A review of the polysaccharide, protein and selected nutrient content of Auricularia, and their potential pharmacological value

Asanka R. Bandara, Sylvie Rapior, Peter E. Mortimer, P Kakumyan, Kevin D. Hyde, Jianchu Xu

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A review of the polysaccharide, protein and selected nutrient content of *Auricularia*, and their potential pharmacological value

Bandara AR[1,2], Rapior S[3], Mortimer PE[4], Kakumyan P[1,2], Hyde KD[1,2,4,5], Xu J[4,5]

[1]*Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[2]*School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[3]*Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, CEFE CNRS – Université de Montpellier – Université Paul-Valéry Montpellier – EPHE – IRD, B.P. 14491, 15 avenue Charles Flahault, 34093 Montpellier Cedex 5, France*
[4]*Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan, China*
[5]*World Agroforestry Centre, East and Central Asia, Kunming 650201, Yunnan, China*
**Authors for correspondence: kdhyde3@gmail.com, jxu@mail.kib.ac.cn*

Abstract

Auricularia is a key genus among edible macrofungi, sourced as either wild or cultivated mushrooms. *Auricularia* species are utilized as nutrient-rich foods and medicinal resources, with particular prominence in Traditional Asian Medicine. Cultivated *Auricularia* species can be grown under a wide range of conditions, allowing for production volumes worldwide. Although *Auricularia* species are used predominantly within the food industry, there is strong potential for their use in the production of therapeutic drugs, thus making it necessary to identify relevant bioactive compounds and further our understanding of its pharmacological properties. Carbohydrates are the major nutritional constituent of edible *Auricularia* species in addition to proteins, fat, fibre, ashes vitamins and minerals. This review discusses polysaccharides as one of the major active compounds found in edible *Auricularia* species in relation to their nutritional value, extraction methods, and pharmacological properties. Current methods of evaluating the pharmacological effects of compounds derived from *Auricularia* include *in vitro* assays, *in vivo* animal models, as well as several human clinical trials. Potential medical applications for these compounds include the production of novel therapeutic drugs for treating diseases such as cancer, diabetes, and cardiovascular disorders.

Keywords – Cancer, Cardiovascular disorders, Constipation, Diabetes, Medicinal mushrooms, Metabolic Syndrome, Polysaccharides

Introduction

Fungi have long been regarded as a source of natural medicine, and mushrooms used for medicinal purposes feature prominently in Traditional Asian Medicine (Hapuarachchi et al. 2018b, Hyde et al. 2019). Over the last four decades, various therapeutic activities have been reported as a result of extensive research on mushrooms using *in vitro* assays, *in vivo* animal models, and, in some cases, human clinical studies; these include but are not limited to: anti-cancer, anti-diabetic, anti-malarial, anti-microbial, antioxidant, anti-tumor, anti-viral, neuroprotective, and hypocholesterolemic properties (De Silva et al. 2012a, b, 2013, Dalonso et al. 2015, Thongbai et al. 2015, Hapuarachchi et al. 2016, Klupp et al. 2016, Wasser 2017, Hapuarachchi et al. 2018a). In the

past mushrooms were primarily used as a food source (Dupont et al. 2017). The aforementioned published reports have changed our perception of mushrooms as a functional food, with mushrooms increasingly viewed as a medicinal resource (Thawthong et al. 2014, Hapuarachchi et al. 2018a). With the use of mushrooms both as a food and medicinal source, global mushroom production has increased more than 30-fold since 1978 (Royse et al. 2017). The increment in global mushroom porudction also coincides with a simultaneous increase (1.7-fold) in the global human population (Royse et al. 2017), presumably due to the importance of mushrooms as a food source (Chang & Wasser 2017). There are two major groups of edible fungi; a) Ascomycetes, which include Discomycetes (Cup fungi) and the famous Tuberales or truffles, and b) Basidiomycetes, which include Phragmobasidiomycetidae encompassing three orders: Auriculariales, Septobasidiales and Tremellales (Alexander 2013). Among these mushrooms, the order Auriculariales (previously known as "Jelly fungi") contain a number of species that have value as both edible and medicinal mushrooms (Alexander 2013).

Auricularia Bull. is an important genus which accounts for approximately 17% of world mushroom production and is the third most cultivated mushroom genus after *Lentinula* (22%) and *Pleurotus* (19%) (Royse et al. 2017). Besides its significance as a major food source, *Auricularia* species can be exploited as medicinal resources for the development of novel therapeutic drugs for many human diseases, including potential for cancer treatment (Wu et al. 2014). The bioactive compounds of *Auricularia* species which primarily include polysaccharides, are known for their anti-tumor, antioxidant, anti-coagulant and immunomodulating properties (Ma et al. 2018). Knowledge of the bioactive compounds of *Auricularia* is crucial for furthering our understanding of their pharmacology and could lead to the development of novel therapeutic drugs. This review provides a synopsis of the biochemistry and pharmacology of *Auricularia* species.

Cultivation of *Auricularia*

Auricularia auricula-judae (known as black fungus or wood ear mushroom) has a history of ~2100 years of cultivation in China (Yao et al. 2018). Currently, *A. cornea* and *A. heimuer* (Fig. 1) are commercially cultivated in China, Indonesia, Malaysia, Philippines, Thailand and Vietnam (Chang & Lee 2004, Duc 2005, Tapingkae 2005, Peng 2008, Reyes et al. 2009, Irawati et al. 2012, Razak et al. 2013, Wu et al. 2014). China is the main producer of cultivated *Auricularia*, and approximately 6.3 billion kg were produced in 2017, which made it the second most widely cultivated mushroom in that country (Chinamushroombusinessnetwork 2018).

Several culture media with different nutritional profiles, as well as optimal temperatures and pH ranges, are currently used to cultivate *Auricularia*, i.e., Czapek-dox, Glucose Peptone, Malt Extract Agar (MEA), Mesangial Cell Medium (MCM), Potato Dextrose Agar (PDA), Yeast Extract Agar (YEA), Yeast Mannitol Agar (YMA), and Leonian medium (Yu et al. 2013, Jo et al. 2014). The biomass production of edible *Auricularia* requires optimal growth conditions for their mycelia. For instance, *A. auricula-judae* was reported to be grown optimally on PDA and MCM at 25–30^{o}C and pH 6–9, but not on Czapek dox or Leonian (Jo et al. 2014). *Auricularia villosula* mycelium can be cultivated on a mix of potato juice, sucrose, soybean powder and 0.5% PO_4^{-3} under optimal temperature (30°C) and pH 8 conditions to produce fruiting bodies that are very similar to those found in nature (Zhang et al. 2018b). Spawn production, generated from the tissue culturing of mycelia, followed by fruiting body production (either on logs, whether wood or artificial, or through the poly bag method), are the main cultivation methods practiced (Priya et al. 2016). Low-cost production of *Auricularia* species is carried out in many Southeast Asian countries by using compost and agro-waste products. For example, compost consisting primarily of corncobs, rice straw, broadleaf tree sawdust, and cottonseed bran with plaster stone, wheat bran, rice bran, and quick lime as supplementary materials, was successfully used to cultivate *A. auricula-judae* in India (Verma & Verma 2017). Similarly, in Taiwan, low-cost agro-waste products, i.e., cotton

waste, rice straw, wood chips, and sawdust are commonly used for the production of *Auricularia* species (Peng 2008).

Optimal culture conditions (temperature, pH) with nutritionally rich supplementation are necessary for increasing the yield as well as commercial quality of edible mushrooms (Carrasco et al. 2018). The substrate supplementation is known to impact the nutritional content of *Auricularia*. For example, the use of 60% sugarcane bagasse resulted in the highest nutrient output (carbohydrates, protein, ash, and fat) from *A. polytricha* in contrast to other agro-waste substrates such as rice straw, and rice husk (Wu et al. 2017). On the other hand, *A. auricula* grown on maize cobs and wheat bran showed higher nutrient content (cellulose, proteins and moisture) in contrast to the counterparts grown on saw dust and rice bran in Kenya (Onyango et al. 2011). The dry biomass weight that represent the content of moisture, crude proteins, ash and carbohydrates has also been reported to be dependent on the type of nutrient medium used for their cultivation. For instance, nitrogen source that was most favorable for *A. polytricha* growth and highest dry biomass production was yeast extract in contrast to reducing impact from tryptone, beef extract and peptone (Hassan & Medany 2012).

The wide range of growing conditions and methods used for production also provides implications on the environmental plasticity of *Auricularia* sp. It also coincides with the high degree of interspecies and intraspecies genetic diversity. Past studies have shown that intraspecies and interspecies genetic diversity is quite high, and this may impact their breeding and cultivation conditions (Yan et al. 2004, Li et al. 2007, Du et al. 2013, Li et al. 2014). In order to address this intraspecies genetic variability, new techniques employing random amplified polymorphic DNA (RAPD) fingerprinting analysis have been shown to be highly successful in determining the genetic diversity of *A. auricula* and *A. polytricha* strains, and have been suggested as an effective method for determining breeding techniques (Yan et al. 2004).

Auricularia as an edible mushroom

Auricularia is a cosmopolitan genus, comprised of eighteen species (Bandara et al. 2017b) of which *Auricularia auricula-judae* (Bull.: Fr.) Quél., [= *A. auricula* (L. ex Hook.) Underw.], *A. cornea* (Ehrenb.) Fr., *A. delicata* (Mont. ex Fr.) Henn., *A. fuscosuccinea* (Mont.) Henn., *A. heimuer* F. Wu, B.K. Cui, Y.C. Dai, *A. thailandica* Bandara & K.D. Hyde and *A. villosula* Malysheva have been reported as edible species (Fig. 1) (Zent et al. 2004, Wu et al. 2014, Sekara et al. 2015, Zhang et al. 2015a, Bandara et al. 2017a, Kamalebo et al. 2018, Zhang et al. 2018b). The characteristic flavor, along with the slippery and crunchy texture of the gelatinous fruiting body of *Auricularia* species accounts for the popularity and high consumption in China and Southeast Asia (Misaki & Kakuta 1995, Cheung 2013, Jo et al. 2014).

Nutrient composition of *Auricularia*

The nutritional content of edible *Auricularia* varies according the species. On average, dried *Auricularia* have an approximate composition of 79.9–93.2% carbohydrates, 6.5–13% crude proteins, 9.9–17.9% total soluble sugars, 0.48–4.5% crude fat (lipid), and 3.5–12.5% crude fiber (Table 1) (Crisan & Sands 1978, Cheung 1997, Mau et al. 1998, Chen et al. 2011, Kadnikova et al. 2015, Bandara et al. 2017a, USDA 2018a). Carbohydrates are the major components in *Auricularia*. For example, proximate composition of *A. auricula-judae* represents carbohydrate: 81.0%, crude protein: 8.1%, crude fat: 1.5%, crude fiber: 6.9%, and ash: 9.4% (Chang & Hayes 2013, Cheung 2013).

Digestible nutrients found in edible mushrooms vary depending on the species, age, and their cultivation methods, and include major nutrients such as carbohydrates, proteins, and minerals (Ca, Na, K, and Mg) (Kadnikova et al. 2015). The digestable carbohydrates are primarily composed of polysaccharides and include water soluble mannans and glucans, pectins, chitin, and cellulose whose diegstibility may vary depending on the percentage of these contents (Kadnikova et al.

2015). The extent of lignification also determine the digestability. However, the non-digestable nurtients such as non-starch polysaccharides represented by fibres are useful as a food source with higher health benefits (Cheung 2013). In addition to these macronutrients, edible mushrooms provide a good source of micronutrients for which most human diets are deficient such as Se, Cu and Zn (Carrasco et al. 2018). Moreover, *Auricularia* species are known to be a source of many minerals that may help to solve human nutrient deficiencies including Mg, Ca, Fe, Zn, Mn, Cu, Ni and Cr (Shin et al. 2007, Kadnikova et al. 2015). As with other wild and cultivated edible mushrooms (Kalač 2010, Kalač 2013, Wang et al. 2014), potassium is the most prevalent macro-element within *Auricularia* species (Afiukwa et al. 2013, Bandara et al. 2017a, USDA 2018a, b), which are typically nutrient-rich with other macro-elements including Ca, Na, Mg, and P (Table 3) (Afiukwa et al. 2013, Bandara et al. 2017a). Among micro-elements, high concentrations of Fe and Zn have been reported in *Auricularia* (50–200 mg/kg), while those of Co, Cr, Cu, Mn and Ni were found to be less than 20 mg/kg of dry weight (Kadnikova et al. 2015, Bandara et al. 2017a). Studies also suggest an intraspecies variability of micronutrients content of Mg, Mn, Zn, Ni, Cr, Sr in *A. auricula* where as the Fe levels were quite similar among all the strains with a maximum concentration of 285 µg/g (Li et al. 2018).

Auricularia is also a rich source of dietary fiber (Cheung 2013). Most of the carbohydrates in *Auricularia* have been reported to be indigestible polysaccharides, such as β-glucans and mannans (Misaki & Kakuta 1995, Mironczuk-Chodakowska et al. 2017). *Auricularia* species have been shown to contain a higher fiber content than other commercially available mushrooms such as *Agaricus bisporus* and *Ganoderma lucidum* (Misaki & Kakuta 1995, Cheung 1997).

Auricularia species have very low soluble sugar content, which is reflected in their flavor (Mau et al. 1998). Furthermore, unlike other edible mushrooms, *Auricularia* have a low fat content (Cheung 2013), and 60% of the fatty acids of harvested wild *Auricularia* are unsaturated (Kavishree et al. 2008). Low-fat diets with a high content of unsaturated fatty acids are recommended for people with high blood cholesterol, making *Auricularia* an ideal food choice for them (Lichtenstein et al. 2006, Chen et al. 2011).

Quantities of crude proteins in *Auricularia* species are lower than in most wild and cultivated edible mushroom species (Fan et al. 2006, Liu et al. 2009, Cheung 2013, Jia et al. 2017). Common edible mushrooms in the market, such as *A. bisporus*, *Lentinula edodes*, and *Pleurotus* spp. contain 10.5–34.8% of crude protein, whereas *A. auricula-judae* contains 8.1% of crude protein (Chang & Miles 2004, Cheung 2013). Edible species of *Auricularia* provide 17–18 amino acids, including eight essential amino acids and three semi-essential amino acids (arginine, cysteine, and tyrosine), all of which are of value for pediatric nutrition (Table 2) (Afiukwa et al. 2015, Bandara et al. 2017a, Ohiri & Bassey 2017). Glutamic acid was the most abundant amino acid present in *Auricularia*, whereas sulfur-containing amino acid (cysteine, methionine) quantities were shown to be relatively low (Table 2) (Afiukwa et al. 2015, Bandara et al. 2017a, Ohiri & Bassey 2017).

Medicinal properties and pharmacological applications of Auricularia

Auricularia species have long been used as traditional remedies in Asia, European folk cultures, African communities, as well as among indigenous groups such as the Māori (New Zealand) and Tzeltal (Mexico) (Fuller et al. 2004, Guzmán 2008, Pala et al. 2013, Sekara et al. 2015, Teke et al. 2018). As an example, *A. cornea* has been used as a traditional medicine to heal poisoning by toxic plants among Māori tribes (Fuller et al. 2004, Fuller et al. 2005), and certain *Auricularia* species have been used by the Tzeltal people to treat anxiety as part of their traditional medicine (Guzmán 2008). However, the application of *Auricularia* for treatment of human disease has moved beyond the domain of traditional medicines and is now being incorporated into Western medicine.

In *A. auricula-judae*, the major polysaccharide constituents of the fruiting body contained water-soluble glucan I with β-D-configuration (Fig. 2.1); alkali-insoluble glucan II with β-D-glucosidic linkages (Fig. 2.2); and acidic heteropolysaccharides, which were a mix of xylose, mannose, glucose and glucuronic acid (1.0: 4.1: 1.3: 1.3) (Table 4) (Sone et al. 1978). Water-soluble glucan I with branched (1→3)-β-D-glucan has been shown to have anti-tumor effects against sarcoma in mice (Table 4) (Misaki et al. 1981).

Numerous studies have reported on the medicinal properties and pharmacological activities of isolated compounds or different solvent extracts from *Auricularia* (Sekara et al. 2015). In particular, the anti-coagulant (Yoon et al. 2003), anti-inflammatory (Ukai et al. 1983a, Kiho et al. 1985, Damte et al. 2011), anti-microbial (Gbolagade & Fasidi 2005), antioxidant (Sun et al. 2010, Bandara et al. 2017a), anti-tumor (Misaki et al. 1981, Ukai et al. 1983b), anti-viral (Nguyen et al. 2012a), and immunomodulatory (Sheu et al. 2004) activities of *Auricularia* have been demonstrated. In addition, *Auricularia* has the potential to treat both hypoglycemia (Yuan et al. 1998a) and hypolipidemia (Reza et al. 2015), as well as lowering cholesterol levels (Table 5) (Misaki & Kakuta 1995, Chen et al. 2008, Zhao et al. 2015). Furthermore, research has shown that *Auricularia* mushrooms have the ability to modulate interstitial microbiomes; for instance, *A. auricula* had a negative impact on Fusobacteriales and a positive impact on Bifidobacteriales and Bacteroidales, two of the most important probiotics (Zhao et al. 2018).

The high fiber content of *Auricularia* has been shown to contribute to preventing gastric disorders (Misaki & Kakuta 1995), such as functional constipation in humans (Kim et al. 2004). Fibers from *Auricularia* have also been used to treat gastric disorders associated with nausea (Khan et al. 2016). In addition to helping with gastric disorders, the dietary fibers in *Auricularia* are known to be bioactive compounds with antioxidant and hypocholesterolemic properties (Cheung 1996).

Previous studies have mentioned the promising effects of *Auricularia* as an anti-cancer and anti-tumor agent (Table 5) (Misaki & Kakuta 1995, Ma et al. 2010, Song & Du 2012a). Due to the growing evidence that *Auricularia* species are able to treat gastric disorders, compounds derived or extracted from *Auricularia* have been used in anti-gastrointestinal cancer therapy, including treatments for colorectal, gastric, liver, pancreatic, and esophageal cancer (Yu et al. 2014, Ma et al. 2018). For example, *A. polytricha* crude polysaccharide extract with a (1→3)-linked-β-D-glucopyranosyl and (1→3, 6)-linked-β-D-glucopyranosyl residue backbone (2:1 ratio) and a terminal (1→)-β-D-glucopyranosyl was shown to inhibit S180 sarcoma cancer transplanted in mice, providing evidence of its anti-cancer properties (Song & Du 2010). Similarly, sarcoma 180 solid tumors could be inhibited by water soluble and ethanol extracts of polysaccharides from *A. auricula-judae* through the inhibition of acinar cell carcinoma proliferation and tumor cell apoptosis mediated by reduced Bcl-2 gene expression and increased Bax expression. A recent meta-analysis provided strong evidence for the use of polysaccharides from *A. auricula* (Huaier) in anti-gastrointestinal cancer therapy (Ma et al. 2018). Fermentation of *A. auricula* followed by hot water extraction gives rise to Huaier cream, which is then mixed with powdered sugar, dextrin, and auxiliary materials to generate Huaier granule. Ma et al. (2018) describes Huaier granules as possessing not only anti-cancer properties but also antioxidant and anti-coagulant activities (Ma et al. 2018). The article cites several studies that have used either the Huaier granule or Huaier cream to treat esophageal, pancreatic, gastric, colorectal and hepatocellular cancers (Ma et al. 2018). Moreover, *A. auricula-judae* ethanol extract has been used to generate a dichloromethane fraction, which contains diazene, a compound known to be used in treating tumors. The dichloromethane fraction was able to inhibit bronchoalveolar cancer and gastric cancer cells by controlling the levels of tumorigenic genes, including p53 overexpression and Bcl-2 downregulation, to induce cytotoxicity and apoptosis, respectively (Reza et al. 2014).

Polysaccharides of *Auricularia*

Polysaccharides are considered to be the most potent group of bioactive compounds found in mushrooms (Friedman 2016, Singdevsachan et al. 2016, Sánchez 2017), serving as the structural components of the cell wall (Zhang et al. 2007, Dalonso et al. 2015) and as carbohydrate reserves in sclerotia (Stone 2009). Polysaccharides seem to be the major constituents of *Auricularia* and are classified as homopolysaccharides or heteropolysaccharides based on their monomeric composition (Sone et al. 1978, Misaki et al. 1981). Homopolysaccharides, composed of β-(1→3)-linked D-glucopyranosyl backbone substituted (1→6)-linked β-D-glucopyranosyl groups, were the most abundantly found macromolecules in most of the edible, medicinal mushrooms (Sone et al. 1978, Misaki & Kakuta 1995, Dalonso et al. 2015, Friedman 2016), including *Auricularia* species such as *A. auricula-judae* (Sone et al. 1978, Misaki et al. 1981, Misaki & Kakuta 1995), *A. polytricha* (Song & Du 2010), and *Auricularia* sp. (Yū ěr) (Kiho et al. 1987, Kiho et al. 1991). Glucan I (Fig. 2.1) and Glucan II (Fig. 2.2) homopolysaccharides were isolated from the fruiting body of *A. auricula-judae* composed of β-(1→3)-linked D-glucopyranosyl backbone substituted (1→6)-linked β-D-glucopyranosyl groups (Sone et al. 1978, Misaki et al. 1981, Misaki & Kakuta 1995). Homopolysaccharides composed of different conformations and different linkages of D-glucopyranosyl backbones were reported from *A. polytricha* AAPS-1 (Fig. 2.3) (Song & Du 2012b), AAFRC (Fig. 2.4) (Song & Du 2012a). Different numbers of (l→6)-D-glycosidic linkages attached to the β-(1→3)-linked D-glucopyranosyl backbones of *Auricularia* polysaccharides were attributed to various chemical, physical and bioactive properties (Table 4).

Polysaccharides consisting of more than one sugar or sugar derivative are called heteropolysaccharides (Enshasy & Hatti-Kaul 2013, Davidson 2018). Heteropolysaccharides composed of α-(1→3)-linked D-mannopyranosyl backbones (Fig. 3.1), with the branches of D-glucopyranosuronic acid, D-mannopyranose and D-xylopyranose residues [i.e. α-(1→3)-glucuronoxylomannans], which contained a small proportion of D-glucopyranose, were reported from *A. auricula-judae* (Sone et al. 1978, Misaki et al. 1981, Ukai et al. 1982, Misaki & Kakuta 1995). Though the α-(1→3)-linked D-mannopyranosyl backbone was reported for several fungal heteropolysaccharides (Sone et al. 1978), α-(1→3)-glucuronoxylomannans were found to be specific to jelly mushrooms (Kakuta et al. 1979, Wasser 2002). α-Configuration of the (1→3)-linked D-mannopyranosyl residues possess a single chain, which leads to the absence of its anti-tumor potential, while the β-configuration, which has a triple helical structure, is necessary to produce anti-tumor and immunomodulating activity (Misaki et al. 1981, Misaki & Kakuta 1995, Enshasy & Hatti-Kaul 2013). Heteropolysaccharides with β-configuration glucopyranosyl residues were also detected from *A. auricula-judae* (Table 4) (Ma et al. 2008, Yang et al. 2011). The water-soluble β-D-glucan isolated from *A. auricula-judae* was composed of β-(1→4)-linked D-glucopyranosyl backbone with β-(1→6)-linked glucopyranosyl side groups at C–6, including glucuronic acid (Ma et al. 2008). A backbone similar to β-D-glucan isolated from *A. auricula-judae* (Fig. 3.2) (Ma et al. 2008) was observed in the polysaccharopeptide Krestin isolated from *Coriolus versicolor* (Ooi & Liu 2000). Although the branching pattern of β-(1→6)-linked glucopyranosyl side groups of β-D-glucan isolated from *A. auricula-judae* and Krestin isolated from *C. versicolor* differed from one another, both polysaccharides acted as potential anti-tumor agents (Ma et al. 2010, Maehara et al. 2012).

The majority of polysaccharides isolated from fungi are water insoluble; among water-soluble polysaccharides, most are heteropolysaccharides, having a complicated structure and thus making it difficult to elucidate their primary and secondary structures (Ma et al. 2008). In general, mushroom polysaccharides are highly diversified in their sugar composition, main chain polymer structure, degree of branching, conformation, molecular weight, and other physical properties, all of which significantly influence the bioactivity and mode of action of the polysaccharide (Enshasy & Hatti-Kaul 2013). It should be noted that different extraction methods and different materials affect the polysaccharide concentration of *A. auricula* (Li et al. 2019). The results showed that the

enzyme-based extraction was better than either ultrasonic-assisted extraction or water extraction technique. The extraction process of polysaccharides affects the bioactive molecule content of the mushroom extracts and then their biological activities, for any therapeutic target (Li et al. 2019).

Bioactive polysaccharide properties of *Auricularia* and potential novel drug therapeutic uses

The following section describes the different bioactive properties of polysaccharides derived from *Auricularia* species, including anti-cancer, anti-tumor, immunomodulatory, anti-bacterial, anti-viral, antioxidant, hypoglycemic, and anti-hypercholesterolemic properties. It should be noted that it is currently unknown whether all *Auricularia* species possess each of these properties. In some cases, the polysaccharides have to be chemically modified (e.g. sulfation) to enhance a certain property, such as the anti-viral properties of *A. auricula* polysaccharides (Nguyen et al. 2012a). Although certain of these compounds are already used in treating disease, there are others that are still in experimental stages of development, determining potential medicinal and pharmaceutical applications in the future. In addition to the different medical properties of these polysaccharides, the factors that may influence their activity are also discussed here. The properties of *Auricularia* polysaccharides are summarized in Fig. 4.

Polysaccharides with anti-cancer or anti-tumor properties

Many *Auricularia* polysaccharides have anti-tumor properties that are mediated through (1→3)-β-D-glucan molecules, which induce cancer cell apoptosis [reviewed in (Meng et al. 2016)]. *Auricularia polytricha* polysaccharides were shown to inhibit cancer activities in A549 human lung cancer cells by inhibiting DNA synthesis, cell cycle arrest, and cell proliferation, thus indicating their potential as a chemotherapeutic agent in combating lung cancer (Yu et al. 2014). The incorporation of these compounds into unique drug delivery systems can enhance their effectiveness. One such example is the use of the Huaier granules for gastrointestinal cancer (Ma et al. 2018). The folic acid conjugated *Auricularia auricula* polysaccharide, *cis*-diaminedichloroplatinum complex, is a good example of an enhanced polysaccharide that utilizes a drug delivery system to increase its anti-tumor potency while reducing its cytotoxicity in treating cervical carcinoma (Qiu et al. 2018). Additionally, water, ethanol, ethyl acetate, dichloromethane, and butanol can be used as solvents for the extraction of bioactive compounds from *Auricularia*, i.e., (1→3)- and (1→6)-β−D-glucan-containing polysaccharides are extracted from *A. auricula-judae* in dichloromethane fraction, which has been identified as an anti-tumor candidate for treating sarcoma 180 cells (Reza et al. 2011). *Auricularia polytricha* polysaccharides have also been shown to have anti-tumor effects against sarcoma (Yu et al. 2009). An *in vivo* anti-tumor test conducted in mice with sarcoma 180 tumors showed that the polysaccharides have anti-tumor effects by activating the functions of macrophages (Yu et al. 2009). A polysaccharide main chain composed of (1→4)-linked D-glucopyranosyl and (1→6)-linked D-glucopyranosyl with a branch at O-6 from *A. auricula-judae* was also shown to have anti-tumor capabilities which are mediated through cancer cell apoptosis [reviewed in (Meng et al. 2016)]. The cytotoxic properties of *A. auricula-judae* are also used to inhibit cancer cells derived from the HCT116 cell line, while depending on the extraction method that renders polarity of the bioactive compounds provided them with additional cytotoxic [non polar (n-hexane) extract], anti-oxidative [ethyl acetate and methanol extracts], and anti-diabetics [ethyl acetate extract] properties (Elkhateeb et al. 2018).

Polysaccharides with anti-bacterial properties

Hot water extraction, followed by alcohol precipitation, was used to collect crude polysaccharides of *A. auricula-judae*, which were then investigated for anti-microbial capacities (Cai et al. 2015). These polysaccharides derived from *A. auricula-judae* were shown to inhibit the activities of *Escherichia coli* and *Staphylococcus aureus*, confirming their anti-microbial activity (Cai et al. 2015). Chitosan is an organic polymer and a linear polysaccharide [β-(1→4)-linked D-

glucosamine and N-acetyl-D-glucosamine] that is commercially available and used to treat many diseases, including obesity and hypercholesterolemia. However, chitosan exhibiting a higher degree of deacetylation compared to commercially available forms was extracted from *Auricularia* species in a recent study (Chang et al. 2019). The work of Chang et al. (2019) showed that the high deacetylation power of *Auricularia* chitosan inhibited the growth of *E. coli* and *S. aureus*, further confirming the anti-bacterial properties of *Auricularia* polysaccharides. Moreover, adding low molecular weight chitosan (positively charged) to *A. auricula* polysaccharides (negatively charged) to create novel polyelectrolyte complex nanoparticles proved to be an ideal delivery system for protein drugs, expanding the potential uses of *Auricularia* polysaccharides in developing novel drug therapies (Xiong et al. 2016).

Polysaccharides with anti-viral properties

Auricularia auricula has been used to extract total *A. auricula* polysaccharides (known as AAPt), which can then be sulfated to enhance their anti-viral activity. The ability of sulfated AAPt in inhibiting the activity of Newcastle Disease Virus on cultured chicken embryo fibroblasts measured using MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay provides evidence for its anti-viral activity (Nguyen et al. 2012a). The authors refer to the sulfated AAPt as "*the component of a new-type anti-viral drug*" (Nguyen et al. 2012a). Studies on anti-viral properties of *Auricularia* species are still rare. However, the proven anti-viral properties of many fungal species within Basidiomycota in inhibiting a wide range of viruses including BoHV-1, H1N12, HBV, HCV, HIV, Influenza, Polio, and Vaccinia highlight the potential for *Auricularia* species to provide similar defenses against these viruses, and their potential applications in treating a wide range of viral diseases (Linnakoski et al. 2018).

Polysaccharides with immunomodulatory properties

The AAPt from *A. auricula* also showcase immuno-enhancing activities when modified by sulfation using the chlorosulfonic acid-pyridine method (Nguyen et al. 2012b). Both *in vitro* and *in vivo* experiments suggest the use of sulfated AAPt (sAAPt) as an "immunopotentiator" (Nguyen et al. 2012b). A submerged culture of *A. auricula-judae* containing exopolysaccharides (CEPSN-1 and CEPSN-2) with (1→4)-α-D-glucose backbones was used by Zhang et al. (2018a) to demonstrate their immunomodulatory activities. Even though the polysaccharide backbone structures differed between the fruiting bodies and the submerged cultures of *A. auricula-judae*, the exopolysaccharides showed potential immunomodulatory activities (Zhang et al. 2018a). This observation suggests that immunomodulatory drugs could be generated using exopolysaccharides from submerged cultures of *A. auricula-judae.*

Inflammation is a natural response to a weakened immune system, and many *Auricularia* species such as *A. polytricha* have anti-inflammatory properties that arise not only from their polysaccharides but also other bioactive compounds such as carotenoids, fatty acids, vitamins, and phenolic compounds (Muszynska et al. 2018). For example, *A. auricular-judae* polysaccharides have shown protective effects in rats who have acute lung injury induced by lipopolysaccharide which was illustrated by the reduced lung edema and lowered levels of inflammatory mediators (Zhuan Yun et al. 2015). Similarly, *A. auricular-judae* polysaccharides were able to inhibit edema (carrageenin-induced) and hyperalgesia (scald-induced) in rats through its anti-inflammatory properties (Ukai et al. 1983a). The medicinal implications of *Auricularia* polysaccharides were further illustrated using a rat model of non-alcoholic fatty liver disease who were treated with a diet containing water extract of *A. polytricha* that is rich in polysaccharides, phenolic compounds and tannins with anti-inflammatory properties. The rats who received the *A. polytricha*-containing diet showed slowed progression of liver disease in association with elevated anti-oxidative markers suggesting the use of *Auricularia* supplementation as a potential remedy (Chiu et al. 2014). As the same disease model showed attenuation of lipid deposition, the study also provides implications of

Auricularia supplementation in treating obesity. The anti-inflammatory as well as antioxidant (discussed below) properties of *Auricularia* polysaccharides may also render cardio-protective properties to them providing insights into using these polysaccharides for cardiovascular diseases (Hao 2014).

Polysaccharides with antioxidant properties

Auricularia species have been reported to possess polysaccharides with antioxidant properties, and examples include *A. auricula* (Xu et al. 2016) and *A. polytricha* (Avci et al. 2016). In addition to different species providing different antioxidants, the potential for antioxidant production can also vary according to the strain of a given *Auricularia* species (Xu et al. 2016). Regardless of the differences observed among varieties, the proven antioxidant ability of *A. auricula* polysaccharides in improving heart functions in a mouse model (Wu et al. 2010) have significant implications for the use of *Auricularia* polysaccharides as pharmaceutical antioxidants for cardiovascular disease treatments. In addition, experimental studies using diabetic mice (*in vivo*) showed that the polysaccharides of *A. auricula* were able to modulate anti-oxidative systems and treat diabetic conditions (Hu et al. 2017). Polysaccharides from *A. polytricha* were extracted using ethanol and distilled water, and an assessment of their antioxidant activity demonstrated that water-based extraction was more efficient for obtaining higher antioxidant activity when compared to ethanol extraction (Avci et al. 2016). These animal model-based studies as well as *in vitro* assays provide further evidence that polysaccharides of *Auricularia* may be used in humans after their advancement into clinical trials. Antioxidants may perform its functions through radical scavenging. For example, sulfated neutral and acidic *A. auricula* polysaccharides showed high radical scavenging abilities which have tremendous implications in disease conditions caused by oxidative stress and generation of free radicals such as Alzheimer's disease and cancer (Zhang et al. 2011). The free radical scavenging properties of *A. auricula* were shown to be useful not only as a medicine but also a functional food when it used as a pickled product (Khaskheli et al. 2015).

Polysaccharides with anti-hypercholesterolemic properties

Polysaccharides extracted from *Auricularia* have the potential to reduce cholesterol, thus indicating the potential for use in the treatment of hypercholesterolemia. Several animals, including rats and mice, have been used in experiments demonstrating the anti-hypercholesterolemic properties of *Auricularia* polysaccharides. For example, a diet-induced hypercholesterolemic rat was orally administered with soluble polysaccharides from *Auricularia polytricha*, resulting in a reduction in blood lipid levels, thereby confirming the natural anti-hypercholesterolemic properties of *Auricularia polytricha* (Table 5) (Zhao et al. 2015). Similarly, hypercholesterolemic rats fed with an *A. auricula* diet in dry powder form had reduced levels of serum LDL cholesterol, as well as lower fecal neutral steroids and bile acids, all of which suggest its effectiveness in reducing hypercholesterolemic activity (Cheung 1996). Further evidence for the hypocholesterolemic capacities of *Auricularia* was provided when hot water-extracted polysaccharides from *A. auricula* were orally administered to hypercholesterolemic mice, and total cholesterol and high-density lipoprotein cholesterol were significantly reduced (Chen et al. 2008).

Polysaccharides with hypoglycemic properties

Bio-compounds with hypoglycemic properties are important in the treatment of diabetes. *Auricularia auricula* polysaccharides-simulated-hydrolysates are bio-compounds with hypoglycemic properties, and intragastric administration of *A. auricula* polysaccharides-simulated-hydrolysates has been suggested as a potential treatment for diabetes mellitus (Lu et al. 2018b). A model incorporating diabetic mice (diet-streptozotocin-induced) was used to test the anti-diabetic capabilities of *A. auricula* polysaccharides. These polysaccharides were reported to reduce blood glucose levels in the mice, producing a result similar to the well-known anti-diabetic medicine

Metformin (Hu et al. 2017); however, further studies are required to verify these results. Moreover, polysaccharides composed of glucose, 2-deoxy-glucose, arabinose, xylose, mannose, and glucosamine from *A. auricula* were shown to effectively treat rats with type 2 diabetes mellitus by inhibiting the oxidative stress response signaling pathway (Lu et al. 2018a), a pathological mechanism associated with diabetes (Tiwari et al. 2013).

Research conducted previously provides evidence that the extraction method of *Auricularia* polysaccharides is critical to their anti-diabetic properties and glucose tolerance (Yuan et al. 1998b). According to the study, in contrast to the anti-diabetic properties of crude polysaccharides and neutral polysaccharide fractions extracted from *A. auricula-judae*, the acidic polysaccharide fraction derived from *A. auricula-judae* had no impact on diabetes when tested on mice. The administration of crude polysaccharides but not neutral polysaccharides improved glucose tolerance (Yuan et al. 1998b). In addition to maintaining blood glucose levels, *Auricularia* has also been associated with the treatment of nerve damage linked to diabetes. Aldose Reductase Inhibitors are a drug used for the treatment of this type of nerve damage and *A. auricula-judae* polysaccharide extracts exhibited similar actions to Aldose Reductase inhibitory activity, suggesting its application as an anti-diabetic drug (Wu & Xu 2015).

Additional bioactive compounds and their functions

Proteins

After polysaccharides, proteins are the most abundant bio-compounds in *Auricularia* species representing its proximate composition. Proximate composition analysis of *A. auricula-judae* has shown that it contains 8.36% of proteins and 33.04% of total proteins are composed of essential amino acids (Afiukwa et al. 2015). Some of the proteins and peptide sequences isolated from *Auricularia* appear to have medical and pharmaceutical properties similar to polysaccharides (Sheu et al. 2004, Agyei & Danquah 2011). The peptide sequences from *Auricularia* species that have pharmacological properties are called bioactive peptides (Agyei & Danquah 2011). An example of a bioactive protein is an immunomodulatory protein extracted from the basidiocarp of *A. polytricha* found to have immunostimulant abilities (Sheu et al. 2004). Further experimentation has shown that the immunomodulatory proteins isolated from *A. polytricha* have properties of pharmaceutical and stable immune stimulants such as tolerance to acids and alkali, heat and freezing, and dehydration (Chang et al. 2007).

Exo-biopolymers

Exo-biopolymers, also known as exopolymers, are biopolymers generated by an organism, such as a fungus, and are secreted into the environment. In particular, *Auricularia* species are known to produce a wide range of biopolymers (Yang et al. 2002, Xu & Yun 2003, Jeong et al. 2007). These compounds have been shown to have potential in the treatment of hyperlipidemia, in a study carried out on hyperlipidemic rats (Yang et al. 2002). In these rats, an exo-biopolymer, isolated from *A. polytricha* and composed of carbohydrates (77.5%) and proteins (22.5%), reduced plasma LDL cholesterol by 70% in rats (Yang et al. 2002).

Melanin

Apart from the major edible and medicinal components of *Auricularia* species, melanin is one compound that has applications in the food industry, as well as the pharmacological, medicinal, and cosmetic fields, with potential for novel claims within these industries (Hyde et al. 2010, Sun et al. 2016). Submerged cultures of *A. auricula* with glucose, tyrosine, peptone, and $CaCO_3$ additives is a reliable method for the large-scale production of natural melanin (Zhang et al. 2015b). Sun et al. (2016) demonstrated that the use of the yeast extracts tyrosine and lactose during the microbial submerged fermentation process of the melanin extraction and purification protocol could

significantly increase the amount of purified melanin by *A. auricula*. The capacity of *A. auricula* submerged cultures (with tyrosine additives) to produce melanin pigments has also been demonstrated (Wu et al. 2018, Zou & Ma 2018). Thus, *A. auricula* also has applications in the food industry as a colorant (Zou & Ma 2018). Additionally, a mix of methanol (1%), peanut oil (0.25%), and stearic acid (1.0%) or palmitic acid (0.5%) has been shown to increase the biosynthesis of melanin 1.53 times that of submerged cultures of *A. auricula* (Wu et al. 2018). Melanin isolated from *A. auricula* also possesses anti-microbial or anti-biofilm properties, as illustrated by the inhibition of biofilms formed by *E. coli* K-12, *Pseudomonas aeruginosa* PAO1, and *Pseudomonas fluorescens* P-3 (Bin et al. 2012).

Adenosine

Adenosine is a nucleoside which is a major constituent of DNA and RNA. Adenosine from *A. auricula* extracted using water, methanol, or butanol showed a potent inhibitory effect for rabbit platelet aggregation and suggests anti-platelet functions (Murata et al. 2004). *Auricularia auricula* and *A. polytricha* with adenosine contents of 133 and 154 µg/g of dry fungus, respectively, have been shown to inhibit ADP-induced aggregation of platelets both on a rat model and in human platelets, thereby indicating their potential for application in humans (Agarwal et al. 1982). These anti-platelet or anti-aggregant properties are of great interest for their capacity to inhibit thrombus formation, and thus can be used as a potential drug therapy for cerebrovascular or cardiovascular diseases.

Polyphenolic compounds

Ethanol extract of *A. auricula* containing polyphenolic compounds [16% (g/g dry weight) without polysaccharides, water-soluble fiber, and proteins] was found to decrease total cholesterol levels and atherosclerosis indexes in mice, indicating the hypocholesterolemic effects of these polyphenolic compounds (Chen et al. 2011). According to the authors, the study provides evidence for the application of *A. auricula* not only to prevent but also to treat cardiovascular disease.

Conclusion and Future Perspectives

Among the bioactive chemicals of *Auricularia* species, polysaccharides are the most promising, possessing anti-cancer, anti-bacterial, anti-viral, immunomodulatory, antioxidant, hypoglycemic, and anti-hypercholesterolemic properties. In addition, exo-biopolymers, melanin, polyphenolic compounds, and proteins have also been recognized as potential pharmacological agents that can be further developed into novel drugs for treating human disease. However, proteins have been shown to be of great potential as a source of novel compounds and treatments, and thus should be more extensively researched in the future. The bioactive compounds of *Auricularia* species hold promise for the development of future drug therapies and supplements for many human diseases. However, much of the research regarding these treatments has been conducted on animal models and thus requires further validation using clinical trials and human patients.

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Figures

Figure 1 – Edible species of *Auricularia* in Asia **a.** *Auricularia cornea* (Phetchaburi, Thailand), **b.** *Auricularia heimuer* (Mudanjiang, Heilonjiang, China), **c.** *Auricularia delicata* (Honghe, Yunnan, China) and **d.** *Auricularia thailandica* (Kunming, Yunnan, China). – Scale bar for a-d: 3 cm. These pictures are copyright of Asanka R. Bandara

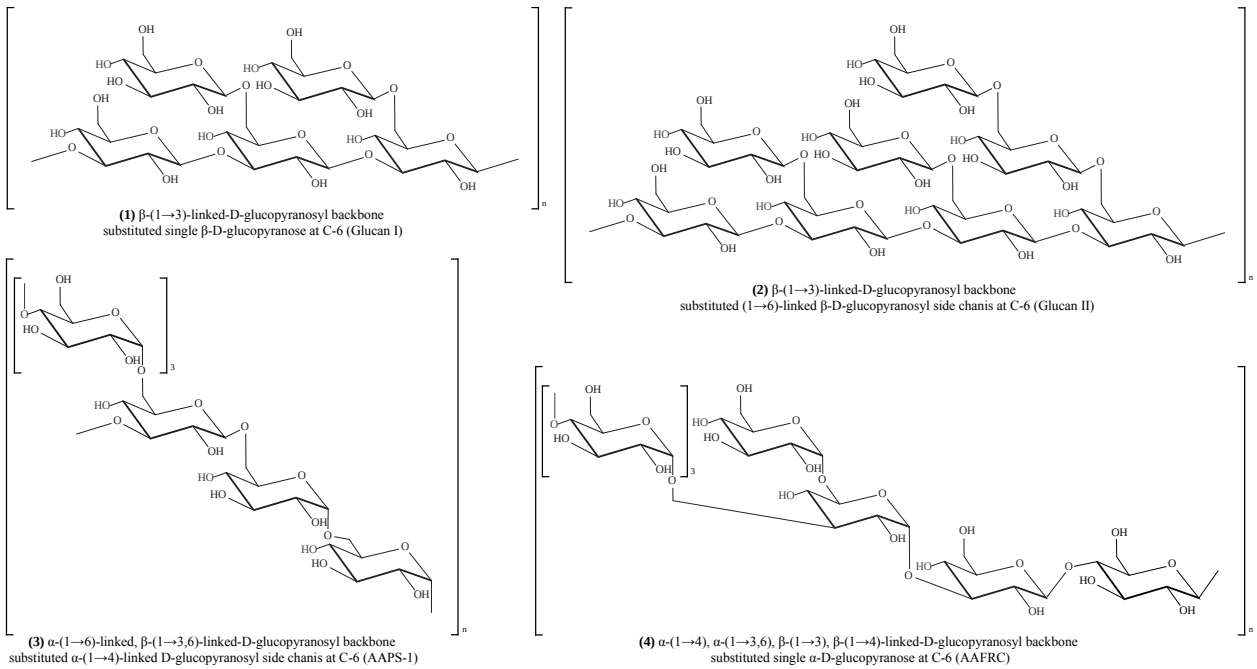
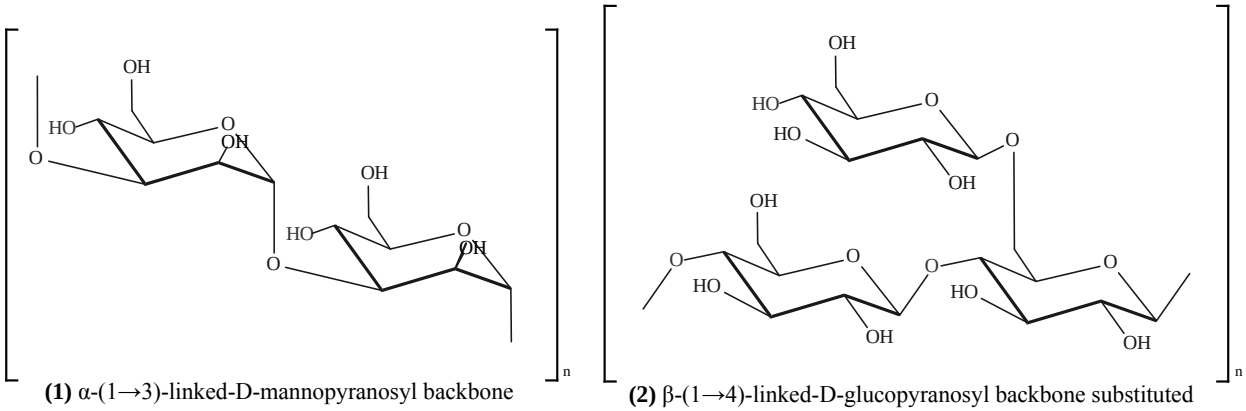


Figure 2 – Chemical structures of various types of homopolysaccharides from *Auricularia* spp. 2.1. Glucan I, 2.2. Glucan II (Sone et al. 1978, Misaki et al. 1981, Misaki & Kakuta 1995), 2.3. AAPS-1, 2.4. AAFRC (Song & Du 2012a, b).



(1) α-(1→3)-linked-D-mannopyranosyl backbone

(2) β-(1→4)-linked-D-glucopyranosyl backbone substituted β-(1→6)-linked glucopyranosyl side chanis at C-6 (AAG)

Figure 3 – Chemical structures of various types of backbones of heteropolysaccharides from *Auricularia auricula-judae*. 2.1. α-(1→3)-linked D-mannopyranosyl backbone (Sone et al. 1978, Misaki et al. 1981, Ukai et al. 1982, 1983a, b), 2.2. AAG (Ma et al. 2008, 2010)

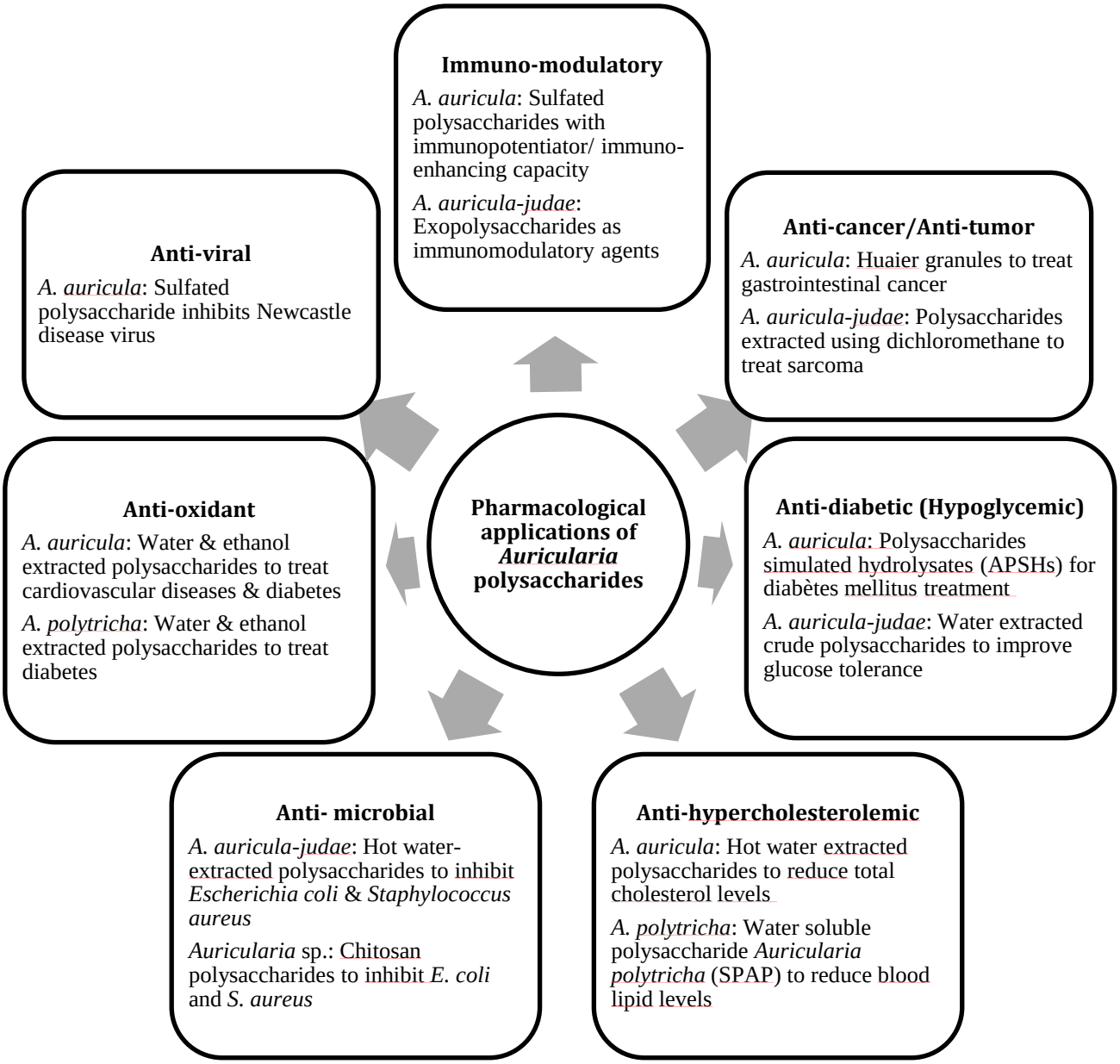


Figure 4 – Bioactive properties of the polysaccharides extracted from different *Auricularia* species. The figure summarizes anti-cancer / anti-tumor (Reza et al. 2011, Ma et al. 2018), anti-diabetic (Yuan et al. 1998b, Lu et al. 2018b), anti-hypercholesterolemic (Chen et al. 2008, Zhao et al. 2015), anti-microbial (Cai et al. 2015, Xiong et al. 2016, Chang et al. 2019), antioxidant (Wu et al. 2010, Avci et al. 2016, Hu et al. 2017), anti-viral (Nguyen et al. 2012a) and immunomodulatory (Nguyen et al. 2012b, Zhang et al. 2018a) properties of polysaccharides from *Auricularia* species.

Tables

Table 1 Nutrient content including total carbohydrate, fat, fibre, protein, free amino acids and total soluble sugars of *Auricularia auricula-judae*, *A. fuscosuccinea*, *A. polytricha* and *A. thailandica* (% of dry weight).

***Auricularia* species**	**Total carbohydrate**	**Fat**	**Fibre**	**Protein**	**Free amino acids**	**Total soluble sugars**	**References**
Auricularia auricula-judae	81.0	1.5,1.7	3.5, 6.9	12.5, 11.5	—	10.2	(Crisan & Sands 1978, Aletor 1995, Kadnikova et al. 2015)
Auricularia fuscosuccinea (b)	71.2,	4.5	11.7	8.6	0.12	9.9	(Mau et al. 1998)
Auricularia fuscosuccinea (w)	68.9	4.5	12.5	12.5	0.07	10.9	(Mau et al. 1998)
Auricularia polytricha	88.1, 91.1	0.48, 0.5	3.6	6.5	0.05	17.9	(Mau et al. 1998, USDA 2018a)
Auricularia thailandica	—	2.93	4.62	12.99	1.13	16.23	(Bandara et al. 2017a)

b: brown strain; w: white strain; nitrogen to protein conversion factor: N × 6.25.

Table 2 Amino acid contents of *Auricularia auricula-judae*, *A. fuscosuccinea*, *A. polytricha* and *A. thailandica* (% of dry weight).

Amino acid	***Auricularia auricula-judae***	***Auricularia fuscosuccinea* (b)**	***Auricularia fuscosuccinea* (w)**	***Auricularia polytricha***	***Auricularia thailandica***
ALA alanine	3.44	0.24	0.04	0.11	0.53
ARG arginine	3.37	0.13	0.08	0.04	0.63
ASP aspartic acid	3.00	0.12	0.06	0.02	0.35
CYS cystine	0.30	—	—	—	0.15
GLU glutamic acid	5.53	0.14	0.16	0.03	0.92
GLY glycine	1.32	0.02	0.02	0.01	0.29
HIS histidine[e]	0.96	—	—	0.03	0.52
ILE isoleucine[e]	1.03	0.04	0.03	0.04	0.40
LEU leucine[e]	1.94	—	—	0.05	0.73
LYS lysine[e]	1.22	0.13	nd	0.13	0.58
MET methionine[e]	0.35	—	—	0.007	0.01
PHE phenylalanine[e]	1.06	0.07	0.02	0.04	0.44
PRO proline	0.20	—	—	—	0.36
THR threonine[e]	1.74	0.16	0.16	0.03	0.58
TRP tryptophan	—	0.02	0.01	0.01	—
TYR tyrosine	1.03	0.03	0.06	0.02	0.21
SER serine	2.07	0.08	0.16	0.02	0.31
VAL valine[e]	1.25	0.06	0.03	0.03	0.97
References	(Afiukwa et al. 2015)	(Mau et al. 1998)	(Mau et al. 1998)	(Mau et al. 1998, Razak 2013)	(Bandara et al. 2017a)

b: brown strain; w: white strain; nd: not detected; [e]: essential amino acid.

Table 3 Mineral contents of *A. auricula-judae*, *A. polytricha* and *A. thailandica* (mg/kg).

Mineral element	***Auricularia auricula-judae* (dw)**	***Auricularia polytricha* (fw)**	***Auricularia thailandica* (dw)**
Ca	16000	160	885
K	12000	430	13780
Mg	2000	250	895
Na	8000	90	113
P	—	140	3880
Cr	≤ 20	—	4
Cu	≤ 20	4	6
Fe	200	5	64
Mn	≤ 20	1	8
Ni	≤ 20	—	7
Zn	60	6	49
	(Kadnikova et al. 2015)	(USDA 2018a)	(Bandara et al. 2017a)

dw: dry weight; fw: fresh weight.

Table 4 Structure elucidated polysaccharides of different *Auricularia* species: extraction process, chemical, physical and pharmacological properties.

***Auricularia* species**	**Extraction**	**Chemical structure**	**Chemical and physical properties**	**Pharmacological properties, Tested living system**	**Routes of administration, Dose**	**Inhibition/ Decreasing ratio**	**References**
Auricularia auricula[*]	Dried fruit body → hot water extraction (100°C/2 times/4h) → supernatant → precipitated with EtOH → AAP → carboxymethylation → CMAAP → purified → CMAAP22**	CMAAP22: β-(1→3)-linked D- mannopyranosyl backbone substituted with β-D-glucose and partially substituted with -CH_2COOH at C-2, C-4, C-6	mannose:glucose = 1.06:1. m.w. 3.4×10^6 Da.	Antioxidant activity *in vitro*: OH^-, $ABTS^+$ radical scavenging activity	2 mg/ml (r.s.: vitamin C)	Scavenging rate 52.1%, ≈ 70%	(Yang et al. 2011)
A. auricula-judae[*]	Dried fruit body → 0.9% $NaCl_{(aq)}$ (24h) → Residue → hot water extract (120°C/20 min.) → precipitated with Cetylpyridinium chloride (CPC) → supernatant → precipitated with EtOH → Glucan I	Glucan I: β-(1→3)-linked D-glucopyranosyl backbone [two out of three glucose residues of the backbone were substituted with single glucopyranose at C–6] (Fig. 2.1).	$[\alpha]_D^{25}$ −10.1° (*c*=1.5, 0.5M NaOH). Neutral, water-soluble. 2.3% yield. m.w. 1.4×10^6	Anti-tumor activity on sarcoma 180 cells implanted ICR-JCR mice	i.p., 8 mg× 10 days	Average tumor weight 96.6%	(Sone et al. 1978, Misaki et al. 1981, Misaki & Kakuta 1995)
A. auricula-judae[*]	Dried fruit body → 0.9% $NaCl_{(aq)}$ (24h) → residue → hot water extraction (120°C/20 min.) → residue → hot alkali extraction (1M NaOH/65°C/2h) → residue → Glucan II	Glucan II: β-(1→3)-linked D-glucopyranosyl backbone [three out of four glucose residues of the backbone were substituted with single glucopyranose units, and (l→6)-D glucosidic linked short side chains (very few) at C–6] (Fig. 2.2).	Water, alkali (2M NaOH) or Me_2SO insoluble. 49% yield. m.w. ~ 6×10^3	Anti-tumor activity on sarcoma 180 cells implanted ICR-JCR mice	i.p., 10 mg× 10 days	Average tumor weight 18.9%	(Sone et al. 1978, Misaki et al. 1981, Misaki & Kakuta 1995)

Table 4 Continued.

***Auricularia* species**	**Extraction**	**Chemical structure**	**Chemical and physical properties**	**Pharmacological properties, Tested living system**	**Routes of administration, Dose**	**Inhibition/ Decreasing ratio**	**References**
A. auricula-judae[*]	Dried fruit body → 0.9% $NaCl_{(aq)}$ (24h) → Residue → hot water extraction (120°C/20 min.) → precipitated with Cetylpyridinium chloride → dissolve 10% $NaCl_{(aq)}$ → precipitated with EtOH → heteropolysaccharide	Heteropolysaccharide: α-(1→3)-linked D-mannopyranosyl backbone (Fig. 3.1) substituted with D-xylopyranose, D-mannopyranose, D-glucopyranosuronic acid residues at C-2 or C-6 and small proportion of (1→3)-linked D-glucopyranose.	$[\alpha]_D^{25}$ −20.3° (0.5M NaOH). Water-soluble. Molar ratio of D-xylose: D-mannose: D-glucose: D-glucuronic acid = 1: 4.1: 1.3: 1.3. 48.7% yield. m.w. 5×10^5	Anti-tumor activity on sarcoma 180 cells implanted ICR-JCR mice	i.p., 10 mg× 10 days	Average tumor weight 18.5%	(Sone et al. 1978, Misaki et al. 1981)
A. auricula-judae[*]	Dried fruit body → $NaCl_{(aq)}$ (24h) → Residue → hot water extraction (120°C/20 min.) → residue → hot alkali (1M NaOH/65°C/2h) extract → precipitated with EtOH → heteropolysaccharide		Alkali soluble. Molar ratio of D-xylose: D-mannose: D-glucose: D-glucuronic acid = 1: 2.1: 1: 0.6.	—	—	—	(Sone et al. 1978)

Table 4 Continued.

Auricularia species	Extraction	Chemical structure	Chemical and physical properties	Pharmacological properties, Tested living system	Routes of administration, Dose	Inhibition/ Decreasing ratio	References
A. auricula-judae[*]	Dried fruit body → hot MeOH extraction → residue → hot 70% EtOH extract (3 times/20h) → purified then fractionated by cetyltrimethylammonium bromide (CTAB) →MEA**	MEA and MHA (heteropolysaccharide): α-(1→3)-linked D-mannopyranosyl backbone (Fig. 3.1) substituted with β-D- glucopyranosyluronic acid at C-2, single residue or short chains of β-D-xylopyranose at C-2, C-6 and small amount of (1→6)-linked D- mannopyranose (and/or D-glucopyranose), branching D-mannopyranose at C-4, C-6.	$[\alpha]_D^{23}$ +31° (*c*=1, H_2O). Me_2SO slightly soluble. Molar ratio D-glucuronic acid: D-xylose: D-mannose = 1: 0.5: 2.8. 4% yield. m.w. 3.7×10^5	Anti-tumor activity on sarcoma 180 cells implanted ddY male mice	i.p., 25 mg× 10 days	Average tumor weight 42%	(Ukai et al. 1982, 1983b)
				Anti-inflammatory activity on Sprague Dawley mice: Carrageenin edema, Scald hyperalgesia	i.p., 50 mg/kg× 2 times immediately and 1h after	Hind paw thickness 0%, Pain threshold 6%	(Ukai et al. 1983a)
A. auricula-judae[*]	Dried fruit body → hot MeOH extraction → Residue → Hot 70% EtOH extraction (3 times/20h) → residue → hot water extract (93–98°C/4 times/4h) → purified then fractionated by CTAB →MHA**		$[\alpha]_D^{23}$ +33° (*c*=1, H_2O). Me_2SO insoluble. Molar ratio D-glucuronic acid: D-xylose: D-mannose = 1: 0.6: 1. 16% yield. m.w. 3×10^5	Anti-tumor activity on sarcoma 180 cells implanted ddY male mice	i.p., 25 mg× 10 days	Average tumor weight 29%	(Ukai et al. 1982, 1983b)
				Anti-inflammatory activity on Sprague Dawley mice: Carrageenan induced edema, Scald induced hyperalgesia	i.p., 50 mg/kg× 2 times immediately and 1h after	Hind paw thickness 33%, Pain threshold 19%	(Ukai et al. 1983a)
A. auricula-judae[*]	Dried fruit body → hot ethyl acetate and methanol → Residue → 70% ethanol/water extract	AAG: β-(1→4)-linked D-glucopyranosyl backbone with β-(1→6)-linked D-glucopyranosyl side groups at C-6 (Fig. 3.2). Glucuronic acid may substitute on two adjacent glucose residues, or substitute alternate with one or more glucose residues.	Water soluble, D-glucose: D-glucuronic acid = 6: 1. m.w. 2.88×10^5	Anti-tumor activity on acinar cell carcinoma *in vitro*, sarcoma 180 cells implanted BALB/c male mice	(1) 0.05 mg/l (2) i.p., 20 mg/kg× 8 days. (r.s.: 5-Fluorouracil)	Average tumor weight 34.1%, 39.1%	(Ma et al. 2008, 2010)

Table 4 Continued.

***Auricularia* species**	**Extraction**	**Chemical structure**	**Chemical and physical properties**	**Pharmacological properties, Tested living system**	**Routes of administration, Dose**	**Inhibition/ Decreasing ratio**	**References**
A. polytricha[*]	Dried fruit body → hot water extract (90°C/3 times/3h) → precipitated with EtOH → purified → APPS → HSCCC separation → AAPS-1, AAPS-2, AAPS-3**	AAPS-2: β-(1→3)-linked D-glucopyranosyl backbone [one out of three glucose residues of the backbone were substituted with single β-D-glucopyranose at C–6]	m.w. 2.59×10^5 Da	Anti-tumor activity on sarcoma 180 cells implanted Swiss albino female mice	p.o., 12 mg/kg/day (r.s.: cyclophos-phamide)	Average tumor weight 40.43%	(Song & Du 2010)
A. polytricha[*]	Dried fruit body → hot water extract (90°C/3 times/3h) → precipitated with EtOH → purified → APPS → HSCCC separation → AAPS-1**	AAPS-1: α-(1→6), β-(1→6)-linked D-glucopyranosyl backbone substituted with α-(1→4)-linked D-glucopyranose side chains (Fig. 2.3)	Water-soluble. m.w. 1.62×10^5 Da	—	—	—	(Song & Du 2012b)
A. polytricha[*]	Dried fruit body → hot water extract (100°C/2h) → centrifuged residual → hot 0.9% NaCl extract (100°C/2h) → centrifuged residual → 0.05M NaOH extract (50°C/2h) → AAFRC**	AAFRC: α-(1→3), α-(1→4), β-(1→3), β-(1→4)-linked D-glucopyranosyl backbone substituted with single α-(1→6)-linked D-glucopyranose at C–6 on every six residues (Fig. 2.4)	Water-soluble. m.w. 1.2×10^6 Da	Anti-tumor activity on sarcoma 180 cells implanted ICR female mice	i.p., 24 mg/kg/day (r.s.: cyclophos-phamide)	Average tumor weight 43.61%	(Song & Du 2012a)
Auricularia sp.[*] (Yū ěr)	Dried fruit body → extracted with hot MeOH, hot 70% EtOH, hot water Successively → residue extracted with NaOH (1M /25°C) → precipitated with EtOH → N-5P**	N-5P: β-(1→3)-linked D-glucosyl backbone substituted with single glucose units at C–6.	$[\alpha]_D^{23}$ +2.3° (*c*=0.51, 0.5M NaOH). Water, acidic insoluble. Alkali, Me_2SO soluble. 1.2% yield. m.w. 5.6×10^5	Anti-tumor activity on sarcoma 180 cells implanted ddY male mice	i.p., 10 mg/kg × 10 days	Average tumor weight 81%	(Kiho et al. 1987)

Table 4 Continued.

***Auricularia* species**	**Extraction**	**Chemical structure**	**Chemical and physical properties**	**Pharmacological properties, Tested living system**	**Routes of administration, Dose**	**Inhibition/ Decreasing ratio**	**References**
Auricularia sp.* (Yū ěr)	Dried fruit body → hot MeOH extract + residue extracted with hot 70% EtOH + residue extracted with hot water (4 times/5h) → Purified, precipitated with EtOH → U-3-EP → fractionated by CTAB → U-3-A precipitated → supernatant → purified → U-3-N**	U-3-N: β-(1→3)-linked D-glucosyl backbone substituted with single β-(1→6)-linked D-glucose.	$[\alpha]_D^{23}$ +1.0° (*c*=0.13, 0.5M NaOH). Water insoluble. Alkali soluble. 1.24% yield. m.w. 6.1×10^5	Anti-tumor activity on sarcoma 180 cells implanted ddY male mice	i.p., 1 mg/kg × 10 days	Average tumor weight 84%	(Kiho et al. 1991)
Auricularia sp.* (Yū ěr)	Dried fruit body → hot MeOH extract + residue extracted with hot 70% EtOH + residue extracted with hot water (4 times/5h) → purified, precipitated with EtOH → U-3-EP → fractionated by CTAB → U-3-A precipitated → supernatant → purified → fractionated eluted by NaCl → U-3-AP_1**	U-3-AP_1: β-(1→3)-linked D-glucosyl backbone substituted with β-(1→6)-linked D-glucosyl residue.	$[\alpha]_D^{23}$ +2.5° (*c*=0.13, 1M NaOH). Water insoluble. Alkali soluble. 0.63% yield. m.w. 6.4×10^4	Anti-tumor activity on sarcoma 180 cells implanted ddY male mice	i.p., 1 mg/kg × 10 days	Average tumor weight 8%	(Kiho et al. 1991)

*commercial strain; **abbreviated name of polysaccharide used in the original reference; i.m., intramuscular injection; i.p., intraperitoneal injection; i.v., intravenous injection; m.w., molecular weight; r.s., reference standard; p.o., oral administration; s.c., subcutaneous injection.

Table 5 Polysaccharides with unknown structure of different *Auricularia* species: extraction process and pharmacological properties.

***Auricularia* species**	**Extraction**	**Pharmacological activities / Tested living system**	**Routes of administration/ Dose**	**Inhibition / Decreasing ratio**	**References**
Auricularia auricula[*]	Dried fruit body → refluxed with EtOH (76°C/2h/2 times) → suspension → purified → supernatant → extracted with EtOH (4°C/24h) → concentrated and lyophilized → crude polysaccharide	Anti-coagulant activity on Sprague–Dawley male rats *in ex vivo*	p.o., 300 mg/kg body weight/ day × 4 weeks (r.s.: aspirin)	Inhibitory effects on platelet aggregation = 39.6%	(Yoon et al. 2003)
A. auricula	Fresh fruit body → hot water extract (100°C/2h) → centrifuged → supernatant → purified → precipitated with EtOH → dialyzed and purified → CAAP**	Antioxidant activity *in vitro* (1) OH^- (2) O_2^{2-} (3) DPPH (4) Fe^{2+} radical scavenging activity	0–2.0 mg/ml (r.s.: vitamin C)	EC_{50} = (1) >2 mg/ml (2) >2 mg/ml (3) 1.62 (4) >2 mg/ml	(He et al. 2012)
A. auricula[*]	Dried fruit body → hot water extract (94 °C/3.4h) → centrifuged → supernatant → precipitated with EtOH → centrifuged → purified → AAFB**	Antioxidant activity *in vitro* (1) Fe^{2+} (2) OH^- radical scavenging activity	0.2–1.0 mg/ml (r.s.: butylated hydroxytoluene)	IC_{50} = (1) 0.43 mg/ml (2) 0.38 mg/ml	(Zou et al. 2015)
A. auricula[*]	Dried fruit body → hot water extract → precipitated with EtOH → purified → Sulfation → sAAPt**	Anti-viral activity on Newcastle disease virus infected SPF chicken embryo *in vitro*	0.244–3.91 µg/ml	Virus inhibitory rate = 70.90%	(Nguyen et al. 2012a)
A. auricula	Dried fruit body → hot water extract (100°C/2 times/2h) → precipitated with EtOH → AARP**	Hepatoprotective activity on CCl_4 induced liver injured mice	50-100 mg/kg/day	Serum aspartate aminotransferase (AST) and malondialdehyde aldehyde (MDA) decreased, superoxide dismutase (SOD) activity increased ($P < 0.05$)	(Guo et al. 2015)
A. auricula[*]	Dried fruit body → Ether extract (r.t./3 times/48h) → residue → hot water extract (100°C/2 times/2h) → precipitated with EtOH → AAP **	Hypocholesterolemic activity on cholesterol-enriched diet fed male ICR mice	p.o., 120 mg/kg/day (r.s.: distilled water)	Concentrations of total cholesterol and low-density lipoprotein cholesterol in blood serum decreased significantly ($P < 0.05$)	(Chen et al. 2008)
A. auricula[*]	Dried fruit body → refluxed with EtOH (80°C/3 times/2h) → suspension → extracted with EtOH (4°C/24h) → concentrated and lyophilized → AAE**	Hypocholesterolemic activity on cholesterol-enriched diet fed male ICR mice	p.o., 150 mg/kg/day (r.s.: distilled water)	Concentrations of total cholesterol in blood serum and hepatic lipids serum decreased significantly ($P < 0.05$)	(Chen et al. 2011)

Table 5 Continued.

***Auricularia* species**	**Extraction**	**Pharmacological activities / Tested living system**	**Routes of administration/ Dose**	**Inhibition / Decreasing ratio**	**References**
A. auricula-judae	Dried fruit body → 80% EtOH extract (100°C/2 times/3h) → residue → suspended with water → Dichloromethane fraction	Anti-inflammatory activity *in vitro* on Murine macrophage RAW 264.7 cells	100 µg/ml (r.s.: —)	73.5%	(Damte et al. 2011)
A. auricula-judae	Dried fruit body → 95% EtOH extract (r.t./72h)	Anti-microbial activity *in vitro* on *Bacillus subtillis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*	10 mg/ml (r.s.: vancomycin)	Minimum inhibitory concentrations (mg/ml): *Escherichia coli*, *Pseudomonas aeruginosa* = 50; *Bacillus subtillis*, *Salmonella typhi*, *Staphylococcus aureus* = 12.5	(Deka et al. 2017)
A. auricula-judae	Ethanol extract – Dichloromethane fraction (Diazane + unidentified components)	Anti-tumor activity on broncheoalveolar cancer NCI H538 *in vitro*	0.01–1.0 mg/ml (r.s.: doxorubicin)	IC_{50} = 57.2 µg/ml	(Reza et al. 2014)
A. auricula-judae	Ethanol extract – Dichloromethane fraction (Diazane + unidentified components)	Anti-tumor activity on Gastric cancer cells SNU1 *in vitro*	0.01–1.0 mg/ml (r.s.: doxorubicin)	IC_{50} = 73.2 µg/ml	(Reza et al. 2014)
A. auricula-judae	Dried fruit body → 70% EtOH extract (100°C/6h)	Anti-tumor activity *in vitro* on (1) P388D1 macrophage, (2) Sarcoma 180, (3) NCI H358 (bronchoalveolar), (4) SNU1 (gastric carcinoma) cells	1 mg/ml (r.s.: doxorubicin)	(1) 42.21%, (2) 65.71%, (3) 69.76%, (4) 68.01%	(Reza et al. 2012)
A. auricula-judae	Ethanol extract – Dichloromethane fraction	Anti-tumor activity on sarcoma 180 tumor cells implanted BALB/c mice	p.o., 100 mg/kg × 10 days (r.s.: doxorubicin)	42.62%	(Reza et al. 2011)
A. auricula-judae[*]	Dried fruit body → 85% EtOH extract (r.t./3 times/48h) → residue → hot water extract (100°C/4 times/3h) → precipitated with EtOH → polysaccharide: FA**	Hypoglycemic activity on male KK-A^y genetically diabetic mice	p.o., 30 g of FA/kg of diet	Plasma glucose, insulin, and urinary glucose significantly decreased ($P < 0.05$)	(Yuan et al. 1998a)

Table 5 Continued.

***Auricularia* species**	**Extraction**	**Pharmacological activities / Tested living system**	**Routes of administration/ Dose**	**Inhibition / Decreasing ratio**	**References**
A. auricula-judae	Dried fruit body → 70% EtOH extract (100°C/6h) → supernatant → concentrated → AAE**	Hypolipidemic activity on male C57BL/6 mice *in vivo*	0.1%, 0.3%, 1% (w/w) × 8 weeks (r.s.: —)	Body weight, weight of adipose tissue and weights of different organs not significantly ($P > 0.05$) different from control group	(Reza et al. 2015)
A. polytricha[*]	Fruit body → 95% EtOH extract (75°C /5h) → residue → hot water extraction (3 times/6h for each) → supernatant → precipitated with EtOH → polysaccharide: APPs**	Anti-cancer activity: (1) Lung cancer cells A549 (*in vitro*) (2) subcutaneous injected A549 cells BALB/c-nu mice (*in vivo*)	(1) 5–200 μg/ml (2) i.p., 1–3 mg/kg × 15 days (r.s.: normal saline)	IC50: (1) 28.07 μg/ml (2) 0.38 mg/ml	(Yu et al. 2014)
A. polytricha	Fruit body → hot water (95°C/3 times/4h) extract → precipitated with EtOH → polysaccharide: SPAP**	Anti-hypercholesterolemic activity on cholesterol enriched diet fed Sprague-Dawley male mice	p.o., 4.5 mg/kg of body weight	Total cholesterol (34.6 ± 7.6%), triglycerides and LDL-cholesterol in blood serum significantly ($P < 0.05$) decreased	(Zhao et al. 2015)
A. polytricha	Dried fruit body → 95% MeOH extract (— °C/7 days)	Anti-microbial activity *in vitro* on *Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*	20 mg/ml (r.s.: —)	Minimum inhibitory concentrations (mg/ml): *Bacillus cereus* = 3.75; *Escherichia coli* = 3.0; *Proteus vulgaris* = 5.5; *Staphylococcus aureus* = 7.0	(Gbolagade & Fasidi 2005)
A. polytricha	Dried fruit body → hot 95% EtOH extract (75°C/3 times/3h) → residue → hot water extract (75°C/3 times/3h) → precipitated with 95% EtOH → APPsA-1**, APPsB-1**, APPsB-2**, APPsC-1**	Antioxidant activity *in vitro* (1) OH^- (2) Fe^{3+} radical scavenging activity	4 mg/ml (r.s.: ascorbic acid, ethylenediaminete traacetic acid)	Scavenging rate APPsC-1, APPsB-2, APPsB 1: (1) ≈70–90% (2) ≈60–80%. APPsA-1: (1) ≈20–30% (2) ≈20–30%	(Sun et al. 2010)
A. polytricha	Dried fruit body → hot water extract (100°C/2h) → centrifuged → supernatant → precipitated with EtOH → centrifuged → residual in water → dialyzed → precipitated with EtOH → purified polysaccharides: APPIIA**	Anti-tumor activity on Sarcoma 180 cells implanted BALB/c albino mice	i.p., 5 mg/kg × 15 days	Average tumor growth inhibition 53.6% ($P < 0.01$)	(Yu et al. 2009)
Auricularia sp.[*] (Yū ěr)	Dried fruit body → hot MeOH extract + residue extracted with hot 70% EtOH + residue extracted with hot water (4 times/5h) → purified, precipitated with EtOH → U-3-EP** → fractionated by CTAB → U-3-A**	Anti-inflammatory activity on Sprague Dawley mice (1) Carrageenin induced edema (2) Scald induced hyperalgesia	i.p., 25 mg/kg (r.s.: phenylbutazone)	(1) Hind paw thickness 50.1% (2) Pain threshold 64.1%	(Kiho et al. 1985)

Table 5 Continued.

***Auricularia* species**	**Extraction**	**Pharmacological activities / Tested living system**	**Routes of administration/ Dose**	**Inhibition / Decreasing ratio**	**References**
Auricularia sp.* (Yū ěr)	Dried fruit body → hot MeOH extract + residue extracted with hot 70% EtOH + residue extracted with hot water (4 times/5h) → purified, precipitated with EtOH → U-3-EP** → fractionated by CTAB → U-3-A**	Anti-tumor activity on Sarcoma 180 cells implanted ddY male mice	i.p., 25 mg × 10 days	Average tumor weight 86%	(Ukai et al. 1983b)
A. thailandica	Dried fruit body → 100% MeOH extract (45°C/40 min.)	Antioxidant activity *in vitro* (1) ABTS (2) DPPH radical scavenging activity	1–5 mg/ml (r.s.: ascorbic acid	EC50 (1) 835 µg/ml (2) 342 µg/ml	(Bandara et al. 2017a)

*commercial strain; **abbreviated name of polysaccharide used in the original reference; i.m., intramuscular injection; i.p., intraperitoneal injection; i.v., intravenous injection; m.w., molecular weight; r.s., reference standard; r.t., room temperature; p.o., oral administration; s.c., subcutaneous injection.