastrodin, a simple glycoside consisting of glucose and 4-hydroxybenzyl alcohol. Additionally, other compounds from gastrodia show potential anticonvulsant activity, specifically 4-hydroxybenzaldehyde, vanillyl alcohol and vanillin (3-methoxy-4-hydroxybenzaldehyde) (Ojemann et al., 2006). Gastrodia and its main constituent gastrodin have been used in China through oral administration, intramuscular injection and intravenous drip in clinical treating of neurasthenia, vertigo and headache (Lu et al., 2002).

G. elata is found primarily in eastern Asia, specifically in the mountainous ranges of China and Korea. Gastrodia had become too rare to meet the demands of herbal medicine use before the discovery of two symbiotic fungi in the host *G. elata* in the 1960s (Xu and Guo, 2000). It requires a fungus *Mycena osmundicola* to sprout the seeds and *Armillaria mellea* to invade the underground tubers without the rootlets to gather up nutrients from the soil. Once these two requirements were met, cultivation of gastrodia became relatively easy. However, the plant grows slowly and the demand has remained high, so it remains one of the more expensive medicinal herbs in China.

More importantly, the medicinal components of gastrodia were found to be mainly the metabolites of the *A. mellea* mushroom (Yang et al., 1984). Modern pharmacological studies showed that *A. mellea* was beneficial to several systems, including the immune, neural and circulatory systems (Liu et al., 2003). The batch fermentation of *Armellaria* mycelia could give the alternative biotechnological way to solve the problem of gastrodia resources. Dozens of investigations have been undertaken to show the chemical constituents, pharmacology and clinical effects of *A. mellea,* but there is a lack of a review paper on this unique symbiotic fungal. The present aim is to review the literature covering culture,

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Figure 1. (A) *A. mellea* seedling and (B) its rhizome (tuber, *Tian ma*) as traditional Chinese medicine.

phytochemical and pharmacological aspects of *A. mellea*. It is hoped that this review will encourage the potential development on the pharmaceutical application of *A. mellea* metabolites.

CULTIVATION

Solid-state culture

Interestingly, the morphology of *A. mellea* was different by a variety of cultivation methods. In solid media, rhizomorphs of *A. mellea* were formed, extending into the agar or on the solid agar surface (Figure 2A). The white fluffy aerial hypha gradually turned brown, finally forming brown crustose aerial hypha (Hannson and Seifert, 1987). The optimum pH and incubation temperature for fungal growth were pH 3.5 and 22°C, respectively (Weitz et al., 2001). The biomass and morphology of *A. mellea* were different in various media. It was reported that the semi-solid media (GPC) containing 2% glucose, 0.6% peptone, 1% corn power, 0.5% agar was suitable for *A. mellea* growth (Cheng et al., 2006a).

Standing liquid culture and shake flask culture

In standing liquid culture, submerged and large clumps of rhizomorphs were formed (Figure 2C). However, the mycelium grew in pellets and no rhizomorphs were foamed in shake flask (Figure 2B). Tan et al. (2002) reported that the optimum initial pH in shake flask cultures was about 5.0 and the optimum nitrogen source were soybean cake powder and wheat bran, respectively. To optimize the submerged culture conditions for the mycelial biomass of *A. mellea,* the optimum media components were suggested (Zhang et al., 2001; Li et al., 2003; Cheng et al., 2007). The optimum concentrations of the media components for the maximum mycelial biomass and polysaccharide production were (w/v): 1.5% silkworm pupa powder, 1.5% soybean cake power, 2.0% sucrose, 1% ethanol, 1.0% glucose, 0.075% $MgSO_4$ and 0.15% K_2HPO_4. In order to enhance the production of mycelial biomass, some elicitors were applied. Ethanol was found to stimulate the mycelial growth (Weinhold et al., 1963).

Fruiting body formation

After asexual growth, aerial hypha of *A. mellea* can differentiate to foam fruiting body (Figure 2D). The fruiting body was induced at 25°C in the dark and then cultured at 18°C (relative humidity 90%). Malt agar medium was the optimal substrate for fruiting body formation (Xue et al., 2004). Fruiting body could also be induced on bran-glucose solid media (bran 3%, glucose 2%, K_2HPO_4 0.3%, $MgSO_4$ 0.15%, agar 1.5%, w/v) (Cheng et al., 2006b).

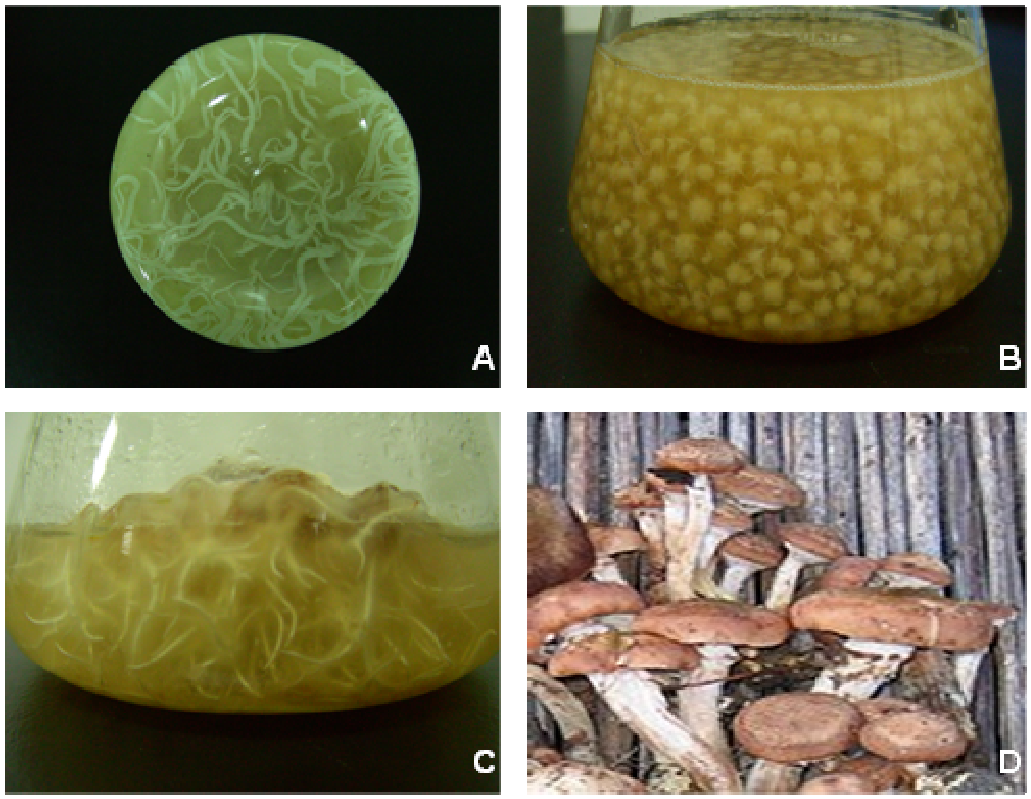

Figure 2. Morphology of *A. mellea* by different cultivation methods. **A.** Rhizomorphs in solid media; **B.** Globular mycelia in shake flask; **C.** Standing liquid culture; **D.** Fruiting body.

CONSTITUENTS

Polysaccharides

A. mellea contains high amount of polysaccharide in the rhizomorph of *A. mellea* which ranged from 4.70% in stable phase to 9.24% in the logarithmic growth phase of the total dry weight (Cheng et al., 2006a). The crude polysaccharide from the cultural mycelium was even more than 10% (w/w) (Zhang et al. 2001). The polysaccharide of *A. mellea* was considered to possess the activities of anti-oxidation (Yang et al., 2007), immunopotentiation (Sun et al., 2009), anti-vertigo (Yu et al., 2006a) and anti-aging activities (Zhang et al., 2001).

In order to analyze the polysaccharides at different developmental stages, *A. mellea* polysaccharides in rhizomorph, fruiting body, mycelia and its fermenting media were extracted, isolated and purified (Chen et al., 2001). They found that the molecular weight of polysaccharides was 10-70 KD by gel filtration. The polysaccharides from mycelia and its fermenting liquor consisted of only glucose, but glucose and xylose in both rhizomorph and fruiting body. Another polysaccharide (about 665 KD) from the fermenting liquor of *A. mellea* was reported to be of D-glucose, D-fucose, D-arabinose, D-mannose, D-rhamnose and D-galactose in a molar ratio of 17.05:0.33:0.36:1:0.42:13.67 (Kong et al., 2003). A polysaccharide (MW 138 KD) in rhizomorph was composed of D-glucose, D-galactose, D-mannose, D-xylose and uronic acid (Shen and Hong, 1998). Moreover, two polysaccharides were isolated from the mycelium of *A. mellea* (Zhang et al., 1995). One was a neutral water-soluble polysaccharide, which composed of mannose, glucose and galactose in a molar ratio of 1.7:5:1. The other was an acidic water-insoluble polysaccharide with a combination of galactose, xylose, arabinose, fucose and rhamnose in a molar ratio of 2.8:2.7:1:1.7:2. Bouveng et al. (1967) isolated two different water-soluble polysaccharides. The first has been shown to be a xylomannan having a backbone of α(1→3)-linked -mannopyranose residues and the second polysaccharide contains D-galactose, D-mannose, L-fucose and 3-*O*-methyl-D-galactose, in a molecular ratio of 6:1:2:2.

Sesquiterpene aryl esters

Sesquiterpene aryl esters are the major components in *A. mellea*. In 1982, a new antibiotic melleolide was isolated from cultured *A. mellea*. It was the first isolated sesquiterpene aryl ester from *A. mellea* (Midland et al., 1982). Since then, more than 37 sesquiterpene aryl esters have been isolated from *A. mellea*. Since armillarin and armillaridin were obtained from *A. mellea* in 1984, many

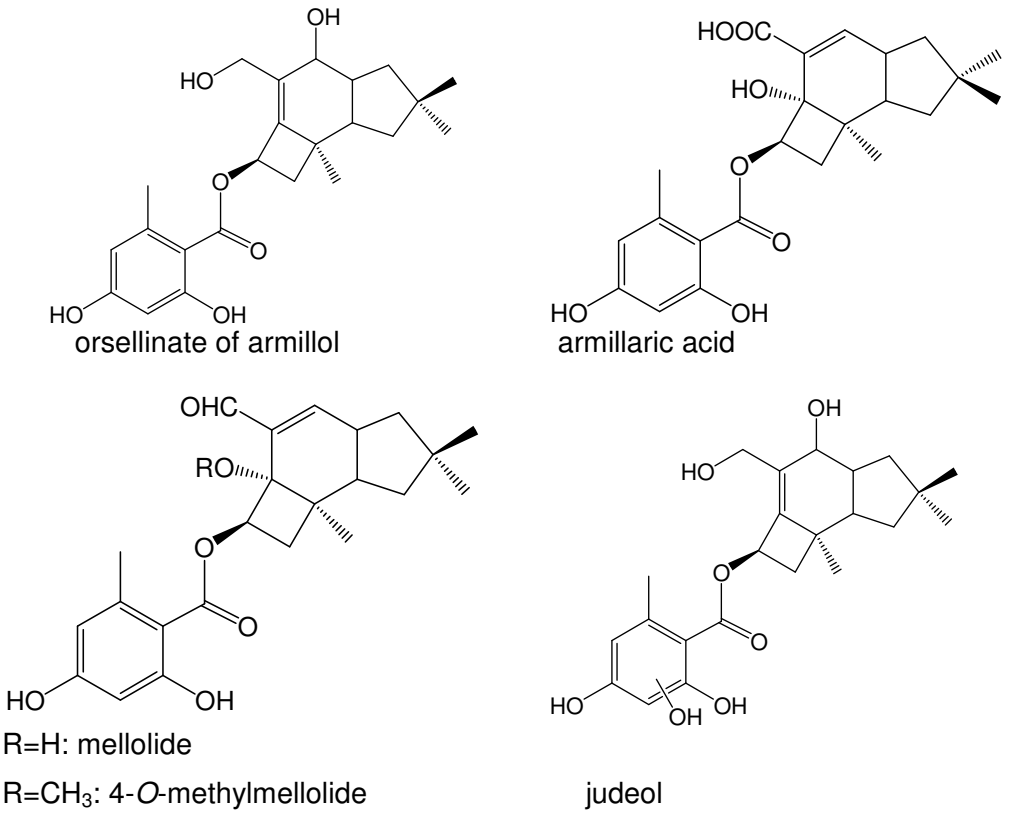

Figure 3. Structures of sesquiterpene aryl esters isolated from *A. mellea*.

sesquiterpene aryl esters incuding armillaricin, armillaribin, armillarigin have been isolated by Yang's group (Yang et al., 1984; Yang et al., 1989a; Yang et al., 1989b; Yang et al., 1990a; Yang et al., 1990b; Obuchi et al., 1990; Yang et al., 1991a; Yang et al., 1991b). Simultaneously, Donnelly's group had analyzed the constituents of *A. mellea* and obtained about 14 new sesquiterpene aryl esters (Donnelly et al., 1984; Donnelly et al., 1985a; Donnelly et al., 1985b; Donnelly et al., 1986; Donnelly et al., 1987; Donnelly et al., 1990a; Donnelly et al., 1990b) (Figure 3). Many of these compounds were shown to have antibiotic and antifungal activity.

Enzymes

A fibrinolytic enzyme, designated as *A. mellea* metalloprotease, was purified from the cultured mycelia of *A. mellea* by ion-exchange chromatography and gel filtration (Lee et al., 2005). This protease hydrolyzed fibrinogen effectively, preferentially digesting the Aα-chain over the Bβ- and r-chains and the enzyme activity was influenced by metal ions. In addition, a lysine-specific proteinase, isolated from the fruiting body of the basidiomycete fungus *A. mellea*, also exhibited potent fibrinolytic activity (Healy et al., 1999). A crude enzyme extract from *A. mellea* showed the polyphenol oxidase activity on 4-methylcatechol (Colak et al., 2007). Laccase, a polyphenol oxidase with copper, was also found in *A. mellea* (Xiao et al., 2002; Wu et al., 2001).

Other constituents

N^6-substituted adenosine (Figure 4) with cerebral protecting activity was isolated from *A. mellea* (Watanabe et al., 1990). A new sphingolipid armillaramide, ergosterol peroxide and ergosta-5, 7-dian-3β-ol were isolated by Gao et al., (2001). Three triterpenes (friedelin, 3α-hydroxyfriedel-2-one and 3-hydroxyfriedel-3-en-2-one) and three steroids (ergosterol, ergosterol peroxide and 6,9-epoxy-ergosta-7,22-dien-3β-ol) were yielded from *A. mellea* (Guo et al., 2007). 5,6-Epidioxyergostan-3-ol was also obtained from the fruiting body of the fungus (Shi et al., 1998).

PHARMACOLOGICAL EFFECTS OF *A. MELLEA*

Effects on nervous system

The fungus, *A. mellea* has been shown to have anticonvulsant properties. The fermentation extracts of *A. mellea* raised the seizure threshold in PTZ-induced

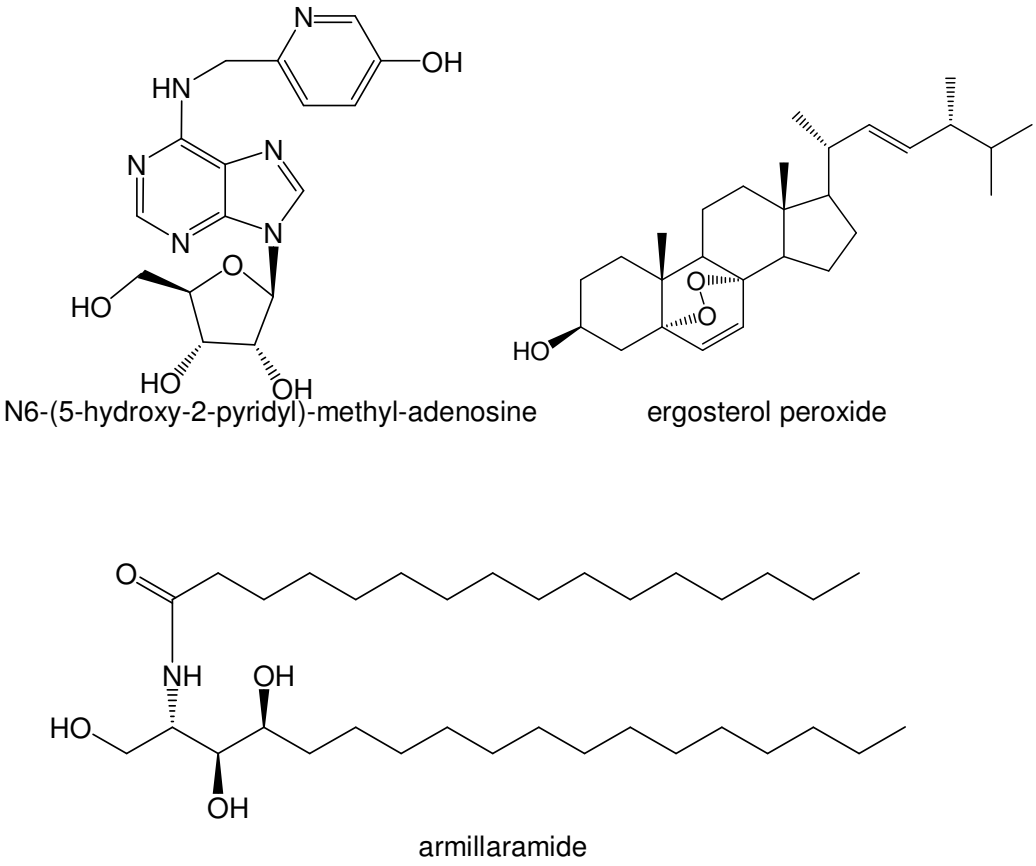

Figure 4. Structures of some other chemical compounds isolated from *A. mellea*.

seizures in mice, as did the aqueous extract of gastrodia (New Drug Group, 1977). *A. mellea* polysaccharides also have therapeutic effect on vertigo induced by machinery rotation (Yu et al., 2006a). N^6-(5-hydroxy-2-pyridyl)-methyl-adenosine (AMG-1) from the mycelia of *A. mellea*, is 1000 times stronger than adenosine in cerebral protecting activity (Watanabe et al., 1990). It has been shown that AMG-1 acts on the presynapse (may be the A1 receptor) to attenuate the release of neurotransmitters. This compound abolished the neurogenic twitch responses induced by electrical field-stimulation, while the responsiveness of rat vas deferens to exogenous acetylcholine was decreased, showing both presynapse and post-synapse depression (Xiong and Huang, 1998).

Effects on immune system

Lin et al. (1988) found that *A. mellea* polysaccride (AP) had immunostimulating activity. AP enhanced the body fluid immunity, increased the production of serum hemolysin and spleen plaque forming cells in normal mice as well as a significant increase in the production of serum hemolysin in cyclophosphamide-induced immunodepressed mice. AP also markedly enhanced Con A-induced lymphocyte proliferation of mouse spleen cells in vitro, but it had no potentiating effect on delayed cutaneous hypersensitivity to 2, 4-dinitrochlorobenzene in normal mice. Moreover, AP increased both clearance rate of charcoal particles and phagocytic activity of macrophages of abdominal cavity in normal mice. Similar results were reported by Dai et al. (2000) and Yu et al. (2001). Exopolysaccharide from *A. mellea* also enhance the immunological function of mice (Kong et al., 2007). Wang et al. (2007) found *A. mellea* polysaccharide improved the immune ability of mice by adjusting the immunocyte and cytokine. Furthermore, Li et al. (2005) reported that the polysaccharide showed protective effects against cyclophosphamide-induced damage to mice bone marrow cells.

Effects on circulation system

Wang et al. (2007) evaluated the effect of compound *A. mellea* tablets on vertebrobasilar insufficiency (VBI). The tablets could reduce whole blood viscosity, anti-platelet aggregation and improve brain blood supply. AMP-1 and AMP-2, the polysaccharides from rhizomorph of *A. mellea*

could obviously decrease the blood glucose in alloxan-induced diabetic mice, while AMP-1 could markedly improve glucose tolerance in normal mice (Yu and Shen, 2002). Additionally, it was reported that *A. mellea* tablets could decrease significantly the blood lipid level in clinical patients (Zhang et al., 1983).

Antimicrobial activity

Many literatures showed that *A. mellea* had antimicrobial activity (Sun, 2004; Yamac and Bilgili, 2006). Sesquiterpene aryl esters could be the candidates of antimicrobial activity. Donnelly et al. (1985) isolated two new sesquiterpene aryl esters, 4-*O*-methylmelleolide and judeol, both of stronge antibacterial activity against grampositive bacteria. Armillaric acid also exhibited marked inhibitory activity against grampositive bacteria and yeast (Obuchi et al., 1990). Momose et al. (2000) isolated three compounds, melleolides K, L and M. Melleolides K were of antimicrobial activity against grampositive bacteria, yeast and fungi. In addition, three antibacterial sesquiterpenoids, melleolides B-D, were yielded from *A. mellea* (Arnone et al., 1986).

Other bioactivities

Yang et al. (2007) reported that the polysaccharides from fruiting body of *A. mellea* have a certain scavenging effect on superoxide anion free radical. Zhang et al. (2002) found that a polysaccharide Am 1 has the function of antimutagenicity in *Drosophilae melanogaster*. The antiaging activity was also found in *A. mellea* extracts (Yu et al., 2006b).

CURRENT FUTURE DEVELOPMENTS

A. mellea is still far from being thoroughly explored, but the information presented here shows a promising fact that *A. mellea* is an unparalleled source for the discovery of new bioactive natural products and development in antiepileptic therapy. Interestingly, the medicinal components of the host, gastrodia, were found to be mainly the metabolites of the *Armellaria* mushroom. It is plausible for the symbiotic fungi to exchange genes with their host plants to produce some common metabolites during long evolutionary process. Genetic research work is needed to reveal the secret of mutulism relationship between host *G. elata* and the *Armellaria* mushroom. Cultivation of *A. mellea* in field is usually avoided as the mycelium is well-known to be capable of causing root rot of many plants (Aguín et al., 2006). The biotechnological approach is a useful alternative for the production of bioactive components of *A. mellea* mushroom. With strain improvement and optimization of culture conditions, the production of secondary metabolites by *A. mellea* culture could be greatly enhanced. The culture in various bio reactors for large-scale production is imperative for the development of *A. mellea* in the industry. On the other hand, compounds that originated from *A. mellea* are offering interesting opportunities for the evaluation of their novel bioactivities in anticonvulsant, immunostimulating properties, among others. It is necessary that the relationship of structure-activity and mechanism of such biological action be further investigated.

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