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REVIEW

Agaricus subrufescens: A review

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Abstract Medicinal mushrooms have currently become a hot issue due to their various therapeutic properties. Of these, *Agaricus subrufescens*, also known as the "almond mushroom", has long been valued by many societies (i.e., Brazil, China, France, and USA). Since its discovery in 1893, this mushroom has been cultivated throughout the world, especially in Brazil where several strains of *A. subrufescens* have been developed and used as health food and alternative medicine. This article presents up-to-date information on this mushroom including its taxonomy and health promoting benefits. Medicinal properties of *A. subrufescens* are emphasized in several studies which are reviewed here. In addition, safety issues concerning the use of this fungus will be discussed.
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Contents

1. Introduction

Mushrooms are popular and valuable foods, which are low in calories but maybe higher in minerals, protein and fiber (Firenzuoli et al., 2008). Their beneficial biochemical properties have also attracted much attention as functional health foods (Gonzaga et al., 2005; Dai et al., 2009). All edible mushrooms are high in vitamin B plus other vitamins such as vitamin C and ergosterol. Mushrooms have historically been used as medicines and tonics (Horm and Ohga, 2008) with *Lentinula edodes* (Berk.) Pegler (Shitake), *Grifola frondosa* (Dicks.) Gray (Maitake), *Ganoderma lucidum* (Curtis) P. Karst (Reishi), *Cordyceps sinensis* (Berk.) Sacc. and *Hericium erinaceus* (Bull.) Persoon being used in traditional Chinese medicine formulations (Dai et al., 2009). Bioactive compounds from edible mushrooms have become new products for health therapy, especially anti-cancer therapies (Ramberg et al., 2010). Studies on anti-cancer properties of mushrooms have revealed the possibility of developing mushrooms as alternative medicines (Ramberg et al., 2010). Wasser (2002) and Ramberg et al. (2010) have reviewed the use of mushrooms as sources of anti-tumor and immunomodulatory compounds. In this paper we focus on *Agaricus subrufescens* Peck and its medicinal properties.

A. subrufescens was called the "almond mushroom" due to its almond taste, and cultivated and consumed in the Atlantic states of the United States from the late 19th to the early 20th century (Kerrigan, 2005). In 1960, this mushroom was discovered again in Brazil, and called the "Piedade mushroom" due to the name of the village, in the Province of Sao Paolo, where it was collected by T. Furumoto who sent it to Japan in 1965 to study its medicinal properties. This mushroom was identified as *Agaricus blazei* Murrill by the Belgian botanist P. Heinemann in 1967. Other common names for this mushroom are Himematsutake in Japan, medicinal mushroom or Sun Mushroom® (Cogumelo do Sol in Portuguese) in Brazil, and Royal Sun Agaricus® in other countries. After the death of Furumoto, the cultivation of *A. blazei* was abandoned in Japan, but because of the interest in the Japanese market, mushroom cultivation commenced in Brazil. *A. subrufescens* had become an important export product for Brazil, fetching higher prices in comparison to other mushrooms (Souza Dias et al., 2004).

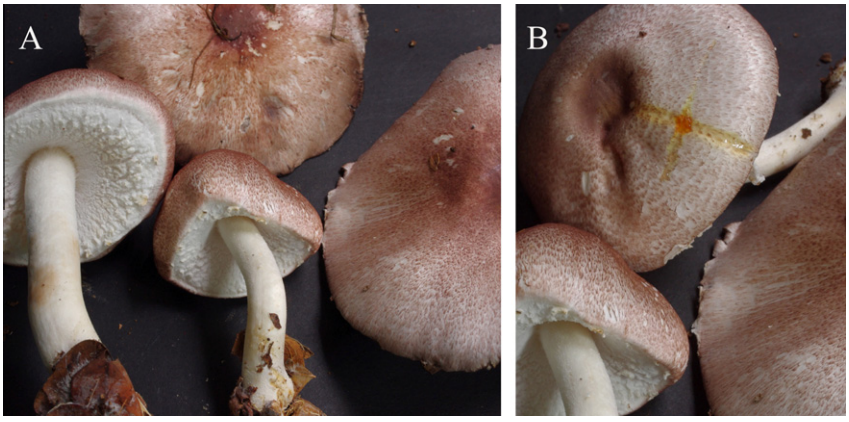

Figure 1 *Agaricus subrufescens*. (A) basidiocarp, (B) Schaeffer's cross-reaction on pileus.

The aim of this present paper is to summarize the available information on *A. subrufescens*, including its taxonomy, phylogeny, distribution, various health benefits and current status of scientific research.

2. Characteristics and taxonomy

A. subrufescens Peck is a gilled fungus belonging to the family of the Agaricaceae in the order Agaricales (Firenzuoli et al., 2008) within the phylum Basidiomycota. It is a saprobe and inhabits rotting leaves often at the borders between forests and parks (Fig. 1). This mushroom is discovered in North America and later South America. However, it has also been found outside America, in Europe, Hawaii, and Thailand where it grows under forest trees and in parks and gardens (Kerrigan, 2005; Arrillaga and Parra, 2006; Dai et al., 2009; Wisitrassameewong et al., 2011).

A. subrufescens has been thoroughly described by Kerrigan (2005) and the illustration and description was also published by Firenzuoli et al. (2008) and by Wisitrassameewong et al. (2011). The basidiomata morphology of this species is variable. Sporocarps can be robust or gracile, both due to the genotype and environmental influences (Kerrigan, 2005). The cap is 20–70 mm broad in button stage and 60–150 mm broad in mature stage hemispherical to convex to plano-convex shape and fleshy. The surface is dry and covered by fibrillose squamulose hairs. The pileus color is somewhat variable and sensible to the light, ranging from brownish-gold, reddish brown, purple brown to brownish orange, more or less pale and sometimes completely white. The basidiospores are chocolate brown (5×4 μm) (Firenzuoli et al., 2008; Wisitrassameewong et al., 2011). The specimens from Thailand differ from those previously reported in terms of size, sturdiness and length of stipe, which were larger, more virgate and fragile than those found in America; their cap color is more reddish than those of the European taxa. The stipe is also highly variable which could be short and firm or more slender and virgate (Kerrigan, 2005). However, the cap shape and lamellae color were in concordance with those reported by Firenzuoli et al. (2008) from Brazil. The small cottony floccules beneath the remnant partial viel are a consistent character of the species (Peck, 1893; Kerrigan, 2005). This important feature is also found in Thai taxa. The morphological variability of this species may be influenced by different climates and ecosystem.

From a nomenclatural point of view, taxonomists agree that the species has been incorrectly referred as *A. blazei* Murrill. Therefore it was proposed by Wasser et al. (2002) as a new species, *Agaricus brasiliensis* Wasser, Didukh, de Amazonas & Stamets, and then synonymised by Kerrigan (2005) with *A. subrufescens* which has priority since it is older. Callac (2007) noted that *A. brasiliensis* was illegitimate because it is a later homonym of *A. brasiliensis* Fr. 1830. For taxonomy and synonymy of this taxon we have followed Kerrigan (2005), Arrillaga and Parra (2006) and Ludwig (2007). *A. subrufescens* Peck was published in 14 March 1894 and has priority over *Agaricus subrufescens* Ellis & Everh. which was published in 29 March 1894 as '*Agaricus* (*Tricholoma*) *subrufescens*' according to a recent updating in Index Fungorum (see http://www.indexfungorum.org/names/Names.asp) and MycoBank (http://www.mycobank.org/MycoTaxo.aspx).

Agaricus subrufescens Peck, Ann. Rep. N.Y. St. Mus. **46**: 25 (1893).

= *Agaricus subrufescens* Ellis & Everh., *Proc. Acad. nat. Sci. Philad.* **45**: 440 (1894) is a homonym of the earlier name.
= *Psalliota subrufescens* (Peck) Kauffman, 1918, *Publications Mich. geol. biol. Surv., Biol. Ser.* 5 26, 239.
= *Agaricus rufotegulis* Nauta, 1999 *Persoonia* 17, 230.

Misapplied names:

Agaricus blazei Murrill, 1945, 8, 193.
Agaricus blazei Murrill *sensu* Heinemann, 1993 Bull Jard Bot Belgium 62, 653.

Illegitimate name:
Agaricus brasiliensis Wasser, M. Didukh, Amazonas & Stamets, in Wasser, Didukh, Amazonas, Nevo, Stamets & Eira, *International Journal of Medicinal Mushrooms* (Redding) **4**(4): 274 (2002) Nom. illegit., Art. 53.1, a homonym of *Agaricus brasiliensis* Fr. 1830.

Wasser et al. (2002) rejected conspecificity between specimens from Brazil belonging to *A. blazei* Murrill *sensu* Heinemann and those from north America belonging to *A. subrufescens* Peck, based on size and shape of fruiting bodies and pileal surface, type of pileal covering, presence of cheilocystidia and spore size. Kerrigan (2005) however, showed that a cultivated specimen originating from Brazil and a wild specimen from USA (California) belonged to the same species based on biological and phylogenetic approaches. Firstly, the two specimens were interfertile and first generation fertile hybrids were produced. Secondly, the north and south American specimens had 100% similarity of ITS1 + 2 sequences, which were produced from the ITS1 and ITS4 primers (White et al., 1990). Kerrigan (2005) also extended the synonymy to *A. rufotegulis* Nauta described for European specimens which also exhibited similar ITS1 + 2 sequences, however, interfertility between European and American specimens has not been tested. Finally, some specimens from Hawaii were also included in *A. subrufescens* but their ITS1 + 2 sequences slightly diverged from those of others (Kerrigan, 2005). Until other sequence data become available and interfertility tests are performed, we exclude neither the existence of infraspecific taxa, nor the fact that *A. subrufescens* might be a complex of species.

This caution however, concerns only the non American populations, because the synonymy between *A. blazei* Murrill *sensu* Heinemann from south America and *A. subrufescens* Peck from north America appears to be indisputable. The major argument that Wasser et al. (2002) used against synonymizing *A. blazei* with *A. subrufescens* was the divergence that appeared in the phylogenetic trees of Geml et al. (2004) in which '*A. blazei*' and '*A. subrufescens*' highly diverge, in contradiction with the results of Kerrigan (2005). However, not only was the identification of the strain W17 used to represent *A. subrufescens* not confirmed because sporophores of W17 were not examined or reported, but also a simple examination of the sequence of W17 used in the study of Geml et al. (2004, GenBank accession number AY484674), showed that its ITS1 region is close to ITS1 sequences of species belonging to the section *Arvenses*, which its ITS2 region is similar (a single different character) to many ITS2 sequences of *Agaricus bisporus* (the button mushroom, see for example GenBank accession number DQ404388, isolate AFTOL-ID 448). *A. bisporus* belongs to the non related section *Bivelares* and differs from any other ITS2 sequence of *A. subrufescens* or *A. blazei* at

Table 1 Bioactive compounds from *Agaricus subrufescens*.

α-(1–4)-; β-(1–6)-glucan	Fujimiya et al., 1998
α-(1–6)-; α-(1–4)-glucan	Mizuno et al., 1990
β-(1–6)-; β-(1–3)-glucan	Mizuno et al., 1990
β-(1–6)-; α-(1–3)-glucan	Mizuno et al., 1990
Lectin	Kawagishi et al., 1990
Riboglucan	Cho et al., 1999
Glucomannan	Hikichi et al., 1999
β-(1–2)-; β-(1–3)-glucomannan	Tsuchida et al., 2001, Mizuno et al., 1999
Ergosterol	Takaku et al., 2001
Sodium pyroglutamate	Kimura et al., 2004
RNA–protein complex	Gao et al., 2007
Agaritine	Stijve et al., 2003
Blazein	Itoh et al., 2008

about 30 positions. The deposited sequence of W17 probably results from an accidental assemblage of sequences from two different strains.

In conclusion, *A. subrufescens* Peck is a species distributed in north and south America. We provisionally include in this species other populations known in Europe, Oceania and Asia. Further studies will be necessary to determine the biological and genetic divergence between these populations. Large phenotypic variability is generally found inside and between populations of some other species of *Agaricus* having a large distribution range across several continents. For example, in *A. bisporus*, three varieties were described based on biological, genetic and morphological traits; these varieties remain interfertile, but have different life cycles (Kamzolkina et al., 2006); however tropical populations of this species, reported by Heinemann (1956) have never been studied. In contrast, *Agaricus subfloccosus* (Lange) Pilat has been first considered as a species complex (Kerrigan et al., 1999); but the two main homothallic lineages being intersterile and having different habitats were finally elevated at the species rank (Kerrigan et al., 2008). These two examples show how the life cycle can have an impact on the population structure, on the speciation processes, on the circumscription of the species, and finally on the taxonomy. In comparison, *A. subrufescens* is poorly known: population genetic and life cycle studies of *A. subrufescens* are almost lacking. For example, a biological approach of Kerrigan (2005) suggested that *A. subrufescens* have an amphithallic life cycle (partly heterothallic and partly pseudohomothallic), while a recent cytological approach (Souza dias et al., 2008) suggested that the life cycle be heterothallic. In such conditions, taking into account that reproductive biology and genetic resources are crucial knowledge for strain improvement in species of interest, and more taxonomic inference seems therefore premature. Instead, one can appreciate the large available variability existing in this species (or species complex), now extended to Thailand, and its potential for human health.

In this review we refer to the mushroom as *A. subrufescens* and treat results of studies on mushrooms named as *A. blazei* and *A. brasiliensis* as this species. However, all the data concerning the medicinal properties reported are based on studies of a limited number of isolates, all or nearly all of them from the local population in Brazil. It is likely that medicinal properties may differ qualitatively or quantitatively among other populations. It appears that the genetic resources of this species remain significantly underutilized.

3. Chemical composition

The fruiting bodies of *A. subrufescens* contain 89–91% water, which is in general less than that of *A. bisporus*. Almost 48% of total dry matter consists of crude protein and 18% of carbohydrates, but the lipid content is only 0.5% (Györfi et al., 2010). The fruiting bodies of *A. subrufescens* contain high levels of valuable minerals, e.g. potassium, phosphorus, calcium, magnesium and zinc. Nevertheless, a minute amount of cadmium was also detectable (Györfi et al., 2010).

Bioactive compounds of mushrooms can be isolated from their fruiting bodies, or culture extraction from pure culture of mycelia (Chang and Miles, 2004). *A. subrufescens* has been reported to produce various bioactive compounds that have potential to treat many diseases (Firenzuoli et al., 2008). This mushroom has been used as a medicinal food for the prevention of cancer, diabetes, hyperlipidemia, arteriosclerosis, and chronic hepatitis, and is known to stimulate the immune system (Takaku et al., 2001).

Several categories of molecules are involved in beneficial effects of *A. subrufescens* and most of them are common to the entire fungal kingdom (Levitz, 2010). Some compounds (such as ergosterol and β-glucans) are considered as biochemical markers for the kingdom and are ubiquitous. β-Glucans are cell wall constituents that can be found in many fungi (Levitz, 2010). A list of bioactive compounds identified in *A. subrufescens* is given in Table 2.

The bioactive compounds isolated from *A. subrufescens* are mainly polysaccharides such as riboglucans (Cho et al., 1999), β-glucans (Gonzaga et al., 2005; Fujimiya et al., 1998; Mizuno et al., 1990) and glucomannans (Hikichi et al., 1999). Chemical analysis of polysaccharides in *A. subrufescens* has been measured using High Performance Liquid Chromatography (HPLC) (Volman et al., 2010). *A. subrufescens* polysaccharide consists of 57.7% glucose, 27.7% galactose, 7.3% mannose/xylose, and 4% fucose (Volman et al., 2010). Structural analysis of β-glucans with 1–4 and 1–6 linkages showed they were presented in a ratio of about 1:1. A small proportion of 1–3 linkages was also detected (Volman et al., 2010). More recently, a new configuration of polysaccharide purified from *A. subrufescens* has been proposed (Liu et al., 2011). The purified polysaccharide had a (1–6)-linked α-D-glucopyranosyl backbone and one terminal (1-)-α-D-glucopyranosyl branch along the main chain in a ratio of 1:1:1 (Liu et al., 2011; Liu and Sun, 2011). The extract probably existed in a triple-strand helical conformation in water (Liu et al., 2011). Alkaline extraction and methylation analysis of purified polysaccharide from *A. subrufescens* showed six types of residues (Liu and Sun, 2011). The extract had a backbone consisting of (1–6) linked-β-D galactopyranosyl, (1–6) linked-β-D glucopyranosyl and (1–3,6)-linked β-D glucopyranosyl residues and branched with three terminal (1-)-β-L-fucopyranosyl, (1-)-β-L-arabinofuranose and (1-)-β-D glucopyranosyl unit at the *O*-3position of (1–3,6)-linked-α-D-glucopyranosyl along the main chain in the ratio of 20:10:10:6:2:2 (Liu and Sun, 2011).

Anti-tumor activity has been found in lipid fractions of *A. subrufescens* (Takaku et al., 2001). The bioactive substance with the anti-tumor effect was purified and then identified as

Table 2 Beneficial properties from *Agaricus subrufescens* that have been published.

Medicinal properties	References
Tumor growth reduction	Mizuno et al., 1990; Takaku et al., 2001; Itoh et al., 2008; Kim et al., 2009; Gonzaga et al., 2009; Niu et al., 2009; Yu et al., 2009; Ohno et al., 2001; Itoh et al., 1994; Ito et al., 1997; Kawagishi et al., 1989, 1990; Kimura et al., 2004, Pinto et al., 2009, Jumes et al., 2010
Immunomodulatory activities	Liu et al., 2008, Chan et al., 2007; Kimura et al., 2004; Niu et al., 2009; Ramberg et al., 2010
Immunostimulatory effects	Kasai et al., 2004; Kaneno et al., 2004; Yuminamochi et al., 2007; Førland et al., 2010, 2011; Johnson et al., 2009; Kozarski et al., 2009; Sorimachi et al., 2001; Endo et al., 2010; Fujimiya et al., 1998
Antimicrobial activities	Bernardshaw et al., 2005; Bernardshaw et al.,2006
Antiviral activities	Bruggemann et al., 2006; Faccin et al., 2007
Anti-allergy effects	Ellertsen and Hetland 2009

ergosterol (Takaku et al., 2001). Ergosterol is functionally an analog of mammalian cholesterol. It is a component of the fungal cell membrane and has been described as the beneficial component in some medicinal mushrooms such as *Lentinus edodes* (Berk) Pegler (Jasinghe and Perera, 2005), *A. bisporus* (J.E. Lange) Imbach (Lindequist et al., 2005; Volman et al., 2010), *Grifola frondosa* (Dicks.) Gray (Lindequist et al., 2005) and *Ganoderma lucidum* (Curtis) P. Karst (Lindequist et al., 2005).

Other bioactive molecules providing medicinal benefits in *A. subrufescens* are sodium pyroglutamate which has anti-angiogenic and has anti-tumor and anti-metastatic properties, as well as immune-modulator activity (Kimura et al., 2004). An RNA–protein complex enriched fraction is cytotoxic by stimulating the apoptosis pathways and has *in vitro* anti-tumor activity (Gao et al., 2007). *A. subrufescens* lectin, a glycoprotein normally associated with β (1-6)-glucan, is characterized by Kawagishi et al. (1990) and claims to have anti-tumor activity. Aromatic hydrazines, especially agaritine and its derivatives also occur in *A. subrufescens* (Nagaoka et al., 2006). The agaritine extracted from *A. subrufescens* exerts an *in vitro* anti-tumor activity in leukemic cells with no significant effects on normal lymphatic cells. The cytotoxicological effect of agaritine has been demonstrated on leukemic cell lines with an IC^{50} of 2.7–16 μg/ml. (Endo et al., 2010; Akiyama et al., 2011). The agaritine concentration is comparable to the concentration obtained from *A. bisporus*, and is approximately 1.8 mg/g dry weight (Stijve et al., 2003; Nagaoka et al., 2006). Blazein (named after the synonym *A. blazei*) is a steroid derivative found in *A. subrufescens* with an *in vitro* anti-cancer activity on human lung cancer LU99 cells without affecting normal human lymphocytes (Itoh et al., 2008).

4. Pharmaceutical properties

A. subrufescens is a well-known medicinal mushroom used in many countries, and thus consumption of this mushroom is used as an alternative way to cure diseases. Various pharmaceutical activities have been found associated with *A. subrufescens* and researches to reveal the function of bioactive compounds are extensive. Recent studies have been performed *in vitro* and *in vivo* to confirm the mushrooms therapeutic properties (Firenzuoli et al., 2008). Identification of (novel) immunomodulating bioactive compounds from the mushroom may also help in new treatments for patients suffering from cancer and immunodeficiency (Ohno et al., 2001).

5. Anti-cancer and tumor suppressive activity

There have been many studies showing the anti-tumor activity of *A. subrufescens* (Firenzuoli et al., 2008). In the last decade, many bioactive substances (Table 1) have been shown to exhibit the potential anti-tumor properties. The major anti-tumor substances from *A. subrufescens* are polysaccharide-enriched extracts and protein–bound polysaccharide complexes (Kawagishi et al., 1989; Itoh et al., 1994; Ito et al., 1997). Mushroom polysaccharides are thought to prevent oncogenesis, have shown an indirect anti-tumor activity against various allogeneic and syngeneic tumors, and prevent tumor metastasis (Kimura et al., 2004). The major polysaccharide fraction of *A. subrufescens* extract is revealed as β-glucan (Ohno et al., 2001). The anti-tumor mechanisms of *A. subrufescens* extracts have been shown by oral administration in different laboratory mice models such as Sarcoma 180-implanted bearing mice (Takaku et al., 2001), Meth-A fibrosarcoma tumor-bearing mice (Kawagishi et al., 1989, 1990; Mizuno et al., 1990, 1998; Itoh et al., 1994; Fujimiya et al., 1998; Takaku et al., 2001) and BALB/c nu/nu mice implanted leukemic model (Kim et al., 2009). Gonzaga et al. (2005) extracted the polysaccharide-complex from *A. subrufescens* and characterized a glucan–protein complex by FTIR, ^{13}C NMR and ^{1}H NMR spectroscopy. The extract exhibits both of α and β glycosidic linkage of glucans. The result indicates that β-glucans are the predominant structures as compared to α-glucans.

A. subrufescens extracts may be cytotoxic to tumor cells *in vitro* and inhibit the growth of human leukemia cells by inducing apoptosis (Jin et al., 2007), which directly inhibit the *in vitro* growth of tumor cells (Jin et al., 2007; Kim et al., 2009). Kim et al. (2009) used the agarose DNA fragmentation assay (DNA laddering assay) and cell death detection ELISA to demonstrate the induction of apoptosis in cancer cell culture. Ethanol/water extracts or *n*-hexane extracts induced *in vitro* apoptosis. Water and ethanol extracts have slight or lacked direct inhibitory effects. The anti-tumor effect on the implanted leukemia mice model may therefore have been due to the enhancement of the host immune system. Based on *in vivo* and *in vitro* results, they conclude that *A. subrufescens* polysaccharides may not possess direct anti-leukemic activity (Kim et al., 2009). Jin et al. (2007) also confirmed

that proteoglycan enriched fraction (ratio of polysaccharides to peptides 74:26) of *A. subrufescens* induced apoptosis in human leukemic U937 cells through down regulation of Bcl-2 and activation of caspase-3 and cleavage of poly (ADP-ribose) polymerase-PARP. By using transfected U937 cells with Bcl-2 containing plasmids (i.e. increasing the Bcl-2 expression level) the major regulators of the *A. subrufescens* extract inducing apoptosis were shown to be Bcl-2 and caspace-3 associated to the dephospholyration of the Akt cascade signal pathway.

A human prostate carcinoma cell line was used as a model to investigate the anti-tumor inhibitory activity of polysaccharide-enriched crude extracts (Yu et al., 2009). The activities of caspase-3 and the DNA fragmentation were most enhanced in treated prostate cancer cell lines. The crude extract containing (1–3)-β-D-glucan probably inhibited the growth of prostate cancer cells via an apoptotic pathway and expressed anti-proliferation and anti-angiogenic mechanisms against prostate tumor growth (Yu et al., 2009). *A. subrufescens* extracts may affect angiogenesis mechanisms of tumor cells. Angiogenesis obtains nutrients for growth and development of tumor cells and thus this may inhibit their growth. Tumor inhibitory rate in s180 xenograft models also increased in a dose-dependent manner using low molecular weight polysaccharides (LMPAB) isolated from *A. subrufescens* (Niu et al., 2009). The anti-angiogenesis effect of LMPAB was found in chicken embryo chorioallantonic membrane (CAM) angiogenesis.

6. Anti-cancer and tumor suppressive activity of polysaccharides

It is well-known that crude extracts of *A. subrufescens* have beneficial medicinal properties, however, they are rather complex and the mode of action of the components of the extracts need to be established. Several studies have characterized the constituents of *A. subrufescens* crude extracts (Gonzaga et al., 2005) and polysaccharides have consistently been shown to be the major ingredients (Gonzaga et al., 2005; Dong et al., 2002; Ohno et al., 2001). Extensive research has been carried out to elucidate the structures of polysaccharides and their relationship with anti-tumor activity (Gonzaga et al., 2009). Most anti-tumor compounds in fungi are generally attributed to polysaccharides with β-(1–3) glucan branches. The main polysaccharides in *A. subrufescens* are from the β-(1–6) glucan protein complex (Ohno et al., 2001). β-Glucan is a well-known component of the polysaccharide crude that is important in tumor cell inhibition.

β-Glucan is a D-glucose molecule linked by glycosidic bond at the β position. β-Glucans are well known as anti-tumor agents that are naturally found in bacteria and fungi (Firenzuoli et al., 2008). Several studies have isolated this bioactive compound from fruiting bodies, mycelium and liquid culture medium of mushrooms (Chang and Miles, 2004; Boonyanuphap and Hansawasdi, 2011). *A. subrufescens* has high content of β-(1–6)-(1–3) glucan (Kozarski et al., 2009) and glucan–protein complex (Gonzaga et al., 2005). β-Glucans are thought to enhance the immune system to suppress or kill pathogens and cancer cells (Ohno et al., 2001), which appear to indirectly inhibit tumor cells via activating different immune responses. The anti-tumor effects of polysaccharides work by specific and non-specific immune response activation. Tumor size of Walker-256 tumor bearing rats was reduced after treating with four treatments of *A. subrufescens* (pure powdered basidiocarp, aqueous, acid and alkaline extracts) (Jumes et al., 2010). β-Glucan–protein complex polysaccharide also shows strong *in vivo* antitumor effect against Sarcoma 180 cell. Moreover, it also can increase the antitumor activity of other chemotherapeutics (Gonzaga et al., 2009).

The β-glucan content has been measured in different stages of *A. subrufescens* fruiting body development (Camelini et al., 2005). Immature fruiting bodies, mature fruiting bodies with immature spores and mature spores were used to elucidate the amount and structural characterization of β-glucan. The yield of β-glucan in mature fruiting bodies with immature spores of *A. subrufescens* was slightly higher as compared to immature fruiting bodies and mature fruiting bodies with mature spores. The important anti-tumor activity was linked to a water-soluble β-(1–6)-(1–3)-glucan (Mizuno et al., 1990; Camelini et al., 2005). However, no significant difference in tumor growth inhibition or in function of the different maturation phases of *A. subrufescens* fruiting bodies was detected (Mourão et al., 2009).

7. Other promising agents

Other bioactive compounds, excluding polysaccharide and protein polysaccharide complex extracts, have also been shown to exhibit anti-tumor effects against tumor cells. Takaku et al. (2001) used a purified lipid fraction of *A. subrufescens* to investigate the anti-tumor activity on sarcoma bearing mice. After purification, the bioactive compound was identified as ergosterol by ^{1}H NMR and mass spectrometry (Takaku et al., 2001). The neovascularization induced by Lewis lung carcinoma cell packed chambers was inhibited by the intraperitoneal administration of ergosterol at doses of 5, 10 and 20 mg/kg for 5 consecutive days (Takaku et al., 2001). Neovascularization is critical for the growth of tumor cells and is mediated by physiological substances produced by the tumors. Therefore, the inhibition of neovascularization is one of the several potential ways for cancer therapy (Takaku et al., 2001).

Blazein has also been isolated from *A. subrufescens*, which can induce DNA fragmentations in *in vitro* culture cell of human lung cancer LU99 and stomach KATOIII cancer cells (Itoh et al., 2008). The formation of a DNA ladder on agarose gel after cultivation in the presence of blazein indicated the occurrence of apoptosis. In contrast, no induction of apoptosis was observed by blazein in normal lymphocytes prepared from healthy volunteers (Itoh et al., 2008). Itoh et al. (2008) suggested that the activity of these compounds is specific for cancer cells and would not be destructive to healthy tissues. However, there is little amount of literature concerning this steroid compound. These findings suggest that the bioactive compounds from *A. subrufescens* extract can inhibit the growth of many types of cancer cells.

More recently, a new promising agent was recognized from *A. subrufescens* as a candidate for cancer therapy (Barbisan et al., 2002;Akiyama et al., 2011). Purified agaritine from *A. subrufescens* exerted the anti-tumor activity against *in vitro* leukemic tumor cells (Endo et al, 2010; Akiyama et al., 2011). However, the action of agaritine differs from β-glucan which indirectly suppresses proliferation of tumor cells. Agaritine has been shown to be weakly cytotoxic to normal lymphatic cells. In addition, mutagenicity test using *Umu salmonella* was negative in contradiction with a previous mutagenicity

study. Agaritine induces moderate apoptosis in leukemic cells by caspase activation via cytochrome c release from mitochondria (Akiyama et al., 2011). However, the direct target of agaritine for apoptosis induction is still difficult to understand.

8. Anti-genotoxicity activities

Some *in vitro* and *in vivo* genotoxic studies (e.g. DNA damage and aberration tests) have been performed on chemical carcinogenesis models (Angeli et al., 2006, 2009; Barbisan et al., 2002; Delmanto et al., 2001). It appears that *A. subrufescens* extracts or polysaccharides enriched fractions have direct preventive effect against DNA damaged induced by known genotoxic chemicals (Menoli et al., 2001; Delmanto et al., 2001). Cyclophosphamide is a clastogenic agent which induces disruption or breakages of chromosomes (Matsumoto and Clus, 2000). It has been used to study in preventive of chromosome aberration test in animal models treated by *A. subrufescens* extracts. An aqueous solution from this mushroom inhibited induction of micronuclei by cylophosphamide in bone marrow and peripheral blood of treated Swiss mice (Delmanto et al., 2001). An aqueous solution obtained from *A. subrufescens* tea product and dried *A. subrufescens* reduced the frequencies of mutagen methyl methanesulfonate (MMS) micronuclei induction in Chinese hamster V79 cell lines (Menoli et al., 2001). The comet assay and GST-P-positive liver foci development were used to evaluate the influence of mushroom aqueous extracts on liver cell DNA damage and the initiation of liver carcinogenesis. *A. subrufescens* extracts protected rat liver cells against diethylnitrosamine induction. Diethylnitrosamine is a genotoxic carcinogen that leads to the initiation of preneoplastic hepatocytes (GST-P positive foci) (Barbisan et al., 2002). Treatment with high concentrations of *A. subrufescens* extract (11.5 mg/ml) significantly reduced DNA damage, indicating a protective effect against different doses of diethylnitrosamine (DEN)-induced liver genotoxicity. Barbisan et al. (2003) observed no protective effect of GST-P-positive liver foci and the extract significantly increased the number of positive foci. More recently, the genotoxic and preventive genotoxic effect of β-glucan enriched extract against chemicals was evaluated *in vitro* in human lymphocyte cells and in human hepatoma cell-Hep G2 cell lines (Angeli et al., 2006; Angeli et al., 2009a, 2009b; Ishii et al., 2011). β-Glucan from *A. subrufescens* extracts reduced DNA damage induced by Benzo-a-pyrene (BaP) and H_2O_2 in the comet test, micronuclei test and in a binding assay (BaP only).

9. Biological responses on the Immune System

Beneficial properties of fungi on the human immune system have been demonstrated for several decades (Lucas, 1957; Chihara, 1969; Chihara et al., 1970; Ooi and Liu, 2000; Wasser, 2002). Many studies stated that the immune system can be stimulated by fungal extracts and their polysaccharides or protein–polysaccharides complexes (Lucas, 1957; Sternberg et al., 1963; Wooles and Di Luzio, 1963; Chihara, 1969).

To protect against infections, the human body uses both innate immunity, including physical barriers, phagocytes and soluble mediators (e.g. complements), and adaptive immunity, including antibodies and cytotoxic T cells, to destroy pathogenic invaders and also prevent re-infections (Chan et al., 2007). It is consensual that cell mediated immunity (CMI) is the main mechanism of defense concerning fungi (Blanco and Garcia, 2008). In cell mediated immunity, the involvement of predominant cell types, such as macrophages and other cell types appears to depend on pathogen associated molecular patterns from fungal genera (Romani, 2011). It also appears that the elimination of fungi by an immune response occurs via the innate immune cells such as antigen presenting cells, monocytes and dendritic cells (Romani, 2011). In fact, bioactive compounds from fungi are derived from cell wall or structural compounds, such as β-glucan, chitin, mannan, gluco-protein and lipids such ergosterol (Romani, 2011). Therefore, many different fungal genera can share a common antigen which is recognized by pattern recognition receptors of host cells. However the fungal composition can also vary with the morphotype which can differ with the morphologic state, i.e. spores versus filamentous fungi, and yeast versus pseudo-hyphae or hyphae in dimorphic fungi (Romani, 2004, 2008; Blanco and Garcia, 2008). Nevertheless, there have been some studies reported that humoral immunity can protect against fungal infection. Antibody production of the humoral immunity has importance for protection against fungi (Blanco and Garcia, 2008). The main actions of antibodies in fungal infection involve prevention of adherence, antibody opsonization, toxin neutralization and antibody-dependent cellular cytotoxicity (Blanco and Garcia, 2008).

The critical point of the first line defense of innate and acquired immunity against fungi is the production of chemokines in order to recruit lymphocytces (phagocytes, T and B cells) at the fungal infection sites (Blanco and Garcia, 2008). Chemokine production is generally activated by an invariant recognition process involving invariant pathogen associated molecular patterns. Pathogen associated molecular patterns can be recognized by a series of pattern recognition receptors of host cells in order to consequentially promote the activation of the immune system e.g. chemokine, cytokine, phagocytosis and defensin (Romani, 2011). The receptors involving pattern recognition receptors consist of Toll-like receptors, C-type lectin receptors, complement receptor 3 and Dectins receptors (Brown, 2006; Romani, 2011). The immunostimulatory *A. subrufescens* extracts also appear to interact with several pattern recognition receptors and occur partly via innate immune cells of cell-mediated immunity (CMI), involving proteo-glucan or glucans or even peptido-glucan and/or mannan residues (Johnson et al., 2009, Førland et al., 2010). Therefore it appears that the stimulatory effect appears to be mainly mediated by pattern recognition receptors including via the lectin-binding site for β-glucans in complement receptor 3 (Czop et al., 1989; Vetvicka et al., 1996; Akramiene et al., 2007; Romani 2011). The β-glucans from *A. subrufescens* extracts may mediate complement receptor 3 through the lectin-binding site (Johnson et al., 2009; Førland et al., 2010) and subsequently stimulated complement 3 which is a crucial component in the complement system. Shimizu et al. (2002) also proposed the activation of the alternative pathway in the complement system by *A. subrufescens* particles.

The immunostimulatory and its responses of water and ethanol extract from *A. subrufescens* have been established in many studies for the last decade. Immunotherapy has increasingly been considered as an alternative way to treat cancers. There are many clinical trials being carried out using fungi and clinical results are showing potential (Ohno et al., 2001;

Hsu et al., 2007; Firenzuoli et al., 2008; Ohno et al., 2011). *A. subrufescens* is rich in biological response modulators, such as proteoglucans (Hetland et al., 2008). Anti-tumor, antibacterial and anti-viral activities have been demonstrated by the extracts from *A. subrufescens* (Ohno et al., 2001; Gonzaga et al., 2009; Bernardshaw et al., 2005; Bernardshaw et al., 2006; Bruggemann et al., 2006; Faccin et al., 2007; Firenzuoli et al., 2008; Hetland et al., 2008). Those biological responses are performed by stimulating or modulating the immunity (Hetland et al., 2008).

Extracts of *A. subrufescens* have been used successfully as adjuvant in DNA vaccine to improve their efficacy against hepatitis B virus (HBV) infection and foot-and-mouth disease (FMDV) (Hetland et al., 2008). Grinde et al. (2006) have used microarray examination to prove the activation of expression level of some modulators in the immune system. The result from hepatitis C virus patients administrated with *A. subrufescens* extract, resulted in the increase of expression of the IFN-α, β receptors. The up-regulation of this gene is involved in cell signaling and cycling, as well as in transcriptional regulation (Grinde et al., 2006).

10. Cytokine induction

A. subrufescens is shown to stimulate cytokine production, such as interleukin-12 (Kasai et al., 2004), interferon-γ (Yuminamochi et al., 2007) and natural killer (NK) activity (Kaneno et al. 2004). NK cells are white blood lymphocytes of the innate immune system that is recognized as important for immune surveillance for tumor cells (Talmadge et al., 1980; Smyth et al., 2002) and pathogen (Biron and Brossay, 2001; Yokoyama and Scalzo, 2002). According to the study of Kasai et al. (2004), IL-12 is a cytokine known to be a critical regulator of cellular immune responses. Cytokine is involved in the activation of NK cells against tumor cells. It is induced after administrated with hemicellulase derived mycelia extract. Oral administration of the fraction also enhances the activity of NK cells in the spleen. These results are consistent with Yuminamochi et al. (2007), powdered dried fruiting bodies and hemicellulase-digested component of *A. subrufescens* augmented NK cells activation through IL-12 mediated IFN-γ production. IL12 exert a potent antitumor activity in different murine tumors models. The IL-12 *in vivo* antitumor activity is found to be mediated by NK and/or NKT (Smyth et al., 2002). IL-12 sustains many biological activities including differentiation of lymphocytes, activating the phagocytes, induction of nitrogen oxide, induction of Th1 responses and NK cells activation (Romani, 2011). The T-cells IL12/IFN-γ driven response is generally considered as central axis for protection against fungi. (Romani, 2008).

The analysis of spleen lymphocytes of mice model after intake with β-glucans enriched fraction (α-1,4 and/ or β-1,6 branched glucans) of *A. subrufescens* demonstrated the stimulation of spleen lymphocyte such as cytotoxic T-cells even in normal mice (Mizuno et al., 1998). Hemicellulases-treated mycelia extract of *A. subrufescens* was also suggested to be involved in immunostimulatory effects in *in vitro* human peripherical mononuclear cells (PBMC). The extract could induce IL-12 production by CD14-positive cell population consisting of monocytes and macrophages. This response was inhibited by anti-CD14 antibodies but interestingly also against anti-TLR4 showing the involvement of Toll-like receptors in the immunostimulatory properties of the *A. subrufescens* extract. Therefore, it is suggested that monocyte and macrophage phagocytes are activated by *A. subrufescens* fractions in a dose-dependent manner on CD14/TLR4 receptor complex and NK activity (Kasai et al., 2004). It is speculated that β-glucan or others components such mannan might be recognized. Circulating dendritic cells do not produce IL-12 and IFN-γ and not involved in the eliciting properties of *A. subrufescens* in the *in vitro* test.

Daily administration of 32 mg or 64 mg of powdered *A. subrufescens* significantly promoted cytotoxicity activity of liver mononuclear cells. The augmentation of NK cell cytotoxicity was dependent on IFN-γ but not T or NKT cells. NK cells suppress tumor cells through a variety of effector mechanisms, including the perforin/granzyme-containing granule-mediated pathway, death-receptor pathway and IFN-γ mediated pathway (Smyth et al., 2002). Immunomodulatory effects of *A. subrufescens* were also evaluated in Balb/cByj mice (Chan et al., 2007). The extract of this mushroom exhibited a significantly increased serum IgG level, T-cell population and phagocytic activity in mice. *A. subrufescens* fraction had been shown to induce macrophages to secrete tumor necrosis factor-alpha (TNF-α), interleukin (IL)-8 and nitric oxide (NO) in *vitro* test (Sorimachi et al., 2001). After treatment with *Agaricus* fractions, northern blot analysis showed that the increases in cytokine and NO secretion were due to an increase in cytokine mRNA or NO mRNAs. Moreover, the study of signal substance secretion after *A. subrufescens* administration exhibited the promotion of synthesis of the proinflammatory, cytokine and chemokine in monocyte-derived dendritic cells (MDDC) (Førland et al., 2010). TNF-α and IL-8 are pro-inflammatory cytokines. It is well known as immunity against fungal pathogen proceeds partly through pro-inflammatory process via cytokines (Romani, 2008; Blanco and Garcia, 2008). Inflammatory processes are usually undesirable but probably necessary to eliminate fungal pathogens. As mentioned above, the mode of immune defense against this type of pathogens proceeds mainly through CMI. Recent advance knowledge on fungal pathogens stated that lymphocytes-Th 17 response played an inflammatory role previously attributed to uncontrolled Th1 responses (Romani 2008; 2011). Furthermore, nitric oxide was known as a stress mediator that included the signal for vasorelaxation, neurotransmission and cytotoxicity. Macrophage reactive nitrogen played an important role in killing micro-organism and fungus (Nahrevanian, 2009). In contrast, it was recently observed in mice *in vivo* model that nitric oxide (NO) negatively regulate the IL-12 mediated tumor regression and blocking the inducible nitric oxide reductase by L-NAME dramatically increases tumor suppression (Egilmez et al., 2011).

Concerning the *A. subrufescens* extracts, it has been shown in *in vitro* and *ex vivo* studies that the extracts could promote the synthesis of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α), but not IL-12 or the anti-inflammatory cytokine IL-10. Johnson et al. (2009) reported the study of Tryggestad et al. (2007) that the transcription factor NF-κB was activated by non-binding to TLR-4 but relative binding to TLR-2 (Tryggestad et al., 2007). TLR-2 was another type of pattern recognition receptors (PRRs) which also facilitate production of pro-inflammatory cytokines (Johnson et al., 2009; Romani 2011).

11. Lymphocyte activation

Generally, dendritic cells activating lymphocyte T and lymphocyte T balance could also allow the elicitation of humoral immunity via activation and recruitment of lymphocyte B cells. The water-soluble proteoglycan and Andosan™, a commercial product from crude *A. subrufescens*, upregulated the *in vitro* maturation of dendritic cells (Kim et al., 2005a, 2005b; Forland et al., 2010). *In vitro* and *ex vivo* experiments on the total of heparinized blood of volunteers were shown that the monocytes-derived dendritic cells from peripherical blood mononuclear cells increased the cytokine and chemokines level. After stimulating by *A. subrufescens*, the most abundant cytokines are pro-inflammatory cytokine and chemocytokines IL-8, G-CSF, TNF-α, IL-1β, IL-6, IL 17 and MIP-1β. The synthesis of IL-2 and IFN γ is also reported but not of IL-12 as previously mentioned. Therefore, only some Th1-type and Th17 type cytokines were up regulated whereas no anti-inflammatory effect and the stimulation of Th2 cytokines in *in vitro* test. Whereas *ex vivo* experiment, there is a release of different cytokines including Th-1, Th-2, pro-inflammatory or anti-inflammatory as well as chemokines and leukocyte growth factor. Therefore *A. subrufescens* extract mainly stimulates pro-inflammatory chemokine and cytokines *in vitro* or *ex vivo*.

Nevertheless, there have been some contradictory results in immune responses of *A. subrufescens* extract. After 12 days of consecutive administration of 20.4 g of Andosan™, a commercial mushroom extract which contains 82.4% of *A. subrufescens*, the significant reduction of pro-inflammatory cytokines, mainly TNF-α, IL-1β, IL-2 and IL 17, are detected in healthy volunteers. In addition, the other findings are slightly reduction of the pro-inflammatory IL-8, IFN-γ or growth factor G-CSF. The cytokine tests rather tend to be constant. The inhibition of pro-inflammatory cytokines appears to be in a dose dependant manner (Johnson et al., 2009). This is contradictory with many studies as mentioned above. This is explained as the capacity of certain glucans to pass the murine intestinal barriers. *Ex vivo* experiment with a healthy volunteer suggests that heparinized blood has shown to be controversial of immune stimulation of all pro-inflammatory cytokines tested. The patients in the clinical trial test were suffered by inflammatory bowel disease (IBD) (Førland et al., 2011). Volunteers' patients with Crohn's disease (CD), widely regarded as an autoimmune disease, and ulcerative colitis (UC) were exposed to oral dose of *A. subrufescens* enriched mixture (Ando-San™). After 12 days repeated ingestion, the *A. subrufescens* extract promotes anti-inflammatory effects without side effects. Most of the pro-inflammatory cytokines and chemokines tested were downregulated and this was demonstrated *ex vivo* with heparinized blood of exposed UC, CD and normal patients. Actually, most of the conclusion on downregulation of the cytokines and chemokines results mainly of the observation made in presence of LPS.

There are contrary effects between in *vivo* and *ex vivo* assays with normal patients, pro-inflammatory cytokines and chemokines are controversial upregulated and downregulated, respectively. This discrepancy was proposed by the antioxidant potential of *A. subrufescens* (Ker et al., 2005; Oliveira et al., 2007). The limited the absorption of large macromolecules complex or bioactive molecule, such as β-glucans, across the intestinal mucosa to the reticuloendothelial system and blood of the gastrointestinal tracts. With CD and UC patients, the same conclusive explanation is proposed, but the intestinal barrier is hypothesized to be presumably selective permeable to certain β-glucans bioactive fragments to body fluids (blood and lymphoid system) as in murine (Førland et al., 2011). This was not verified whether the presence of molecules which differed from polysaccharides or PAMPS exerts immunosuppressive effect on the innate immune system.

According to the above mentioned *A. subrufescens* extract, it is rather confirmed that the extract could exert both immunostimulatory and immunosuppressive effects. β-Glucan derivatives, proteoglucan, peptide-glucan appeared to be preferably immunostimulating responses and indirectly promote anti-cancer effects. Whether immunosuppression is realized by the same categories of molecules such as more or less branch polysaccharides or proteoglucans remains unclear. However, because of the immunosuppressive potential, safety assessment of dietary edible commercial *A. subrufescens* should be evaluated.

12. Antimicrobial activity

A. subrufescens has been extensively used in folk medicine against life-threatening diseases worldwide. The protein-bound polysaccharide, "proteoglucan", is a valuable compound that enhances defense against invasive organisms (Hetland et al., 1998). There are many reports of the effect of *A. subrufescens* against bacterial infection (Bernardshaw et al., 2005, 2006). β-Glucan is the main polysaccharide naturally found in aqueous extracts of *A. subrufescens*. Hetland et al. (1998) proposed that β-glucan could protect against the infection of bacillus Calmette-Guerin (BCG) and *Streptococcus pneumoniae* (Klein) (2000). Extracts from *A. subrufescens* are able to prevent *S. pneumoniae* infection in mice (Bernardshaw et al., 2005) and could reduce plaques formation of virus in cell culture (Bruggemann et al., 2006). The extract exhibited anti-viral activity in poliovirus type 1 (Faccin et al., 2007) and herpesvirus (Bruggemann et al., 2006). Therefore, it is likely that *A. subrufescens* extracts have therapeutic effects against bacterial and viral infection. Thus intake of *A. subrufescens* extract may be able to serve as an alternative to antibiotics.

13. Anti-allergy effect

There are a few studies in the anti-allergic test of *A. subrufescens* extract (Ellertsen and Hetland, 2009). Most studies of this mushroom involved the antitumor and anticancer tests in cell or animal model, generally using mouse. Ellertsen and Hetland (2009) used ovalbumin (OVA) as the model allergen. Anti-OVA IgE levels, either treated with *A. subrufescens* extract before and after OVA immunization, were reduced in the serum of mice. The extract may prevent the development of IgE-mediated allergy when given before and after allergen immunization. Furthermore, there was a tendency to reduce Th2 relative to Th1 cytokine levels in the specimens. Previously, pure β-glucans from yeast and fungi have been used to study in allergic response with OVA. The results found increased specific anti-OVA IgE levels in serum (Ormstad et al., 2000; Instanes et al., 2004). Therefore, we speculated that β-glucans or glucan protein or peptide complex from the mushroom extract might promote anti-allergic effect against allergen. *A.*

subrufescens given by oral route to mice also appeared to enhance Th1 response and had antiallergenic properties. This process underwent via activation of macrophage by epithelial cells that promote the differentiation of naïve lymphocyte-T cells into Th1 cells without apparently pro-inflammatory consequences in mice (Bouike et al., 2011). Although, the recent studies cannot fully answer in anti-allergic responses of *A. subrufescens* extract. The mushroom extract may have potential to use as a therapeutical substance against allergy (Ellertsen and Hetland, 2009).

A. subrufescens extract has also shown to have an anti-diabetic activity in diabetic rats (Kim et al., 2005a, 2005b; Oh et al., 2010). Purified β-glucan from this mushroom was shown to have anti-hyperglycemic, anti-hypertriglyceridemic, anti-hypercholesterolemic and anti-arteriosclerotic activities indicating overall anti-diabetic activity (Kim et al., 2005a, 2005b), which was also confirmed in β-glucan removed semi-purified fractions of *A. subrufescens* (Oh et al., 2010). An ethyl acetate fraction of hot-water extract from this fungus was tested on Streptozotocin-induced diabetic rats. Hypoglymeric action indicated the fungus could be useful in the treatment of diabetes mellitus (Oh et al., 2010).

14. Production

Due to its nutritional and medicinal importance, *A. subrufescens* is widely cultivated, although there is a huge demand for this mushroom as health foods and in the pharmaceutical industry (Souza Dias et al., 2004). Brazil is the largest commercial producer of *A. subrufescens* (Souza Dias et al., 2004; De Mendonca et al., 2005). Expansion of cultivation has therefore occurred due to the high price on the international markets (De Mendonca et al., 2005). However, the consumption of this fungus in Brazil is low and has been restricted to small communities or higher cultural status groups (Souza Dias et al., 2004). With its noticeable medicinal properties of *A. subrufescens*, Brazilian mushroom growers are interested in growing and exporting mushroom to many countries such as Australia, Bolivia, Germany, Korea, India, Japan, South Africa, Thailand, and the USA (De Mendonca et al., 2005). Several Brazilian publicly funded institutions are engaged in research on *A. subrufescens* concerning biochemical, physiological, genetic aspects as well as the optimization and selection of compost, soil, casing techniques and growing conditions for this fungus (Souza Dias et al., 2004). The production of this mushroom is increasing worldwide due to their important medicinal values and exotic slightly almond-like taste (Györfi et al., 2010).

A. subrufescens normally requires temperatures of 25–28 °C for optimal mycelial growth and 22–25 °C for fruiting (De Mendonca et al., 2005). Temperature is a very important factor in *A. subrufescens* growth and fruiting because it is very sensitive to extreme temperatures out of the optimal range (De Mendonca et al., 2005). Higher temperatures decrease yields due to the faster development of pathogens, while fruiting body formation stops at low temperatures (De Mendonca et al., 2005). Fruiting body formation is induced by providing fresh air, lowering the temperature below 25 °C, providing enough humidity and performing heavy irrigation of the compost. The optimal humidity of the compost is approximately 60–70%. Whereas a humidity of 80–85% should be maintained in the growing house. The subsequent harvesting periods may require additional 30–60 days depending on environment conditions (De Mendonca et al., 2005).

The productivity of *A. subrufescens* is related to the quality of compost and choice of soil used as casing material (De Siqueira et al., 2009). Agricultural wastes such as rice straw, wheat straw, sawdust, rice bran, sugarcane bagasse and animal manure have been used as substrates for mushroom cultivation because they are cheap and readily available (Horm and Ohga, 2008). Various studies have attempted to optimize the composition of compost and casing material for mushroom cultivation. The casing layer is one of the most important phases of *A. bisporus* and *A. subrufescens* cultivation (Colauto et al., 2011). It covers the compost layer and is responsible for the induction of fructification. The function of the casing layer is to protect the compost from drying, pest and disease (Colauto et al., 2011). Depth of casing layer generally ranges from 3 to 5 cm, depending on the environmental variability of the growing house. The pH recommendation for casing soil for *A. subrufescens* is 7.0 and 7.5 (Calvalcante et al., 2008). Zeid et al. (2010) reported that the texture of different soils and environment directly influenced precocity in *A. subrufescens* yield and the production phase had a tendency of being longer when soil + charcoal + calcitic lime were used as casing layer. Colauto et al. (2010) proposed alternative materials instead of peat to use in the casing layer. The increase in use of peat use has resulted in a rapid depletion in nature and is a component of the greenhouse effect due to the abundant liberation of CO_2 through the aerobic decomposition of carbon. Furthermore, some production areas in the world, especially in the southern hemisphere, there are no large peat sources available (Colauto et al., 2011). However, peat is predominantly used as a casing layer worldwide. Increased macroporosity of the casing layer provides better productivity. Lime schist is a promising alternative for mushroom production and it increases productivity up to 92% compared with the control (Colauto et al., 2010). The use of lime schist gave a good result in production flush and accumulated mass of fresh mushrooms when compared to other casing layers (Colauto et al., 2011). The use of clay soil as a casing layer decreases productivity, due to rapid compaction and low water retention capacity. Cattle and sugarcane compost supplemented with sawdust and rice bran also promoted the development of *A. subrufescens* (Horm and Ohga, 2008). The high amount of organic matter in the casing soil might increase undesirable microorganisms. Soybean fiber is also an appropriate substrate for *A. subrufescens* development. It is more porous, has high water absorption capacity and high nutrients for mycelial growth. Soybean fiber has high N content, which is an essential nutrient to promote the growth of mycelium (Junior et al., 2010). Some minerals in compost stimulate the mushroom fructification such as zinc, copper and manganese. They have an important role in the elimination of quinine, which is a substance that inhibits fructification (Calvalcante et al., 2008). Thus we concluded that various factors should be considered for *A. subrufescens* cultivation such as temperature, humidity of substrate and growing house, and composition of compost and casing layer. Nutrients and minerals are essential for promoting the mycelial growth and fructification.

More widespread international cultivation of *A. subrufescens* would reduce the price of this mushroom, although it would reach more consumers. The price would be cheaper in Brazil where it is presently mostly produced and therefore could

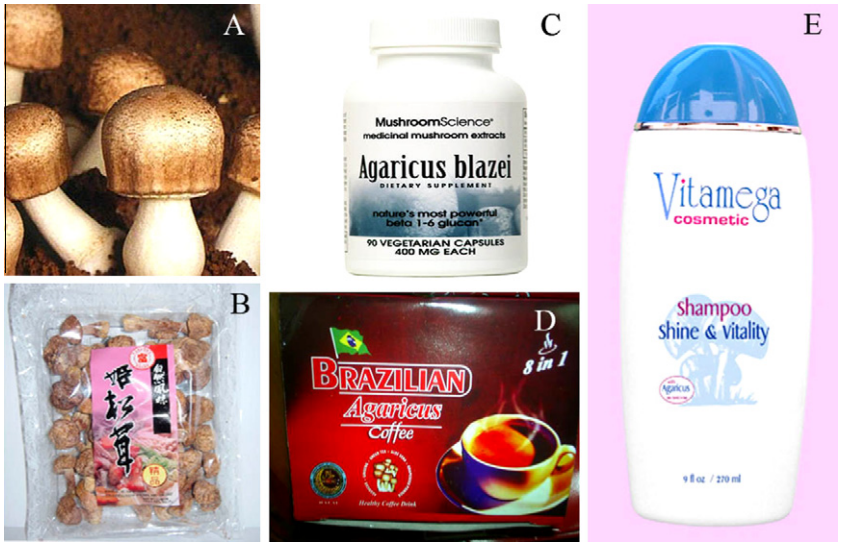

Figure 2 Products from *Agaricus subrufescens*. (A) fresh mushroom (Http://www.diabetichealthyresources.com/can-a-mushroom-from-brazil-be-the-secret-to-brain-health/, 2010), (B) dried mushroom (Http://www.sunwahfoods.com/subpages/home.php, 2010), (C) nutrient supplement (Http://www.sunfood.com/buy/1/10/Agaricus-blazei-Mushroom-Science-90-veg-ca, 2010), (D) coffee (Http://quezon-city.olx.com.ph/brazilian-coffee-8-in-1-slimming-coffee-iid-6, 2010) and (E) shampoo (Http://www.vitamegacosmetic.com/, 2010).

be used by Brazilian consumers (De Mendonca et al., 2005). Presently Brazil exports *A. subrufescens*, including as fresh, dried, sliced or powdered mushrooms. The development of *A. subrufescens* products would increase the product value in the market (De Mendonca et al., 2005). It is used as nutrient supplement because of the high content of β-glucan (Ohno et al., 2001). Consumers can find the product of this mushroom in variable forms. Beside fresh fruiting bodies, this mushroom is sold in a pulverized form, which is supplied in capsules or pills or as an extract produced using hot water (Györfi et al., 2010). Furthermore, many medicinal mushrooms, including *A. subrufescens*, have been used as additive ingredients in cosmetic products. Hyde et al. (2010) reported on some cosmetic products containing mushrooms. Vitamega is a Brazilian cosmetic company that used *A. subrufescens* as the main bioactive ingredient of their products. Some products from *A. subrufescens* mushrooms on international markets are illustrated in Fig. 2.

15. Toxicological problems

Although eating cultivated mushrooms or their extracts is thought to provide medicinal and other benefits, there are few studies on the safety of this practice. *A. subrufescens* has long been consumed as a culinary and supplementary food and has not been reported to be directly toxic or carcinogenic to humans. Nevertheless, we need to be cautious and consider if there are any deleterious effects from eating *A. subrufescens*. The content of poisonous microelements in fruiting bodies of this fungus has been reported by Györfi et al. (2010). The content of Cobalt (Co), Molybdenum (Mo), Selenium (Se), Vanadium (V) are under detectable limits which are about 0.1–0.2 mg/kg of dry matter. However, the detectable amount of cadmium (Cd), is about 2–17 mg/kg of dry matter (Györfi et al., 2010) and must be considered. According to the result, the amount of Cd found is likely reflected in the actual Cd accumulation in *A. subrufescens*.

The major concern over the use of *A. subrufescens* is the problem of the hydrazine content. *A. subrufescens* has been studied and assessed for possible side-effects of agaritine and its derivatives (Stijve et al., 2003; Nagaoka et al., 2006) as these are suspected to be genotoxic and possible carcinogenic or tumorigenic agents. This molecule is thought to be capable of binding to the DNA of organs after administration to mice models (Shephard and Schlatter, 1998). The genotoxicity of agaritine is however, very limited. The cumulative lifespan of cancer risk obtained from agaritine consumption is probably estimated at $10^{(-5)}$. The possible deleterious effect of the accumulative hydrazine was first reported from populations living in industrial settings with rocket fuel contamination. Toth (1996) believed that most hydrazines and derivatives (more than 80%), including fungal hydrazine, are probably harmful due to being carcinogens. Agaritine is a substrate for gamma-glutamyl transpeptidase and is converted into a highly reactive diazonium ion derivative that could bind to protein which promotes apparent mutagenicity and gives a positive response in the Ames test (Walton et al., 1997a,b). However, the results from Walton et al. (1997a,b) contradict those of Paparaskeva-Petrides et al. (1993) and Endo et al. (2010), who showed that agaritine did not activate the *umu* gene of *Salmonella*, which reacted to carcinogens (Endo et al., 2010). 4-methylphenylhydrazine hydrochloride, a derivative of agaritine was reported to induce 24% fibrosarcomas in Swiss mice administrated with this compound (Toth et al., 1977; Toth and Nagel, 1981).

In 2006, the Ministry of Health, Labour and Welfare of Japan assessed the safety of products containing *A. subrufescens*. The results showed that agaritine and derivatives were found in dried *A. subrufescens* fruiting bodies and canned mushroom products containing *A. subrufescens* (Nagaoka et al., 2006). Firenzuoli et al. (2008) also speculated that fungal hydrazine (hydroxyphenyl hydrazine) probably stimulated proteolysis giving rise to hydrazine-mediated DNA strand

scissions and metabolized toxic intermediates capable of damaging cellular macromolecules. The incubated hydrazine in hemolysate caused strand scission of DNA as well as an increase in the proportion to protein concentration of the hemolysate (Runge-Morris et al., 1994) Runge-Morris et al. (1994) also suggested that oxygen-relative organic free radicals played a dominant role in hydrazine-mediated DNA strand scission. Hydrazine and its derivatives had been found in *A. subrufescens* and its products using HPLC with Fluorescence Derivatization (Nagaoka et al., 2006).

Mukai et al. (2006) had reported on side effects of *A. subrufescens* and stated that the mushroom might induce severe hepatic dysfunction in cancer patients. Three patients with severe hepatic dysfunction were taking the mushroom extract. After treatment with the mushroom extract, one of the three patients developed deterioration of the liver function. It was, however, difficult to affirm the cause of deterioration of liver function in these patients. It should be noted that concurrent use of the mushroom extract made differential diagnosis complicated. The long-term intake of feed supplement with *A. subrufescens* did not result in visible alterations in the kidney function indices (Stutz et al., 2009). Lee et al. (2008) had shown no carcinogenicity or other adverse health effects in rats after intake of *A. subrufescens* extracts over 2 years. *A. subrufescens* fruiting body extract was also studied to test its potential in modifying tumor development in a medium-term multi-organ carcinogenesis bioassay (Doi et al., 2010). No modifying potential on tumor development resulted.

There are only limited reports on toxicological and epidemiological effects of intake of fungal hydrazine as well as mechanisms of action where reports are contradictory. Although hydrazine compounds are regarded as toxic, many patients use these compounds for disease treatment. For example, procarbazine is an anti-neoplastic chemotherapy drug used for the treatment of Hodgkin's lymphoma and brain cancers. Procarbazine is well-known as causing gonadotoxic, teratogenic and hepatic disorders. This is however, characteristic of many cancer drugs. More recently, there have been some contradictory reports concerning the effects of agaritine from *A. subrufescens*. Agaritine purified from this mushroom has a direct anti-tumor activity against leukemic tumor cells *in vitro* (Endo et al., 2010) and moderately induced apoptosis through caspase activation in the leukemic cell line U937 (Akiyama et al., 2011). The beneficial or toxicological effect of a bioactive substance often depends on its concentration; it will occasionally have beneficial properties at low concentrations but could be toxic at high concentrations. However, to ensure the safety of long-term using of *A. subrufescens*, further research may be required to find the optimal method to remove or reduce the content of agaritine in *A. subrufescens* extracts (Sorimachi and Nakamoto, 2011). Further toxicology studies still need to completely confirm the safety of the use of *A. subrufescens*.

16. Conclusion

A subrufescens has been widely used as a food over many decades. Because it has potential medicinal properties, the mushroom has become a hot topic in many research areas. Scientific studies have confirmed various medicinal benefits of the mushroom, such as anti-cancer, anti-microbial and biomodulatory effects. Several bioactive compounds that can be developed and used as therapeutic agents have been identified and characterized from this species. Clinical trial data are however, still necessary to determine whether *A. subrufescens* provides real medicinal benefits (Firenzuoli et al., 2008). Toxicological problems may also be an issue. Toxicology data should be assessed especially with known bioactive compounds such as agaritine, blazein and *A. subrufescens* fruiting bodies, but overall epidemiological data are needed linking intake with any possible side effects. Safety issues in particular should focus on the toxicity and carcinogenicity of agaritine and its derivatives in this mushroom. It would be beneficial to the success of *A. subrufescens* if it was shown not to give rise to any toxicological effects. *A. subrufescens* should be an excellent future alternative medicine.

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