PURIFICATION AND CHARACTERIZATION OF POLYSACCHARIDES EXTRACTED FROM *Tremella fuciformis* AND *Auricularia auricula*

Ratchanee Khamlue [1], Anan Ounaroon [1], and Nuttawut Saelim [2*]

[1] Department of Pharmaceutical Chemistry and Pharmacognosy,
Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand
[2] Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences,
Naresuan University, Phitsanulok 65000, Thailand

*e-mail: nut456zz@yahoo.com

Abstract

For decades, many studies reported and suggested the therapeutic uses of polysaccharides extracted from various sources, especially from wild mushrooms. *Tremella fuciformis* and *Auricularia auricula* are edible fungi commonly abundant in Thailand, and the major component found both fungi is polysaccharides. *T. fuciformis* and *A. auricula* have long been studied for its medicinal purposes such as anti-oxidant, anti-tumor property, anticoagulant activity, hypoglycemic activity, memory improvement, etc. Even though some studies reported potential pharmacological activities of the polysaccharides extracted from *T. fuciformis* and *A. auricular*. The chemical composition and efficiency of extraction processes of polysaccharides in *T. fuciformis* and *A. auricula* were not completely clear. We, therefore, purposed this study to purify and characterize the polysaccharides isolated from *T. fuciformis* and *A. auricula*. The polysaccharides purification process for *T. fuciformis* and *A. auricula* were performed by using the method suggested by Yoon et al. (2003) with some modification until purified polysaccharides obtained. Proteins contamination was determined by BCA protein assay and SDS-PAGE gel. Nucleic acids contamination was measured by using UV–Vis spectrophotometer at 260 nm wavelength. Using this method, our results showed that purified polysaccharides from both fungi were white in color indicating no pigments contamination. The yields of purified polysaccharides from *A. auricula* and *T. fuciformis* were around 0.84% and 2.0% (w/w) with around 80% and 91% polysaccharides purity (expressed as glucose/xylose and mannose/xylose combined), respectively. The proteins and nucleic acids contaminants were small amounts, less than 0.25% and around 0.8% in *A. auricular*, and less than 0.25% and around 3.0% in *T. fuciformis.* In summary, we successfully obtained the purified polysaccharides from *T. fuciformis* and *A. auricula* with high purity and low in pigments, proteins and nucleic acids contaminants.

Keywords: *Tremella fuciformis*, *Auricularia auricula*, Polysaccharides, Fungus, Mushroom

Introduction

Mushrooms have been valued throughout the world as both food and medicine for thousands of years. The main compounds commonly observed were various types of polysaccharides. Polysaccharides are large complex branched chain-like molecules built from many single units of monosaccharides. Previous studies reported and convinced that polysaccharides extracted from various mushrooms were one of obvious choices for investigation in search for their therapeutic uses. There were many pharmacological studies using polysaccharides isolated from some mushrooms, and proved to have antitumor and

immuno-stimulating properties, antibiotic activity, antiviral properties, serum lipids and sugar levels reduction. Fungal polysaccharides showed benefit for supporting some or all of the major systems of our body, including nervous, hormonal, and immune systems as well as regulatory functions that help the body to adapt to various environmental and biological stresses. For examples, Hobbs (2000) reported that polysaccharides, lentinan, isolated from *Lentinula edodes* mycelia, provided adjuvant effectiveness in certain cancers(Hobbs, 2000), and Song *et al.* (2010) reported polysaccharides isolated from *Auricularia polytricha* inhibited sarcoma cancer *in vivo* (Song et al., 2010).

Tremella fuciformis (snow fungus, white jelly mushroom) has been appreciated as an edible mushroom. It has a long history of medicinal uses and its various traditional indications including clearing heat and dryness, nourishing the brain and enhancing beauty, etc. *T. fuciformis* is rich in polysaccharides suggested the main bioactive component. The chemical structure of the polysaccharides consists of a linear backbone of (1→3) α-D-mannan with side chains composed of glucuronic acid, xylose and fucose. The estimated ratio of mannose, fucose, xylose, and glucuronic acid is 9:1:4:3 that makes glucoronic acid accounted for 17.6% of the polysaccharides content in *T. fuciformis* (Wu et al., 2008; Kakuta et al., 1979). *T. fuciformis* has been studied and reported for its medicinal purposes such as anti-oxidant (Wu et al., 2008), hypoglycemic (Cho et al., 2007), memory improvement and anti-aging (Kim et al., 2007), suppressing fat accumulation and lowering plasma cholesterol (Kiho et al., 2000).

Auricularia auricula (Wood ear, Jews ear, jelly ear fungus) is an edible black–brown mushroom with high content of carbohydrates, proteins and minerals (Fan et al., 2006). The main monosaccharides composition of *A. auricula* polysaccharides is glucose (72%), mannose (8%), xylose (10%) and fucose (10%) [(9)].The polysaccharides backbone chain is (1→3) β-D-glucans (Misaki et al., 1981). In recent studies, polysaccharides from *A. auricula* has found to have potential biological activities such as antioxidant activity (Acahrya et al., 2004), anti-tumor property (Misaki et al., 1981) , hypoglycemic activity (Pisuena et al., 2003; Zhang et al.,1995), hypolipidemic activity (Takeuchi et al., 2004), anti-inflammatory (Ukai et al.,1983), anticoagulant activity (Yoon et al., 2003) and cardio-protective effect (Wu et al., 2010)

Due to common availability of *T. fuciformis A. auricula* in Thailand, we focused on isolation and basic characterization of the polysaccharides obtained from both mushrooms.

Methodology

1. Polysaccharides extraction and purification

The polysaccharides extracts of *T. fuciformis* and *A. auricula* were isolated by method suggested by Yoon et al. (2003) with some modification until purified polysaccharides obtained. In brief, fruit bodies of *T. fuciformis* and *A. auricula* were initially dried at 70ºC and ground using mortar. Dried powders were suspended and refluxed in methanol and thereafter the suspensions were filtered to remove the methanol-soluble materials such as colored materials, phenolic compounds and lipids. The filtrates were collected, suspended and refluxed in sterile water. Clear supernatants were collected after low speed centrifugation. Afterwards, the protein contaminations were removed by Sevag method. The polysaccharides in the concentrated supernatants were precipitated with absolute ethanol,

subsequently re-suspended in sterile water, and dialyzed by using 10kDa cut off membrane. The purified solutions were finally lyophilized [15].

2. Polysaccharides characterization by colorimetric (Phenol-Sulfuric acid method)

The content of polysaccharides in the extracts was determined by Phenol-Sulfuric acid method. Two milliliters of standard grade sugar solutions (ranging from 10 and 100 µg/ml concentrations of sugar) and 2 ml of purified polysaccharides from *T. fuciformis* and *A. auricula* at 100 µg/ml concentrations were pipetted into a test tube, and 1 ml of 5% phenol solutions were added. Then 5 ml of concentrated sulfuric acids were added rapidly. The tubes were shaken and placed in water bath at 30º C before readings procedure was taken. The colorimetric analysis of all solutions was carried out using a UV–Vis spectrophotometer. The absorbance of the characteristic yellow-orange color was measured at 490 nm for hexose monosaccharide and 480 nm for pentose monosaccharide and uronic acid. Blanks were prepared by substituting distilled water for the sugar solution. The amount of polysaccharides in fungal extracts were determined and expressed as amounts of hexose and pentose sugars by using constructed standard curves of each standard sugar(Dubois et al., 1956).

3. Determination of the contamination in the purified polysaccharides extracts

3.1 The protein contamination

Protein contamination in purified polysaccharides extracts were measured by bicinchoninic acid (BCA) protein assay and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE gel). Proteins on SDS-PAGE were stained with Coomassie blue. Bovine serum albumin (BSA) protein was used as a standard protein. Amount of protein contamination in purified polysaccharides extracts were determined and compared with by Bio Rad Quantity One 1-D Analysis software.

3.2 The nucleic acid contamination

The nucleic acid contamination in polysaccharides extracts were measured with UV–Vis spectrophotometer at 260 nm. Amount of nucleic acids were determined and compared with reference absorption (absorption of 1 OD was equivalent to approximately 50µg/ml).

Results and discussion

1. Polysaccharides extraction and purification

By using the protocol stated above, the appearance of purified polysaccharides from *T. fuciformis* and *A. auricula* were white, sponge-like powders with around 2.0% and 0.84 % yields respectively.

2. Determination of the content of polysaccharides by Phenol-Sulfuric acid method

There is no one direct measurement of polysaccharides since there are mixed complex and combination of variety of monosaccharides. Phenol sulfuric acid method is a colorimetric method widely used to determine the total concentration of carbohydrates. Monosaccharides, oligosaccharides and polysaccharides rearrange themselves to furfural derivatives by the action of sulfuric acid at elevated temperature and furfural derivatives then react with phenol to give colored compounds. The absorbance of the characteristic color was measured at 490 nm for hexose monosaccharide and 480 nm for pentose monosaccharide and uronic acid [17].

The UV-Vis spectrograms showed the reactant reagents (5% phenol and conc. sulfuric acid) absorbed wavelength at 296 nm (Figure 4). Hexoses (mannose and glucose) absorbed wavelength at 490 nm (Figure 5 and 6) and xylose absorbed wavelength at 480 nm (Figure 7). The results confirmed the Phenol-Sulfuric acid method to determine the amount of hexoses at 490 nm and pentoses at 480 nm. In Figure 8 and 9, 100µg/ml purified polysaccharides extracts from *A. auricula* and *T. fuciformis* absorbed maximum visible light wavelength at 487.5 nm rather than 480 or 490 (representing pentose and hexoses), indicating the mixture between hexoses and pentoses in the extracts with more hexoses than pentoses because the shift of maximal absorption wavelength towards 490 nm.

The results showed, the amount of polysaccharides purified from *T. fuciformis* (Figure 9) was 53.37% expressed as hexoses by using constructed standard curve of mannose (Figure 1, 5), and 37.85% expressed as pentoses by using constructed standard curve of xylose (Figure 3, 7). So, the estimated polysaccharides content in purified extracts of *T. fuciformis* was 91.22%. From previous study, the chemical structure of the polysaccharides of *T. fuciformis* consists of a linear backbone of (1→3) α-D-mannan and the major ratio of mannose, fucose, xylose and glucuronic acid is 9:1:4:3 [3, 4]. In *T. fuciformis,* the major hexose sugar content is mannose and the major pentose sugar is xylose, so the amount of hexose and pentose sugars should be expressed as mannose and pentose, respectively. The amount of polysaccharides purified extracts of *A. auricula* (Figure 8) was 46.95% expressed as hexoses by using constructed standard curve of glucose (Figure 2, 6) and 32.48% expressed as pentoses by using constructed standard curves of xylose (Figure 3, 7). So, the total polysaccharides content in purified extracts of *A. auricula* was estimated at 79.43%. In previous report, the major monosaccharide units in *A. auricula* polysaccharides was glucose (72%), mannose (8%), xylose (10%) and fucose (10%) [9], so the amount of hexose and pentose sugars should be expressed as glucose and pentose, respectively.

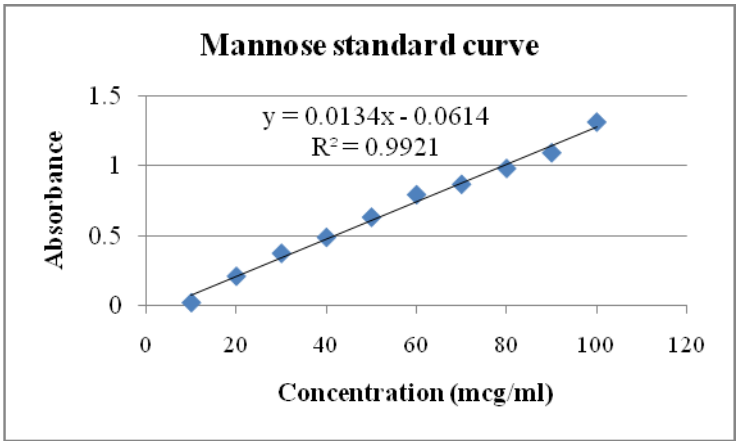


Figure 1 Standard curve of mannose (ranging from 10 to 100µg/ml concentrations of mannose sugar)

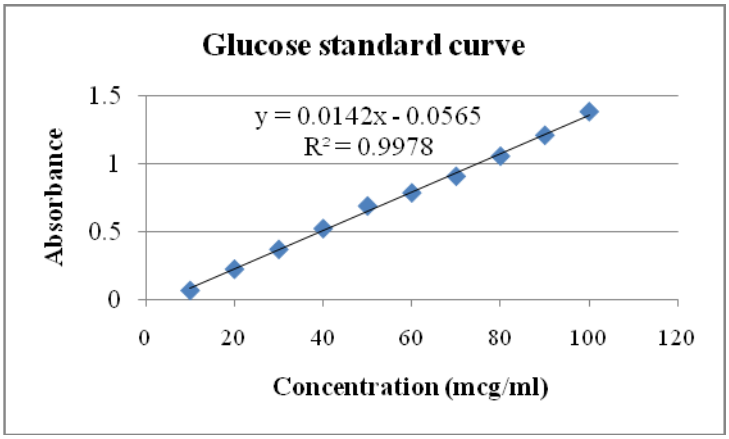


Figure 2 Standard curve of glucose (ranging from 10 and 100µg/ml concentrations of glucose sugar)

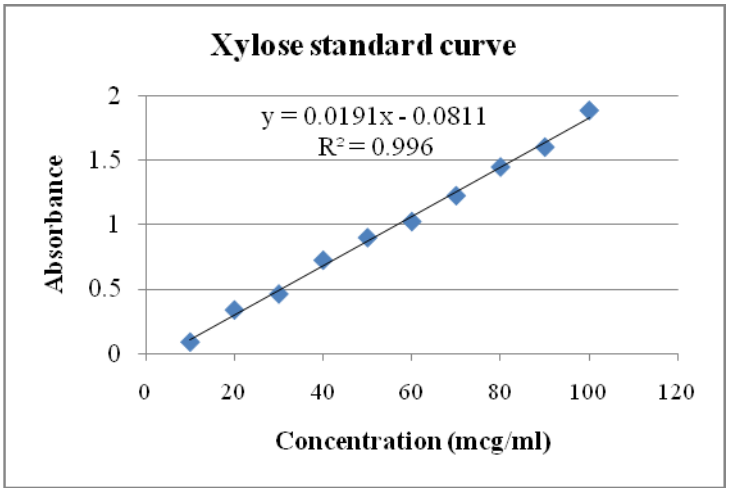
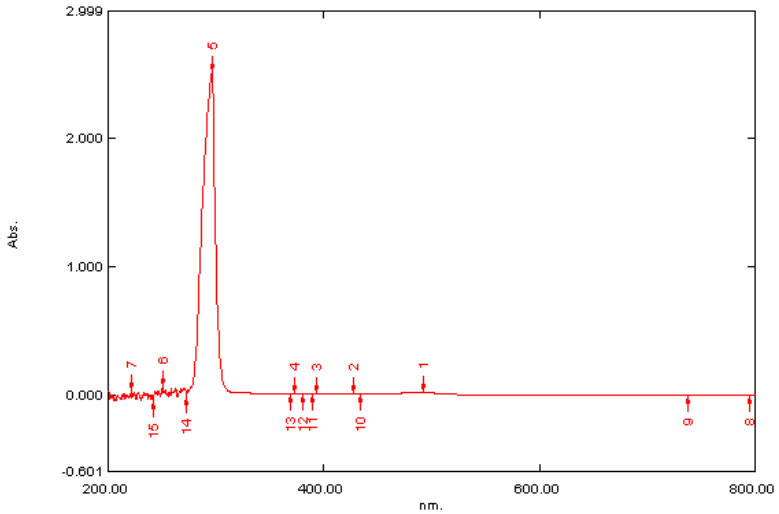
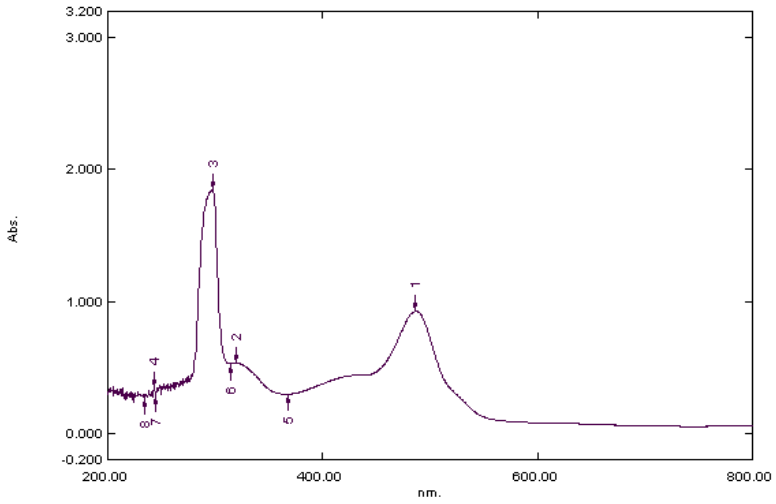


Figure 3 Standard curve of xylose (ranging from 10 and 100µg/ml concentrations of xylose sugar)



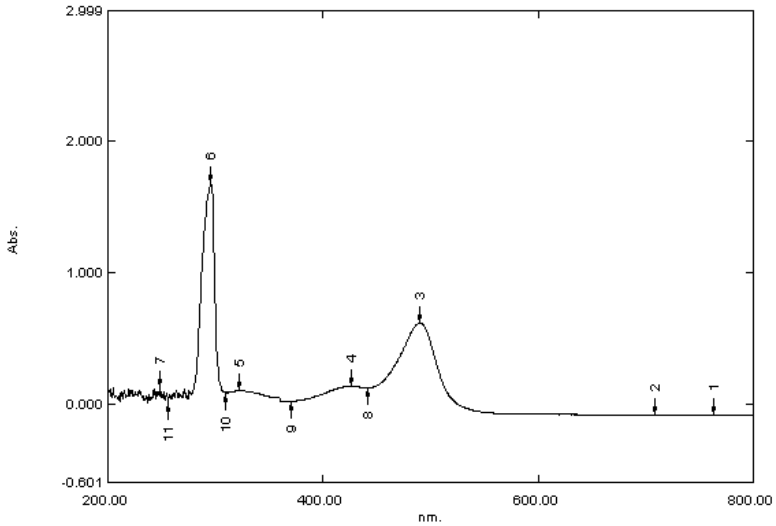
No.	Wavelength (nm)	Absorbance
5	296	2.514
6	250.5	0.058

Figure 4 The absorption pattern of the reagent in phenol-sulfuric acid method (5% phenol and conc. sulfuric acid) when plan scan at 200 to 800 nm by using a UV–Vis spectrophotometer



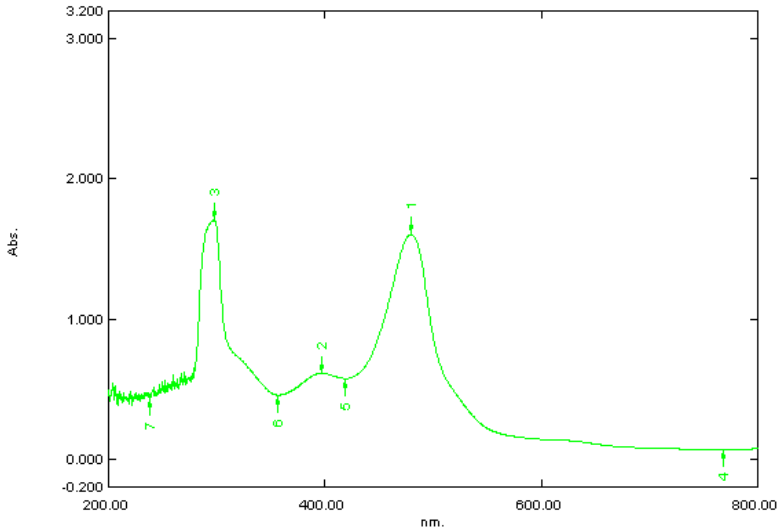
No.	Wavelength (nm)	Absorbance
1	490	0.925
2	320	0.531
3	296	1.847
5	368	0.296

Figure 5 The absorption pattern of 100µg/ml mannose was reacted with phenol-sulfuric acid method when plan scan at 200 to 800 nm by using a UV–Vis spectrophotometer



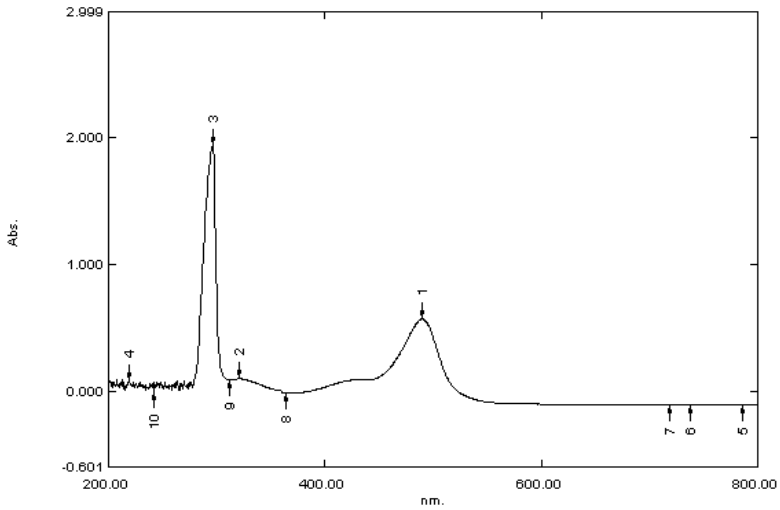
No.	Wavelength (nm)	Absorbance
3	490	0.617
4	426	0.132
5	320	0.101
6	296	1.679

Figure 6 The absorption pattern of 100µg/ml glucose was reacted with phenol-sulfuric acid method when plan scan at 200 to 800 nm by using a UV–Vis spectrophotometer



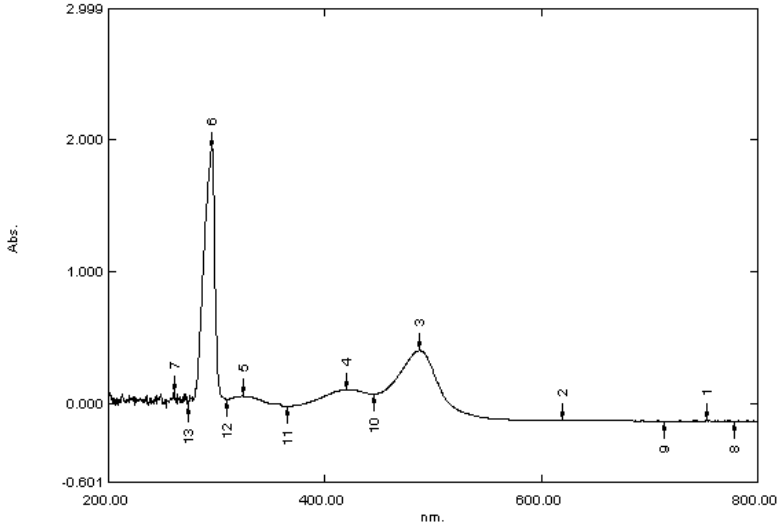
No.	Wavelength (nm)	Absorbance
1	480.0	1.604
2	397.00	0.611
3	296.00	1.706
6	356.00	0.456

Figure 7 The absorption pattern of 100µg/ml xylose was reacted with phenol-sulfuric acid method when plan scan at 200 to 800 nm by using a UV–Vis spectrophotometer



No.	Wavelength (nm)	Absorbance
1	487.5	0.568
2	320	0.097
3	296	1.941

Figure 8. The absorption pattern of 100µg/ml purified polysaccharides extract from *A. auricula* was reacted with phenol-sulfuric acid method when plan scan at 200 to 800 nm by using a UV–Vis spectrophotometer



No.	Wavelength (nm)	Absorbance
3	487.5	0.400
4	420.5	0.105
5	320	0.054
6	296	1.932

Figure 9 The absorption pattern of 100µg/ml purified polysaccharides extract from *T. fuciformis* was reacted with phenol-sulfuric acid method when plan scan at 200 to 800 nm by using a UV–Vis spectrophotometer

3. Determination of the proteins and nucleic acids contamination in the purified polysaccharides extracts

As showed in Table 1, the protein contamination in both purified polysaccharides extracts was less than 0.25% by SDS-PAGE protein gel analysis. Notably, using BCA protein analysis method results in a higher amount of protein content (3.02 and 1.98% in purified polysaccharides extracts from *T. fuciformis* and *A. auricula*, respectively). The reducing ability of some polysaccharides in the purified extracts may cause this interference. The nucleic acid contamination was 3.02% in purified *T. fuciformis* extracts of and 0.79% in *A. auricula*.

Table 1 Characteristics of the purified polysaccharides extracts from *T. fuciformis* and *A. auricula*

Characteristics	*T. fuciformis*	*A. auricula*
Appearance	White sponge	White sponge
%yield (w/w)	2.0%	0.84%
Proteins contamination - BCA protein assay - SDS-PAGE protein gel	 3.02% < 0.25%	 1.98% < 0.25%
Nucleic acids contamination	3.02%	0.79%
Polysaccharides determination	91.22%	79.43%

Conclusion

The method for polysaccharides purification from *T. fuciformis* and *A. auricula* was developed successfully. The purified polysaccharide extracts from *T. fuciformis* and *A. auricula* were approximately achieved at the yields of 2.0% and 0.84% w/w of raw dried mushroom, and the purity of polysaccharides at 91.22% and 79.43%, respectively, with small amount of nucleic acids and proteins contaminations. Notably, the difference in terms of amounts, types, and branch chains and backbone of polysaccharides in both purified fungal extracts may contribute to their different variety and intensity of biological activities reported in previous studies. Therefore, only well purified and characterized polysaccharides products should be applied in further investigations and clinical uses to assure the right conclusions.

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