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Bioactive Analysis of *Auricularia Delicata*: Extraction, Purification, and Characterization of Polysaccharides from *Auricularia Delicata*-Morocco Forest

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Abstract

Auricularia mushrooms are bioactive compounds which are mostly polysaccharides. Many studies have shown the use of *Auricularia* from wild mushroom for medicinal purposes such as antioxidant, antidiabetic, antitumor, hyperlipidemic, immunomodulatory, hepatoprotective and anticoagulant effect. Despite the fact that some studies showed pharmacological functions of polysaccharides extracted from *Auricularia Delicata,* the efficiency and the chemical process of extraction processes of extraction of polysaccharides from *Auricularia* were not clear. Therefore, this study proposes a method to purify and characterize the polysaccharide extracted from *Auricularia* through a method in Yoon *et al.* (2003). Polysaccharides characterization was done by colorimetric (Phenol-Sulfuric acid method). UV–Vis spectrophotometer was used to take colorimetric analysis of all solutions. 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. The yield of purified polysaccharides from *Auricularia* was 0.88% with 80.4% purity expressed as glucose/xylose. We successfully obtained the purified polysaccharides from *Auricularia* with high purity.

Keywords

Auricularia, characterization, mushroom, polysaccharides, purification

1. Introduction

Auricularia delicata is being used today across the globe as food and serves myriad functions in the human body. As such, it is a class of fungi that is edible and plays a key role in the human diet[1]. It's a black-brown mushroom delicious and has a high content of proteins, carbohydrates, and minerals. Examples of *A. delicata* includes Wood ear, jelly ear, and Jews ear fungus. Additionally, *A. delicata* polysaccharide composition is made of glucose, xylose, fructose, xylose, and mannose. In recent research, it has been found that mushrooms help in the growth and development of human body especially when integrated in the diet [2]. *Auricularia delicata* is a basidiocarp fungus that is found in most parts of the world especially the tropics. During its fruiting period, *A. delicata* grows in clusters especially in moist seasons of the year. The mode of dispersal of *A. delicata* is through allantoid spores [3]. Furthermore, it's an edible fungi that most communities or cultures use across the world for therapeutic purposes. There are a number of studies

that have been done on *Auricularia delicata* based on their proximate nutritional composition. The fruiting bodies of *A. delicata* contained 93.2 moisture content, 7.30 crude protein, 1.40 crude fat, 6.90 crude fiber and 4.60 total mineral ash [4]. Its high protein value has triggered researchers to study other nutritional parameters which include protein, carbohydrates, and macronutrient content.

Carbohydrates have been used over time as a key source of energy in our diets. Fats and proteins are other biological sources of energy. Despite the fact that fats and proteins can be used as a source of energy, their energy output is relatively low compared to carbohydrates. In this regard, Carbohydrates, proteins, and fats supply 90% of the dry weight of the diet and 100% of its energy [4]. The energy in calories that is provided by the three components in 1 gram of food include, 4 calories for carbohydrate, 4 calories for protein, and 9 calories for fat. However, carbohydrates are the quickest to supply energy that fats [5].

Carbohydrates can be classified into either simple or complex depending on the size of the molecules. Simple carbohydrates are small molecules that can be easily broken down and absorbed by the body. They act as the quickest sources of energy in a human body and include sugars such as glucose and sucrose [6]. As a result, they quickly elevate the level of blood glucose. On the other hand, complex carbohydrates are composed of long strings of monosaccharide units. In regard to this, complex carbohydrates being larger molecules are first broken down into simple carbohydrates before they can be absorbed in the human body [7]. Therefore, carbohydrates tend to provide energy to the body more slowly than simple carbohydrates but still more quickly than protein or fat. Because they are digested more slowly than simple carbohydrates, they are less likely to be converted to fat. Furthermore, they increase blood sugar levels more slowly [8]. Complex carbohydrates include starch and fibers, which occur in wheat products, grains, beans, and root vegetables such as potatoes and sweet potatoes.

The body needs proteins to maintain and replace tissues, to function well, and grow. Protein is not usually used for energy. However, if the body is not getting enough calories from other nutrients or from the fat stored in the body, protein is used for energy [9]. If more protein is consumed than is needed, the body breaks the protein down and stores its components as fat. Adults need to eat about 60 grams of protein per day that is; 0.8 grams per kilogram of weight or 10 to 15% of total calories. Fats on the other hand are the slowest source of energy but the most energy-efficient form of food. Each gram of fat supplies the body with about 9 calories, more than twice that supplied by proteins or carbohydrates [10]. Because fats are such an efficient form of energy, the body stores any excess energy as fat. The body deposits excess fat in the abdomen (omental fat) and under the skin (subcutaneous fat) to use when it needs more energy. The body may also deposit excess fat in blood vessels and within organs, where it can block blood flow and damage organs, often causing serious disorders [11].

Due to an influx in the number of species, including humans that need carbohydrates for energy, it is evident that there is an exhausting if not a competition for the available sources of carbohydrates with other species. Several researches over the past decades have been carried out on the extraction and characterization of polysaccharides and significance of carbohydrates from natural sources such as *A. delicata.* Most reports from literatures have shown how most people are affected by lack of vital dietary components that can directly be derived from growing fungi (mushroom) [12]. There are a number diseases that emanate from improper functioning of the major organs in our bodies. As a result, most people have been found to suffer a number multifunction of hormonal, nervous, and immune systems. Also, the regulatory systems that are responsible for averting several biological and environmental stresses have posed a challenge to people and this required expensive treatment plan. *A. delicata* is an important resources that serves as both food and medicinal (therapeutic) function to the residents of Morocco and most people across the globe. A need for more natural, readily available, and a cheaper source of carbohydrate is therefore needed.

Mushroom as food

Auricularia delicata (mushroom) have been used as food in most parts of the world for a long period of time by different groups of people globally. It's considered as a black-brown mushroom which is mostly edible and has a high content of proteins, carbohydrates, and minerals. In this regard, the main monosaccharides composition of *A. auricula* polysaccharides is mannose (8%), glucose (72%), fucose, and xylose (10%) (10%) [13]. Furthermore, (1→3) β-D-glucans is the polysaccharide backbone chain that is contained in it [14]. In most of the fungi especially mushroom, the building blocks in the

polysaccharide chain (complex) consist of simple units called monosaccharides.

Auricularia delicata, synthesize vitamins B and D with varying amounts of trace minerals that aid metabolic activities in the human body [15]. Major mineral constituents in mushrooms are K, P, Na, Ca, Mg and the minor elements include Mo, Zn, Cu, Fe, Cd. On the contrary, mushrooms also have the ability to accumulate heavy metals such as Pb, Cd, Ni, Cr, Cu, and Hg [16]. The wild mushrooms are considered to have high mineral content than cultivated ones. As such, the macronutrient constitution of *Auricularia delicata* especially iron helps in body in the production of blood cells through making of proteins [17]. The vital proteins include myoglobin and hemoglobin that are key in the transportation of oxygen throughout the body cells [18].

Mushroom as medicine

The use of *Auricularia delicata*, therapeutic foods that plays a key role for human health. The medicinal function of mushrooms is attributed to their chemical composition and the dietary fiber especially the beta glucans and chitin [19]. In recent studies, polysaccharides from *A. delicata* has found to have potential biological activities such as hypoglycemic activity, antioxidant activity, anti-tumor property, hypolipidemic activity, anticoagulant activity, anti-inflammatory, and cardio-protective effect [20]. The diseases that are found to be prevented by consumption of mushroom include hypercholesterolemia, hypertension, atherosclerosis and cancer which emanate from the chemical make-up. The medicinal properties such potential to reduce elevated sugar levels and possession of antiviral, antitumor, immunomodulating, and antithrombotic properties [21].

Based on the research on mycology, a number of diseases have been researched and mushrooms are play a grave role in chronic catarrh diseases of the hinges and breasts, reduce the cholesterol level of blood, remedy for night sweating, improves circulation, in tuberculosis, gout, rheumatism, jaundice, intestinal worms, dropsy, and have anti-tumor, anti-viral and anti-cancer agents [22].

Also, the free radical busting property that aid in Vitamin B-2 supports the immune system and enhances the body's ability to tolerate stress. This riboflavin helps the body to convert carbohydrates to glucose that fuels the body keeping the skin, hair, and eyes functioning and consequently healthy. This is an anti-oxidant function of B-2 that eliminates the build-up of radicals which may lead to premature aging and high chances of heart attack or cancer in a human body[23].

2. Methods

2.1. Sample Collection

Samples of *Auricularia delicata* were randomly collected by hand picking and storing them in sterile paper bags from the Morocco forest in Morocco. They were later transported to Mohammed V Rabat University – Chemistry Department research laboratory for further analysis.

2.2. Sample Preparation.

The samples were then crushed and dried under shade for about 2 weeks. After this the sample was ready for the extraction of polysaccharides.

2.3. Polysaccharides extraction and purification

The polysaccharides extracts of *A. delicata* were isolated by method suggested by Yoon et al. (2003). This was done until purified polysaccharides obtained. The fruit bodies of *A. delicata* were dried at 68ºC and ground using mortar. The dried powdered particles were suspended and refluxed in methanol. The suspensions were filtered to remove the methanol-soluble materials such as phenolic compounds, lipids and colored materials. The filtrates were collected, suspended and refluxed in deionized water. Clear supernatants were collected after low speed centrifugation. Afterwards, the protein contaminations were removed by Sevag method [18]. The polysaccharides in the concentrated supernatants were precipitated with absolute ethanol and further re-suspended in deionized water. The purified solutions were finally lyophilized.

2.4 Polysaccharides characterization by colorimetric (Phenol-Sulfuric acid method)

2.4.1 Reagents

The reagents used included; 5% Phenol, 96% reagent grade Sulphuric acid, Standard glucose, Glucose Stock solution (prepared by dissolving 100mg of standard glucose in 100mL of distilled water), Glucose working standard (prepared by diluting 10mL of the glucose stock solution to 100mL with distilled water) and 5mL of 2.5 N HCL.

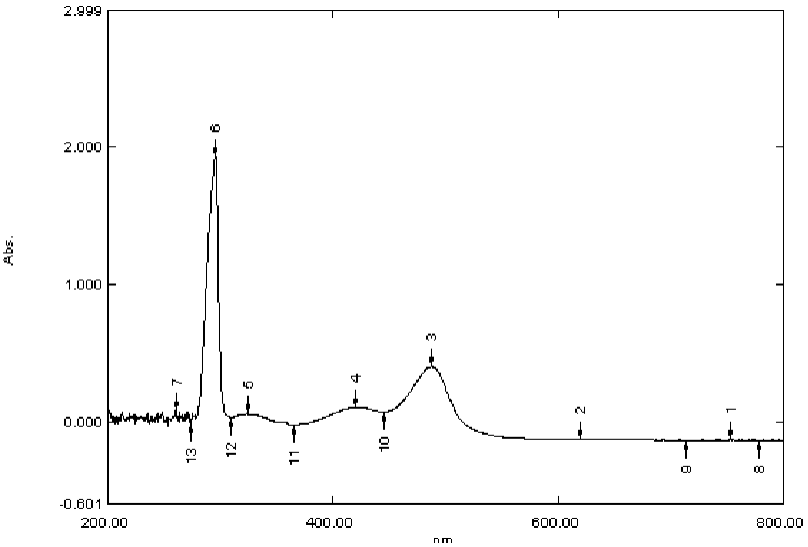
2.4.2 Experimental procedure and total polysaccharide content determination

Based on this method, the content of polysaccharides in the extracts was determined by

Phenol-Sulfuric acid method. 2ml of standard grade sugar solutions with a range of 10-100 µg/ml concentrations of sugar and 2 ml of purified polysaccharides from *A. delicata* at 100 µg/ml concentrations were pipetted into a test tube, and 1 ml of 5% phenol solutions were added. This was followed by rapid addition of 5 ml of concentrated sulfuric acids. The tubes were shaken and placed in water bath at 30º C before readings procedure was taken. UV–Vis spectrophotometer was used to take colorimetric analysis of all solutions. 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.

3. Results and Discussion

The results based on the purification and characterization of *A. delicata* was given in terms of the total carbohydrate content and absorbance based on the standard solutions prepared. The absorbance for the characteristic color was measured at 490nm (for hexose) and 480nm (uric acid and pentose monosaccharides). The UV-Vis spectrograms showed that the reactant reagents absorbed wavelength at 298nm, 480nm for xylose and 490nm for hexoses (manse and glucose). According to the results, the of polysaccharides purified extracts of *A. auricula* was 45.85% expressed as hexoses by using constructed standard curve of glucose and 30.56% expressed as pentoses by using constructed standard curves of xylose. So, the total polysaccharides content in purified extracts of *A. auricula* was estimated at 80.40%. In previous report, the major monosaccharide units in *A. auricula* polysaccharides was glucose (74%), mannose (6%), xylose (10%) and fucose (10%) , so the amount of hexose and pentose sugars should be expressed as glucose and pentose, respectively.



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

Figure 1: The absorption pattern of 100µg/ml purified polysaccharides extract from *A. auricula* at 200 to 800 nm by using a UV–Vis spectrophotometer


Figure 2: Diagrams of wild mushrooms

4. Conclusion

The method for polysaccharides purification from *A. auricula* was developed successfully. The purified polysaccharide extracts from A. auricula were approximately achieved at the yields of 0.88% w/w of raw dried mushroom, and the purity of polysaccharides at 80.40%, respectively, with small amount of nucleic acids and proteins contaminations. Notably, the difference in sterms 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 conclusion.

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Competing interests

The authors declare they have no competing interests.

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