

ANTIOXIDANT AND GLUCOSIDASE INHIBITION ACTIVITIES OF THE MUSHROOM: “*PHAEOGYROPORUS PORTENTOSUS*”

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Abstract

The methanol, ethanol and water extracts of the mushroom, *Phaeoglyphus portentosus*, were evaluated for antioxidant activity using (2,2-diphenyl-1-picrylhydrazyl) DPPH, for free radical scavenging activity (RSA), total phenolic content (TPC) and ferric reducing antioxidant power (FRAP). The methanol extract only of *P. portentosus* was also investigated for its ability to inhibit some commercially available glucosidase enzymes like alpha-D-glucosidases, alpha-D-galactosidase, alpha-D-mannosidase and beta-D-glucosidase. Results revealed that of all extracts tested for antioxidant activity, methanol extract possessed the highest RSA with an IC_{50} of 0.62 ± 0.008 mg/mL and the highest concentration of TPC 2.73 mg GAE/g of extract. But water extract showed the highest FRAP value with IC_{50} of 755.87 ± 23.83 mg/mL. The methanol extract also showed the greatest inhibitory activity against alpha-D-glucosidase enzyme isolated from *Bacillus sterothermophilus* (84.5%) more than all the other glucosidase enzymes tested. The results showed that methanol extract of *P. portentosus* may act as a natural antioxidant agent and alpha-glucosidase inhibitor (AGI).

Keywords: *Phaeoglyphus portentosus*, alpha-glucosidase inhibitor, DPPH, FRAP, TPC

Introduction

Medicinal mushrooms are fleshy spore bearing fruiting bodies of fungi which produce significant metabolites for health benefits. Mushrooms on earth have been estimated to 140,000 yet only 14,000 (i.e 10%) are known [1]. A large number of the species of mushrooms whose health promoting properties are unknown grow in Africa and probably in Nigeria.

Mushrooms are as old as mankind. In recent years, projects have been undertaken to discover more therapeutic potentials such as alpha-glucosidase inhibition, antioxidant and antiviral properties from natural sources, this is due to the highly abundant compounds and

promising biological activities exhibited by natural products extracted from mushrooms [2]. Recently researchers discovered the bioluminescent or glowing ghost mushroom, *Neonothopanus gardneri*, which reappeared in Brazil, after 170 years. Mushrooms are generally called “Olu” meaning King, something to be worshipped, in Yoruba language, Western part of Nigeria. They can be grouped as edible, medicinal and toxic. The most popular edible mushroom in Nigeria is the *Pleurotus tuber-regium* (Fr.) Singer (also called tiger milk or sclerotium-forming mushroom) locally known as “Ofuako” in Eastern Nigeria, meaning teeth cleaner. It is eaten as food and/or used as food supplement [3] in addition, its powder is

used as a very useful disintegrant in tablet formulation [4]. *Ganoderma lucidum*, called “*Tuwon biri*” in Northern Nigeria and well known in China as the “herb of longevity” is another popular mushroom, it is known to lower blood glucose level in diabetic patients through its insulin releasing activity [5].

Mushrooms as antidiabetic agents

Medicinal mushrooms such as *Ganoderma lucidum* contain micronutrients that contribute to antioxidant property as well as bioactive compounds which help in the treatment of chronic diseases like diabetes mellitus, especially in Nigeria. Hypoglycaemic effects (reduction of blood glucose level) in mushrooms like *Inonotus obliquus* have been reported [6].

Diabetes mellitus (Hyperglycaemia) is an abnormal rise in plasma glucose level. It is mainly due to a disorder in glucose metabolism over a long period of time. Diabetes mellitus (DM) is the most leading cause of mortality, after cancer in the world, especially in Africa. Following predictions, that over 18.2 million Africans including an estimated 4.8 million Nigerians, may become diabetic by 2030. World Health Organisation (WHO) called for prioritisation of actions to prevent people from becoming overweight and obese. One therapeutic approach to treat DM is to retard the absorption of glucose by inhibiting the alpha glucosidase enzymes responsible for digestion [6].

Alpha-Glucosidase Inhibitors (AGIs) help in the management of diabetes by competitively blocking the activity of the glucosidase enzyme, thus the digestion of carbohydrates by alpha glucosidases is reduced [7]. AGIs fall under the third category of oral hypoglycemic agents [8]. Many commercially available α -glucosidase inhibitors (acarbose and miglitol) have been used in the management of this disease but due to the cost and various side effects exhibited by these drugs, interest in natural alpha-glucosidase inhibitors in mushrooms has increased [9]. Prolonged hyperglycaemia has been found to lead to increased generation of reactive oxygen

species (ROS) and alteration of endogenous antioxidants [10]. Oxidative stress and free radicals have been implicated in type 2 diabetes [11]. Numerous mushrooms have shown free radical scavenging or antioxidant activity [12] implying that they can be used to combat type 2 diabetes. As a result, interest in natural antioxidants from mushrooms has greatly increased.

Phaeogyroporus portentosus is a rare, large mycorrhizal mushroom in the order boletales and division basidiomycetes, it belongs to the genus of mushroom producing fungi called *Boletus*, which contains species whose extracts are known to inhibit tumor proliferations. Traditionally known in yoruba language (Western Nigeria) as “*Olu ekiika*” meaning giant or amazing mushroom, Calabar language (Eastern Nigeria) “*Akamba udip*”. It was previously known by scientific names such as *Boletus marginatus*, *Phlebopus marginatus* in 1845, until scientific names such as *Phaeogyroporus portentosus*, *Boletus portentosus* were given. This group of fungi have also been reported as good sources of medicines by the Nigerian traditional doctors [13,14,15]. In Northern Thailand, it is reported as source of food after proper boiling and a source of antioxidants for the pharmaceutical and food industry [16].

This study therefore seeks to determine the antioxidant and alpha-glucosidase inhibition properties of the extracts of this mushroom, *P. portentosus* which has not yet been reported from Nigeria.

MATERIALS AND METHODS

Collection and Extraction

A large fruiting body of the mushroom, *Phaeogyroporus portentosus*, was hand picked from the University of Lagos premises, Akoka, Lagos State, Nigeria. It was authenticated, weighed, macerated using mortar and pestle, then homogenized with a blender, until an even fluffy mesh was obtained. The mesh (1.765kg) was extracted with methanol (MeOH), ethanol (EtOH) and water (H₂O) separately for 3 days. The

methanol and ethanol extracts were filtered and concentrated using a rotary evaporator (40°C) and dried in an oven at 40°C while the water extract was concentrated using a freeze dryer.

Antioxidant assay

Dpph free radical scavenging activity

The free radical scavenging activities (RSA) of the extracts were determined according to the method of Benzie and Strain described by Aquino *et. al.*, (2001) [17] with slight modifications using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. To a solution of 0.05 ml of each mushroom extract of various concentrations (0.4, 0.6, 0.8, 1.0 mg/ml). 1.95 ml of methanolic DPPH (0.1mM) was added and mixed thoroughly. It was then left to stand in the dark for 45 minutes, at room temperature, after which free radical-scavenging ability of the extracts determined by elimination of DPPH radicals was read at 520 nm using a UV-VIS spectrophotometer (Ultrospec 400, UV/vis Spec Pharmacia. Biotech.). Ascorbic acid was used as control, while the mixture of 1.95 ml (0.1mM) DPPH and 0.05 ml methanol served as blank. All tests were carried out in triplicates. The radical scavenging activity was calculated relative to the control using the formula:

$$\% \text{ Inhibition} = \frac{[\text{Absorbance of Control} - \text{Absorbance of sample}]}{\text{Absorbance of Control}} \times 100\%$$

Absorbance of Control

Extract concentration providing 50 % inhibition (IC_{50}) was calculated from the graph plotted i.e % Inhibition against extract concentration.

Determination of Ferric Reducing Antioxidant Power (FRAP)

The total antioxidant power of each mushroom extract was determined using Benzie and Strain method described by Ahmed *et. al.*, (2015) [18]. To each extract (1 mL), was added 3.0 mL of freshly prepared FRAP reagent containing 10 volumes of 300 mM acetate buffer, (pH 3.6) + one volume of 10 mM TPTZ (2, 4, 6-

tripiryridyl-s-triazine) in 40mM HCL and one volume of 20 mM $FeCl_3 \cdot 6H_2O$ in the ratio 10:1:1. The mixture was placed in a water bath for 5 minutes at 37°C. The absorbance of the reaction mixture was measured at 593nm spectrophotometrically. Ferrous sulphate heptahydrate, ($FeSO_4 \cdot 7H_2O$) was used as standard. A standard curve of six concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mM) was plotted and the absorbance values were measured as for sample solutions. IC_{50} of mean FRAP values were calculated. All tests were carried out in triplicates.

Determination of Total Phenolic Content (TPC)

The total phenolic content of all extracts was determined according to the Folin-Ciocalteu method described by Chew *et.al.*, (2009) with slight modifications[19]. To 200 μ L of each extract, 1.0 mL of folin-ciocalteu reagent and 0.8 mL Sodium bicarbonate, (Na_2CO_3 , 7.5%) was added. Incubation was done at 30°C for 1 hour. Absorbance was measured at 765 nm. TPC was measured using the following equation $C = cV/m$, where C is the total phenolic content measured in milligram gallic acid equivalent expressed as mg GAE/g of dry extract, c is the concentration of gallic acid obtained from the calibration curve mg/ml, V is the volume of extract in millilitres and m is the weight of extract in grammes. All tests were carried out in triplicates. Gallic acid was used as standard.

Alpha-Glucosidase Inhibition assay

The methanol extract of *P. portentosus* was screened against a range of commercially available glucosidase enzymes to investigate its potential activity as an enzyme inhibitor. Enzyme assays were carried out using a modified version of the method described in Griffiths 1998. The incubation mixture for assays using Para nitrophenyl alpha-D-glucopyranoside (PNP-glucopyranoside) substrates consisted of 10 μ L of enzyme solution, 10 μ L of 1 mg mL^{-1} test compound solution, and 50 μ L of 5 mM substrate solution. Reaction mixtures were incubated at 27 – 29 °C for 10 to 20 minutes, after

which the reactions were stopped by the addition of 70 μL of glycine solution. Enzyme activity was measured spectrophotometrically using synthetic (PNP-pyranoside conjugated) substrates, and compared against suitable controls. Absorbance was read at 405 nm. Assays were carried out in triplicate, and average absorbance values were calculated using values from the three replicates. Inhibition was calculated as the percentage difference between mean absorbance in the control and test reactions. Standards used were deoxynorjirimycin, (DNJ) for α glucosidases, castanospermine for β -glucosidase and swainsonine for mannosidases. Results were expressed as % inhibition at 1 mg mL⁻¹.

RESULTS

Antioxidant activity

The result for the antioxidant assay tested for the methanol, ethanol and water extracts of *P. portentosus* using DPPH is presented in Figure 1.

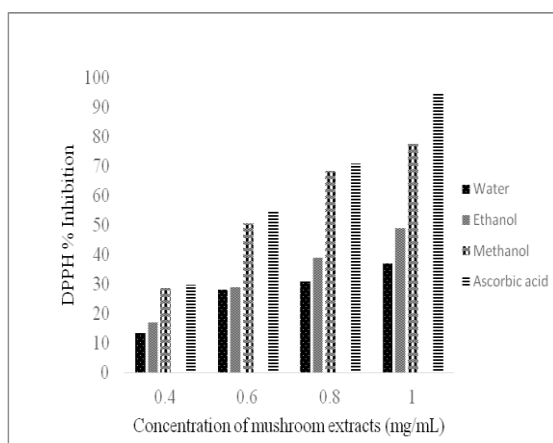


Figure 1: DPPH Inhibition activity comparison between three mushroom extracts (methanol, ethanol and water). Data shows the mean \pm SEM, n=3.

Total Phenolic Content

Phenolic compounds have been reported as major naturally occurring antioxidant components of mushrooms. They prove the importance of antioxidant behaviour. This folin-ciocalteu procedure has been used as a

measure of total phenolics in natural products for many years. This method gives a perspective about phenolic content in a sample. Table 1 shows that the methanol extract of *P. portentosus* possessed the highest phenolic content (2.73 in mg of GAE/ g of dry extract).

Ferric Reducing Antioxidant Power

The FRAP assay method is used to assess the presumable medicinal properties of plants [20]. It is based on the reduction of the ferric tripyridyltriazine (Fe (III) -TPTZ) complex to ferrous tripyridyltriazine (Fe (II) -TPTZ) at a low pH. Table 1 revealed that all of the extracts showed a considerable antioxidant effect from IC₅₀ of FRAP values of 755.87 \pm 23.83 (water extract) to 1246.77 \pm 24.94 (methanol extract) of *P. portentosus*.

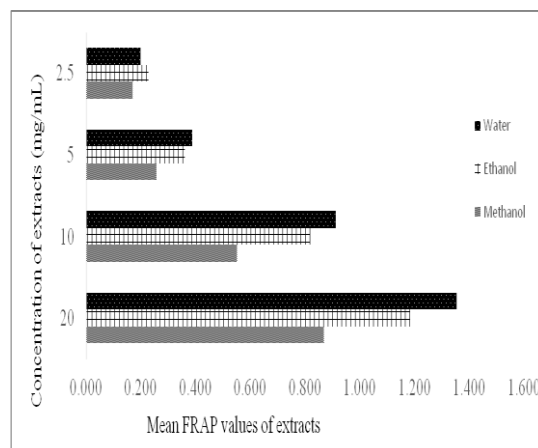


Figure 2: comparison of mean FRAP values between 3 extracts (methanol, ethanol and water) of mushrooms at different concentrations. Data shows the mean \pm SEM, n=3

Glucosidase Inhibition

The methanol extract of *P. portentosus* was run beside commercially available glucosidase enzymes. Figure 3 shows the methanol extract of *P. portentosus*, against α -D-glucosidase from (*Bacillus sterothermophilus*), N-acetyl β -D-galactosidase from (Jack bean), α -D-galactosidase from (green coffee beans), α -D-mannosidase from (Jack bean) and β -D-glucosidase (almond). Among all the enzymes tested for glucosidase inhibition, the methanol

extract of *P. portentosus* exhibited the highest inhibitory activity on α -D-glucosidase enzyme from *Bacillus sterothermophilus* (84.5%), as reported in Table 2.

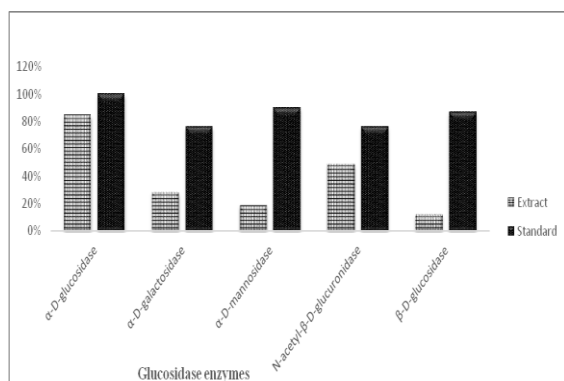


Figure 3: Glucosidase Inhibitory activity on methanol extract of *P. portentosus*

DISCUSSION

The DPPH assay was used to test for the free radical scavenging ability of the extracts and determine their potentials as antioxidants. This assay was also used to determine which of the mushroom extracts has the most potent ability to inhibit the oxidation of other molecules *in vitro*. Since oxidation involves the loss of electrons which produce free radicals. Antioxidant compounds in the mushroom will prevent oxidative (electron or hydrogen transfer) damage to cells by scavenging these radicals. From the extraction procedure, a total yield of 58.5g, 34.37g and 21.26g of methanol, ethanol and water extracts respectively was obtained and used for this research. From Figure 1 above, all the extracts of *P. portentosus* were able to scavenge DPPH radical, in the order methanol > ethanol > water. The change in colour from deep-violet to light yellow was used as a marker for antioxidant capacity. In comparison, methanol extract of *P. portentosus* possessed the highest radical scavenging activity (RSA) among all the extracts, with an IC_{50} of 0.62 ± 0.008 mg/ml, very close to the control, ascorbic acid IC_{50} value of 0.58 ± 0.020 mg/ml as shown in Table 1.

In addition, the methanol extract of *P. portentosus* is seen to possess the highest

phenolic content (2.73 in mg of GAE/ g of dry extract) in Table 1. This might account for the strong antioxidant activity also found in the methanol extract of *P. portentosus* when tested on DPPH radical hence DPPH and TPC are well correlated in this study.

Table 1 also revealed that all of the extracts showed a considerable antioxidant effect from IC_{50} of FRAP values of 755.87 ± 23.83 (water extract) to 1246.77 ± 24.94 (methanol extract) of *P. portentosus*. The water extract possessed the highest mean FRAP value as shown in Figure 2.

This is supported by Puttaraju [21] who reported that water extracts of mushrooms revealed better antioxidative reducing power ability than all other extracts. This means that the water extract of *P. portentosus* has the highest ability to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) due to the electron-donating antioxidants present within the mushroom while using $FeSO_4 \cdot 7H_2O$ as standard. This is different from the results obtained for DPPH and TPC. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

Several medicinal mushrooms have been found to lower elevated blood glucose levels in humans. The causes of these high /elevated blood sugar levels over a long period time (diabetes mellitus) is mainly due to a disorder in glucose metabolism by glucosidases. Inhibitors of these enzymes are known as Alpha glucosidase inhibitors (AGI), and are needed to lower blood sugar levels in patients. Inhibition of these enzymes (AGIs) helps to suppress hyperglycaemia (abnormal rise of glucose in the blood) in patients. AGIs limit the absorption of glucose in the blood by inhibiting alpha glucosidase enzymes[6].

Results in Figure 3 above showed that the methanol extract of *P. portentosus* inhibited alpha-glucosidase activity in the enzyme *in vitro*; this mushroom extract exhibited the highest inhibitory activity on α -D-glucosidase enzyme from *Bacillus*

sterothermophilus (84.5%), as reported in Table 2. The inhibition of the enzyme could slow down the breakdown of disaccharide to simple glucose, hence reducing the amount of glucose absorbed in the blood [22].

Table 1: Results of IC₅₀ values of DPPH (mg/mL), FRAP (mmol of FeSO₄/ 100 g dry weight of mushroom sample). TPC values in mg of GAE/ g of dry extract on extracts of *P. portentosus*. Data expressed as mean ± sem of three samples analysed separately, n=3. NA=not applicable.

	DPPH	TPC	FRAP value
Methanol extract	0.62±0.008	2.73	1246.77±24.94
Ethanol extract	0.98±0.025	2.69	903.05±31.44
Water extract	1.29±0.003	2.48	755.87±23.83
Ascorbic acid	0.58 ± 0.020	NA	NA

Table 2: Table of results of glucosidase Inhibition enzyme activity on methanol extract of *P. Portentosus*

No	Project type	Glucosidase enzymes	% Inhibition
1.	<i>In vitro</i> anti diabetic	β-D- glucosidase , (almond)	12.1
2.	<i>In vitro</i> anti diabetic	α-D-glucosidase, (<i>B. sterothermophilus</i>)	84.5
3.	<i>In vitro</i> anti diabetic	α-D-galactosidase , (green coffee beans)	28.1
4.	<i>In vitro</i> anti diabetic	α-D-mannosidase , (Jack bean)	18.9
5.	<i>In vitro</i> anti diabetic	N-acetyl –β-D- glucuronidase, (Jack bean)	48.8

The methanol extract of *P. portentosus* may act as a natural alpha-glucosidase inhibitor (AGI) and can serve as a template for the synthesis of a host of more beneficial antidiabetic analogues. However, the antidiabetic drugs (acarbose, glucophage) currently used, have been reported to exhibit side effects such as flatulence, diarrhoea and abdominal distention. So, the strong inhibition of alpha-D-glucosidase (84.5%) revealed in this research can be of great pharmacological importance in addressing some of these side effects associated with the synthetic antidiabetic drugs.

CONCLUSION

Many medicinal mushrooms are rich sources of bioactive compounds that are remarkably free from undesirable side-effects and display powerful pharmacological actions. This study revealed that the methanol extract of the mushroom, *Phaeogyroporus portentosus* exhibited the strongest antioxidant activity, high total phenolic contents and effective inhibition against the alpha-glucosidase enzyme. Its antioxidant and antidiabetic potential may help in the treatment of type 2 diabetes. This natural product can be used as an alternative to synthetic oral hypoglycemic drugs with less or even no prominent side effects. In addition, the methanol extract of *P. portentosus* can be an alternative source of polyphenolics with potent antioxidant activities.

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