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Fungal Polysaccharide (Glucan) Contents of Three Edible Mushrooms as Potential Support Bioactive Compound Relevant to Human Health

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ABSTRACT

Fungi polysaccharides, also known as glucan is a naturally-occurring substance found in man's diet involving mushrooms. Mushrooms are a great source of various biologically active compounds implicated as anti-oxidants, immune-regulation, dietary fibers, glucans, vitamins etc and the biological active nature of glucan is a multi-vector based on many factors evidenced in human health quality. This study was intended to biochemically determine three edible mushrooms (*Pleurotus tuber regium*, *Pleurotus ostreatus* and *Auricularia auricular*) for their polysaccharide (glucan) contents. The chemical analysis for the glucan adopted the method of the Association of Official Analytical Chemists (AOAC) while the quantification of glucan was carried out using High Power Liquid Chromatography (HPLC). Results obtained indicated that all the samples contained appreciable quantity of glucan. It also showed that higher values of glucan were concentrated more in *A. auricular* while the quantity of glucan in *Pleurotus tuber regium* and *Pleurotus ostreatus* samples were apparently on the edge. Altogether, however, the implication is that these three edible mushrooms are promising candidates for potential support bioactive compound relevant to promoting human health.

Keywords: Mushrooms, polyaccharide (glucan), biological active compound, anti-oxidant, immuno-regulation, human health.

Introduction

Recently, it has been shown that naturally occurring substances are contained in human daily diets and they are important in preventing many chronic diseases. One of these important substances is polysaccharide; often refered to as glucan. It is naturally occurring in plant cells, such as oats, barley, wheat, yeast and mushrooms.

Mushrooms have gained great values which are implicated as immune-modulatory, medicinal and pharmaceutical reinforcements, over the years. Presently, mushroom derived-polysaccharides have been reported over those values (Kothari *et al.*, 2018; Jiang *et al.*, 2020; Charkraborthy *et al.*, 2021). Mushrooms contain many bioactive compounds but, of all, polysaccharides are the most potent, widely distributed and most studied. These polysaccharides are responsible for the bioactivities of most mushrooms.

Polysaccharides are the major classes of biomolecules and are considered as one of the most important bioactive components in fungi. As a basic structure, fungal polysaccharides are also known as glucan which are polymers consisting of glucose monomers (Young *et al.*, 2001; Ahmad *et al.*, 2012a and b).

Mushroom polysaccharides are structurally diverse group of a macromolecule with a common β-linked glucose backbone (Nakashima *et al.*, 2018). Glucans are the very common polysaccharides present in the fungal cell wall, in the form of cellulose and it is the potent antitumor polysaccharide (Pandy *et al.*, 2018; Choutanya *et al.*, 2019). Among several polysaccharides, beta-glucans are the most studied bioactive compound.

Beta glucans are polysaccharides derived from D-glucose and are linked by glycosidic bonds, bearing different molecular weight, density and 3-dimensional structure (Frioui *et al.*, 2018).

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Glucan is a dietary fiber which functions are attributed to a number of beneficial health properties, such as treatment and prevention of certain digestive diseases and supporting the immune system (Ciecierska *et al.*, 2019).

The biological activities of glucans is reported as a multi-vector that depends on many factors; primarily on the type and configuration of bonds between constituent sugar residues, degree of branching of the side chains of biopolymers; moleculare weight of the polysaccharides and the solubility in water (Thornton *et al.,* 1996).

A mushroom glucan is a carbohydrate polymer derived from the cell wall of mushrooms and its beta-glucan is known as Biological Response Modifier (BRM), which refers to the ability to up-regulate and down-regulate the response of biological systems (Brown and Gordon, 2003; Nakamichi *et al.,* 2016). Mushroom glucans such as schyzophyllan, Ganoderan, lentinan and pleuran are the components of mushrooms and other fungal cell wall. They consist of glucopyranose molecules linked through β(1 – 3), β(1 – 4), β(1 – 6) linkages (Lindequist *et al.,* 2005; Chan *et al.,* 2009; Novak and Vetvicka, 2009). Beta-glucans are also present in many other mushrooms, such as *Auriculana auricular*, *Calocybe indica* as (Calocyban), *Pleurotus abalones* etc (Lindequist *et al.,* 2005; Villares *et al.,* 2012; Zhu *et al.,* 2015). It is also reported that bracket fungi, such as *Trametes versicolor*, and *Boletus edulis* contain more than 50% beta glucans (Sari *et al.,* 2017). Ozcan and Ertam (2015) reported that in most wild mushrooms, the beta-glucan content is higher in the stipe than in the Pileus (cap).

Mushroom polysaccharides with beta linkage have been demonstrated as a boost in the human immune system and the modulation of the immunological response under certain conditions; thus they are often termed as Biological Response Modifier (BRM). Based on this, activation of the human immune system, the polysaccharides show significant, anti-tumour, anti-viral and anti-microbial activities, besides other effects (Villares *et al.,* 2012).

A number of studies have been carried out on beta glucans with health enhancing effects in various important ways such as anti-tumor and immune-modulation (Borchers *et al.,* 2004; Moradali *et al.,* 2007), as Cardiovascular (Wasser and Weis, 1999; Manzi and Pizzoferrato, 2000); liver protection and anti-inflammation (Lindequist *et al.,* 2005); anti-oxidant etc (Deng *et al.,* 2012). Basically, their health promoting ability is influenced by the molecular mass modification of the polysaccharides (Ren *et al.,* 2012). In terms of biological activity, beta – 1, 3 – D – glucans and beta 1, 6 – D – glucans contained in oyster, shitake, split gell and basidiomycetes are considered to be the most effective. However, sufficient work has not been done on the polysaccharides of *Pleurotus tuber-regium*, *Pleurotus ostreatus* and *Auricularia auricular.*

Based on the fore-going therefore; the present study was intended to determine the fungal polysaccharides or glucan contents inherent in *Pleurotus tuber-regium*, *Pleurotus ostreatus* and *Auricularia auricular* which edible mushrooms, viewing the polysaccharide (glucan) as potential support bioactive compound relevant to healthy human life.

Materials and Methods

Stud Area

This study was carried out in the screen house of the Department of Plant Science and Biotechnology, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt and the Biology Laboratory of Ignatius Ajuru University of Education, Rumuolumeni. Both Nkpolu-Oroworukwo and Rumuolumeni are situated in Rivers State which lies in the coordinates of 4^0 20^1 5^0 50^1 N (Lat) and 6^0 20^1 and 7^0 35^1 E (Long.); bounded on the South by the Atlantic ocean, to the North by Imo and Abia States, to the East by Akwa-Ibom and to the West by Bayelsa State.

Source of Mushroom Samples and Preparation for Analysis

Auricularia auricular was obtained from the wild from Omuanwa in Ikwerre Local Government Area of Rivers State in the Niger Delta within the Rain Forest belt. *Pleurotus tuber-regium* was raised in the screen house of Plant Science and Biotechnology and *Pleurotus ostreatus* was procured from Dilomat farm and services, Rivers State University, Port Harcourt. The mushroom samples were air-dried at room temperature and ground to powder form for analysis.

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Determination of Glucan (Polysaccharide)

The chemical analysis for glucan was carried out according to the methods adopted by AOAC (2006). Glucose was measured using the method developed by Nelson (1944) and Somogyi (1945) in which the total sugar was calculated by autrona method as shown below.

$$\text{Glucan} = \frac{\text{Amount of glucose in sample}}{\text{Amount of the powdered sample}}$$

Analysis by Quantification of Glucan Using High Performance Liquid Chromatogrophy (HPLC)

For chromatographic analysis, the samples were obtained as described previously. The samples were transferred from storage at 20ºC to 10ºC and were kept at 10ºC for 12 hours and then were left at room temperature for approximately 4 hours before being analyzed. After temperature stabilization, 25ml of each of the sample was diluted by a factor of 20 with ultra pure water which had been filtered through an ultra filter durapore membrane with a 0.2µm pore size (millipore). A 20µl volume of the diluted sample was injected into the chromatograph for analysis. Values were determined using the AOAC (1992) techniques as modified by Schwan *et al.* (2001) and Shimadu (1998). A high performance liquid chromatography (HPLC), model LC – 10Ai (Shimadzu corp, Japan) was used. This was equipped with refraction index detectors (Model R.D – 10A). For measurement of glucose, a cationic exchange column was used (Shim-pack SCR-10IH) (7.9mmin diameter x 30cm in length).

For carbohydrate measurement, the column was operated at room temperature with a mobile phase of ultrapure water adjusted to pH 2.1 and a flux of 0.6ml min-1.

Quantification was done by comparison with a glucose standard curve which was made using certified standards (Supelco-sigma Aldrich, St Louis, MO, USA). The results read on the HPLC were analyzed using the following equation 2: 0.2; where G is the glucose concentration in mg/ml, determined using a standard curve; factor 0.9: factor that accounts for the proportion of glucose coming from glucan; Factor 250:e conversion factor to convert from µg ml-1 to gkg-1, once the extraction volume is 250ml.

Results

The results of this study are presented in Tables 1 and 2. The results revealed that fungal polysaccharide (glucans) are present in the studied mushroom samples; *Pleurotus tuber-regium, Auricularia auricular* and *P. ostreatus.* The results presented in Table 1 indicated that the glucans are high in *P. ostreatus* and the concentration was maintained in the first and second replications but dropped slightly in the 3rd replication. In the same manner, *Pleurotus tuber-regium* recorded high values of the gucans in all the replications such that the 3rd replication was slightly higher than 1 and 2. The results in Table 2 also indicated that all the three edible mushrooms tested are well loaded with the fungal polysaccharide in the form of glucan for bioactive support of human health.

Table 1: Total Glucan in 3 Edible Mushrooms in (g/100kg)

Replication	Mushroom Species		
	Pleurotus tuber-regium	***Auricularia auricular***	***Pleurotus ostreatus***
A	13.66	17.38	23.28
B	12.60	13.33	23.16
C	13.78	16.79	22.99

The results presented in Table 2 showed the mean average commulation of glucans in 3 replications of the biochemical screening of the test mushrooms. *P. ostreatus* recorded the highest content of glucans, followed by *A. auricular.* However, in the context of this study, the concentration of glucan in *P. tuber-regium* is also high. The results showed that all the samples tested for glucans are promising bioactive support to boost human health, based on the value contents of the glucans as obtained from this study.

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Table 2: Mean Value Contents of Glucan in 3 Edible Mushrooms Tested

	Mushroom Samples		
	Pleurotus tuber-regium	***Auricularia auricular***	***Pleurotus ostreatus***
Mean content value of glucan	13.68	17.24	23.14

Discussion

The results obtained from this study revealed that mushroom polysaccharides or glucans are present in the three mushroom samples studied (*Pleurotus tuber-regium, P. ostreatus* and *Auricularia auricular*); and that the concentrations of the glucans were of relatively high values in all the replications. However, the study report also showed more or higher quantity of glucan in *A. auricular* than other samples.

The quantity of glucans revealed from this study indicated that the glucans in the three edible mushrooms stand as important bioactive and nutraceutical support in sustaining human health and this finding is in line with the report of Manzi and Pizzoferrato (2000) who suggested that glucan have effects on lipid and glucose metabolism and by implication can manage diabetes. The study also showed that the bioactive and the nutraceutical components in the mushrooms can exhibit antioxidant properties by scavenging reactive oxygen species, thereby reducing the risk of diseases such as atheroslerosis, cardiovascular diseases, neurodegenerative diseases, diabetes and cancer of various forms. These are also in line with the suggestions of Hozova *et al.* (2004). According to them, the bioactive and nutraceutical potentials especially in the form of glucan, like other fiber supplements can boost fiber intake, improve blood sugar level, promote digestive health regularity and also support weight management.

The findings of this study rightly align with the report of Hozova *et al.* (2004) to indicate that if these mushrooms are consumed directly or as supplements, the glucans in them can enhance human heart – health in which the report of Ciecierska *et al.* (2019) and Bulam *et al.* (2018), suggested that the glucans in mushrooms play important role in the proper functioning of gastrointestinal tract and preventing inflammation as well as colon cancer.

Also in support of their report are Ahmad *et al.* (2012a and b) who suggested that edible mushrooms are important for their dietary value and biological active and health promoting compounds such as glucan. Additionally, they reported that mushroom glucans in edible mushrooms are not digested in the gastrointestinal track, hence they are considered as a potential source of prebiotics.

The importance and contents of glucans in edible mushrooms cannot be underestimated by reactions from which suggestions have long established the essence of consumptions of the mushrooms, considering their values as antioxidant, bioactive and nutraceutical; hence the reasons for their mode of actions are relayed by researchers. This is in line with the submission of Villares *et al.* (2012) who reported that mushroom polysaccharides (glucans) have been demonstrated as boost in human immune system and in the modulation of the immunological response in certain conditions and this is likely the reason why they are often termed as Biological Response Modifers (BRM). As a result of the activation of the mushroom user's immune system, the glucans show significant anti-tumour, anti-viral and antimicrobial potentials, besides their other effects.

The great impact of glucans on human immunity may be due to how they are attached in the immune system which agrees with the suggestions of Ciecierska *et al.* (2019) who reported that the immune-stimulatory activity of glucans occur due to their attachment to specific receptors present on the immune cell surface. As a result of that, consumption of the studied mushroom samples in whole or part as supplement will boost human health system.

The narrative of the importance of mushroom glucan to human health is unending hence their biological activities are encompassing. This finding agrees with the report of Thornton *et al.* (1996) who submitted and suggested that the biological activity of glucans especially in mushrooms is a multi-vector.

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Additionally, the report of Lindequist *et al*. (2005) is in line with this finding, hence they reported that glucan regulates the up and down of biological system to boost human health.

In conclusion, this study revealed varied concentrations of the content of glucans in *Pleurotus tuber-regium, P. ostreatus* and *Auricularia auricular*; an indication that the mushrooms contain the potential bioactive compound to support human health hence it has been shown to have the potential nutraceutical properties that can be explored in the food and pharmaceutical industries, and might present different functional properties upon modification through suitable means and continuity of detailed clinical studies for the convenience of consumers.

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