ANTI-DIABETIC ACTIVITY OF THREE SPECIES OF OYSTER MUSHROOM

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Abstract
The aim of this research work is to determine the inhibitory effect of three species of oystermushrooms namely; Pleurotus sajur caju, Pleurotus ostreatus and Pleurotus florida extracts on two key saccharides hydrolysing enzymes; α-amylase and α-glucosidase. Sorghum grains were used as growth support in spawn production, while sawdust supplemented with rice bran was used as cultivation substrate for the production of the oyster mushrooms species. The produced mushroom species were dried at 60^{o}C for 7hrs the resulting dried samples were subjected to analysis. Several inorganic therapeutic drugs are available in the markets that have been used in the treatment of chronic diseases, such as cancer, diabetics and hypertension. However, most of them are toxic, costly and promote negative effects on the patients; moreover, these drugs fail to alter the course of the complications. Mushrooms represent one of the world's greatest untapped resources of functional food. The result reveals that the mushroom extracts inhibits the hydrolysing enzymes in a dose dependent in the range Pleurotus sajur caju > Pleurotus ostreatus > Pleurotus florida. Therefore, the present study concluded that oyster mushrooms can function as a therapeutic diet can be an alternative to the use of therapeutic drugs in the treatment of Type-2-diabetes.

Keywords – mushrooms, diabetes, saccharide, enzymes, *in-vitro*.

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1. INTRODUCTION

Diabetes mellitus is a life threatening chronic disorder caused by lack of insulin and insulin dysfunction characterised by high level of sugar in the blood (Prabu and Kumutha, 2014). It is characterised by hyperglycaemia and alteration in carbohydrates, protein and lipid metabolism caused by defects in insulin production or action (King *et al.*, 1998 and Rushita *et al.*, 2013).Therefore, control of blood sugar is critical in the treatments or management of diabetes mellitus, in particular type -2 diabetes and in reducing chronic vascular complications (Ortiz-Andrade *et al.*, 2007). The inhibition of enzymes involved in the digestion of polysaccharides such as α-amylase and α-glucosidase is one of the therapeutic approaches for managing or controlling hyperglycaemia (Shim *et al.*, 2003). Several inorganic therapeutic drugs are available in the markets that have been used in the treatment of chronic diseases, such as cancer, diabetics and hypertension. However, most of them are toxic, costly and promote negative effects on the patients; moreover, these drugs fail to alter the course of the complications (Choi et al., 2001).

Mushroom is the fleshy, spore bearing fruiting body of a fungus, typically produced above ground on soil or on its food source (Rosli *et al*, 2012) like all fungus, mushroom is not a plant because it does not exhibit photosynthesis. Oyster mushrooms are a diverse group of saprotrophic fungi belonging to the genus *Pleurotus* (Kong, 2004). Mushrooms represent one of the world's greatest untapped resources of nutritious food. Presently, there is significant interest in the use of edible mushrooms extracts as dietary supplements based on the fact that they have a lot of bioactive compounds. Pharmaceutically bioactive mushroom constituents continue to be the main focus of most scientists, including chemical structures, isolation and efficacy experimentations *in vivo* or *in vitro*. Mushrooms have been linked with various medicinal and pharmacological properties by both western and eastern medicinal groups.

They range from strengthening the immune system against diseases including viral ones, lowering blood pressure, reducing cholesterol,

improving liver function and combating tumors (Reguła et al ., 2007). *In vitro* tests play a very important role in the evaluation of antidiabetic activity of drugs as initial screening tools ant they might provide useful information on the mechanism of action of therapeutic agents (Laar *et al.*, 2005). The objective of this study is to determine *in-Vitro* the inhibitory effects of aqueous extracts of three species of oyster mushroom on some two key enzymes (α-amylases and α-glucosidase) connected to type-2 diabetes.

2. MATERIAL AND METHOD

Materials for Substrate Production

The following raw materials were used; Spawns of *Pleurotus oestreatus, Pleurotus florida and Pleurotus sajur-caju* were obtained from FIIRO at Lagos and was dully identify by Mr Akinyemi (FIIRO) and Dr Idowu (NIHORT) both of whom were botanist. Sawdust, rice bran, $CaCO_3$, gypsum and ingredients such as the substrate containers that were used for the production / cultivation of oyster mushroom were procured from a local market in Ondo town. The Oyster mushrooms (*Pleurotus oestreatus, Pleurotus florida and Pleurotus sajur caju*) were cultivated using saw dust and rice bran substrate.

Mushroom Cultivation / Production

Production of bottled spawn

Wheat grains (2 kg) was cleaned and into a bowl and 40 g of lime was added. The mixture was soaked in water for 16 hours. The soaked wheat grains were then washed thoroughly, the broken grains removed, the whole grains rinsed and drained. About 100 g of gypsum was then added and mixed properly with the cleaned wheat. The gypsum mixed whole wheat grain was put in a cleaned jam bottle and it was tightly sealed. The filled bottles were then placed in an autoclave for sterilization at 121^{o}C for 2 hours. After cooling, inoculation was carried out. The flow-chart for production is as shown on Figure 1. It remained in the inoculating chamber for a period of 2 weeks for the mycelium to grow, after which it was transferred into the heated sawdust bags (Substrate).

Preparation of substrate for mushroom cultivation

The process of preparing substrate for mushroom cultivation is as shown on Figure 2. Mixtures of both rough and fine sawdust that were free of splinters were moistened to enhance water absorption.

Mixing

The ratio 4:1 of saw dust to Wheat bran and rice bran were cleaned separately, 200g of both lime and 100g gypsum were thoroughly mixed together before water was added to ensure

Heating

The heating of the substrates were quickly carried out in turn to destroy any competing micro-organisms for about 6hrs. An oil drum that can withstand high heat treatment was used for heating the substrate. Wooden racks were placed at the bottom of the oil drum at a height of about 20 cm; it was then filled with water up to the rack (20 cm). Each of the substrate bags was then placed on the rack inside the oil drum; the lid was then pulled on and steamed for 6 hours by heating the drum with firewood. On the lid was a hole which allowed for steam to escape. The heated substrates were then cooled below 30^{o}C before being spawned with the *Pleurotus oestreatus, Pleaurotus florida and Pleurotu sajur caju*.

Spawning

This involves the transferring of the 3 species of *Pleurotus* grown mycelium from the bottle into the sterilized and cooled substrate wheat in the bags. This was performed by removing the plugs from the bags containing the substrate (i.e. opening the bag) and small amount of the spawn was put into the opened bags at each end at a time after which new cotton was plugged back at the ends of the bags and they were tightly sealed again. This was the moment at which contamination was most likely to occur, thus it was ensured that the time of

opening the bag was short. During this spawning period, the incubation room was cleaned and misted with H_2O_2 to prevent contamination. After spawning, the bags were then placed on the shelves in the incubation room.

Spawn run

During spawn run, the mycelium grew through the substrate and humidity was maintained at 90 – 95%. The inoculating room was kept dark. After certain period, fresh air was allowed to pass through the room.

Fruiting / Cropping

The spawn bags arranged on the rack in the incubation were opened as soon as the mycelium covered the substrate completely. At this stage, the cotton plugs and a part of the polyethylene were removed at one end to allow for the mushroom to develop, which took 3 to 5 days to form after the opening of the bags. When the mushroom started growing out they were watered by applying water at the root only.

Harvesting

The mushrooms were ready for harvest after 5 days of bag cutting and this was done by gently pulling the mushroom from the substrate to ensure that only mushrooms were pulled out, to facilitate proper second flush which occurred another 5 to 9 days. In total, 3 to 4 flushes were harvested.

Drying of *Pleurotus oestreatus, Pleurotus florida and Pleurotus sajur caju* Species

Fresh mushroom was dried at 60^{o}C for 8 hours. The dried mushroom was cooled and hygienically packed in high density polythene bag until require for analysis. Plates 2, 4 and 6 shows dried oyster mushroom species. The flow chart was as shown on Figure 3.3.

Preparation of aqueous extracts of *Pleurotus oestreatus, Pleurotus sajur caju, and Pleurotus florida*

Aqueous extract preparation

The inedible portions of the mushroom samples were removed from the edible portions. The edible portions were subsequently washed in distilled water, chopped into small pieces by table knife, air dried and milled. An amount of 5 g of the milled samples were soaked in 100 ml distilled water for about 24 h, the mixture was filtered and later centrifuged at 358 g for 10 min to obtain a clear supernatant which was then used for subsequent analysis (Oboh *et al.* 2007).

Determination of the diabetes therapeutics of *Pleurotus oestreatus, Pleurotus sajur caju, and Pleurotus florida* mushrooms and the suplemented biscuits:

α-Amylase inhibition assay

The aqueous extracts dilution (500 µl) and 500 µl of 0.02 mol·l-1 sodium phosphate buffer (pH 6.9 with 0.006 mol·l-1 NaCl) containing hog pancreatic α-amylase (EC 3.2.1.1; 0.5 mg·ml-1) were incubated at 25 °C for 10 min. Then, 500 µl of 1% starch solution in 0.02 mol·l-1 sodium phos- phate buffer (pH 6.9 with 0.006 mol·l-1 NaCl) was added to the reaction mixture. Thereafter, the re- action mixture was incubated at 25 °C for 10 min and stopped with 1.0 ml of dinitrosalicylic acid (DNSA). The mixture was then incubated in a boiling water bath for 5 min, and cooled to room tem- perature. The reaction mixture was then diluted by adding 10 ml of distilled water, and absorbance was measured at 540 nm in a UV-Visible spectro- photometer (Model 6305; Jenway, Barl oworld Scien tific, Dunmow, United Kingdom) (Bernfield. 1951).

α-Glucosidase inhibition assay

Appropriate dilution of the aqueous extracts (50 µl) and 100 µl of α-glucosidase solution (1.0 U·ml-1) was incubated at 25 °C for 10 min. Thereafter, 50 µl of 5 mmol·l-1 p-nitrophenyl-α-D- glucopyranoside solution in 0.1 mol·l-1 phosphate buffer (pH 6.9) was added. The reaction mixture was then incubated at 25 °C for 5 min, and then absorbance was measured at 405 nm in the

spectrophotometer. The α-glucosidase inhibitory activity was expressed as percentage inhibition Apostolidis, 2007.

Statistical Analysis

The experimental results were expressed as mean ± standard Deviation (SD) of three replicates. Data obtained were statistically analysed using one way Analysis of Variance (ANOVA), a tool in Statistical packages for Social Science (SPSS 14.0). The level of significance was set at $p < 0.05$. Means were separated with Duncan Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

Antidiabetic Propreties of *Pleurotus saju-cajur, Pleurotus ostreatus* and *Pleurotus florida*

Diabetes mellitus is a life threatening chronic metabolic disorder caused by lack of insulin and insulin dysfunction, characterised by high levels of glucose in the blood. Therefore, inhibition of enzymes involved in the digestion of polysaccharides, such as α-amylase and α-glucisidase and this is one of the therapeutic approaches for managing hyperglycaemia (Shim *et al.,* 2003) and this may be achieved by consuming foods (fruits and vegetables) that have the ability to slow down the digestion of carbohydrates such foods include the species of oyster mushroom in this study. The result of the ability of the three oyster mushroom species namely *Pleurotus sajur-caju, Pleurotus ostreatus and Pleurotus florida* to inhibit α-amylase and α-glucosidase are presented in Figures 1and 2.

α-amylase inhibitory activity of *Pleurotus sajur-caju, Pleurotus ostreatus and Pleurotus florida*

The result of this study shows that all the three species of oyster mushroom extracts inhibited α-amylase in a dose dependent manner (in the range of 0.75-3.25 µg/ml). The IC_{50} values obtained were as follows *Pleurotus sajur-caju* (1.83); *Pleurotus ostreatus* (1.96) *and Pleurotus florida* (2.49) all in (µg/ml). However, comparison of the three species of the extracts IC_{50} (extracts concentration causing 50% enzyme inhibition) values reveals that significant difference did exist among the IC_{50} of the three extracts, with *Pleurotus sajur-caju* having a higher inhibitory activity than the other two species, while the *Pleurotus florida* have the least inhibitory activity. It could be noted from the figure 1, that the IC_{50} value is lowest in *Pleurotus sajur-caju,* followed by *Pleurotus ostreatus* and the highest value was recorded in *Pleurotus florida.* The lower the IC_{50} value the more effective the inhibitory activity. Thus the *Pleurotus sajur-caju* is more effective in terms of inhibitory activity. The curve of inhibition is as presented in Figure 1. The result of the α-amylase inhibitory activity of the three species of oyster mushrooms compares favourably with the report various researchers such as; Inhibitory aqueous extracts of two varieties of ginger (Oboh *et al.,* 2010); Inhibitory effects of *Alium spp.* on α-amylase activity (Nickavear, B. and Yousefian, N. (2009) and antidiabetic activity of *Calocybe indica* mushroom, (Prabu and Kumutha, 2014).

α-glucosidase inhibitory activity of *Pleurotus sajur-caju, Pleurotus ostreatus and Pleurotus florida*

Also, the ability of the mushroom extracts to inhibit α-glucosidase enzyme *in vitro* was examined and the inhibition curve is as presented in Fig.2. The values obtained where as shown in table 2. The extracts inhibit the α-glucosidase enzymes in a dose dependent manner. However, the α-glucosidase enzyme inhibitory activity of the extracts was significantly higher than that of their inhibitory activity of α-amylase. This is in agreement with the report of various workers such as Oboh *et al.,* (2010) and Kwon *et al.,* (2007) that plant inhibitors are mild inhibitors of α-amylase and strong inhibitor of α-glucosidase. This makes natural diets a better therapeutics in the managements of diabetes when compare to the use of the synthetic drugs such as glinides, phenformin and acrbose. Several inorganic therapeutic drugs are available in the markets

that have been used in the treatmet of chronic diseases, such as cancer, diabetics and hypertension. However, most of them are toxic, costly and promote negative effects on the patients; moreover, these drugs fail to alter the course of the complications (Choi *et al.*, 2001). This may be due to the fact that these synthetic drugs are known to have higher inhibitory effects on α – amylase than α –glucosidase. It has been suggested that the adverse effect of the synthetis drugs might be due to excessive inhibition of the abdorminal bacterial fermentation of undigested saccharides in the colon (Oboh *et al.*, 2010; Bischoff, 1994 and Horii *et al.*, 1987).

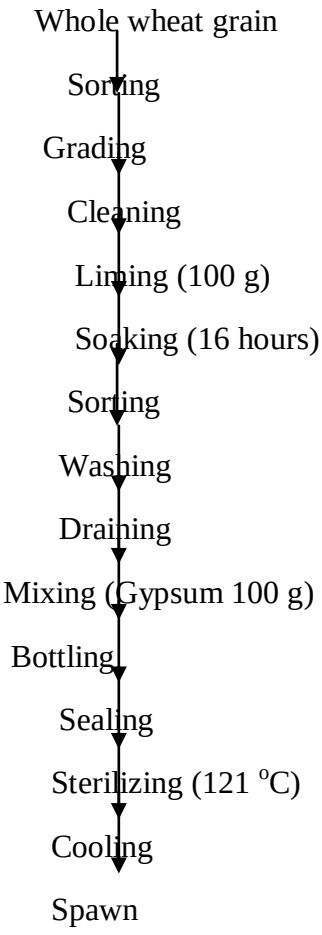

Fig. 1: Flow Chart for the Inoculation of Oyster Mushroom *(Pleurotus oestreatus)* Bottled Spawn

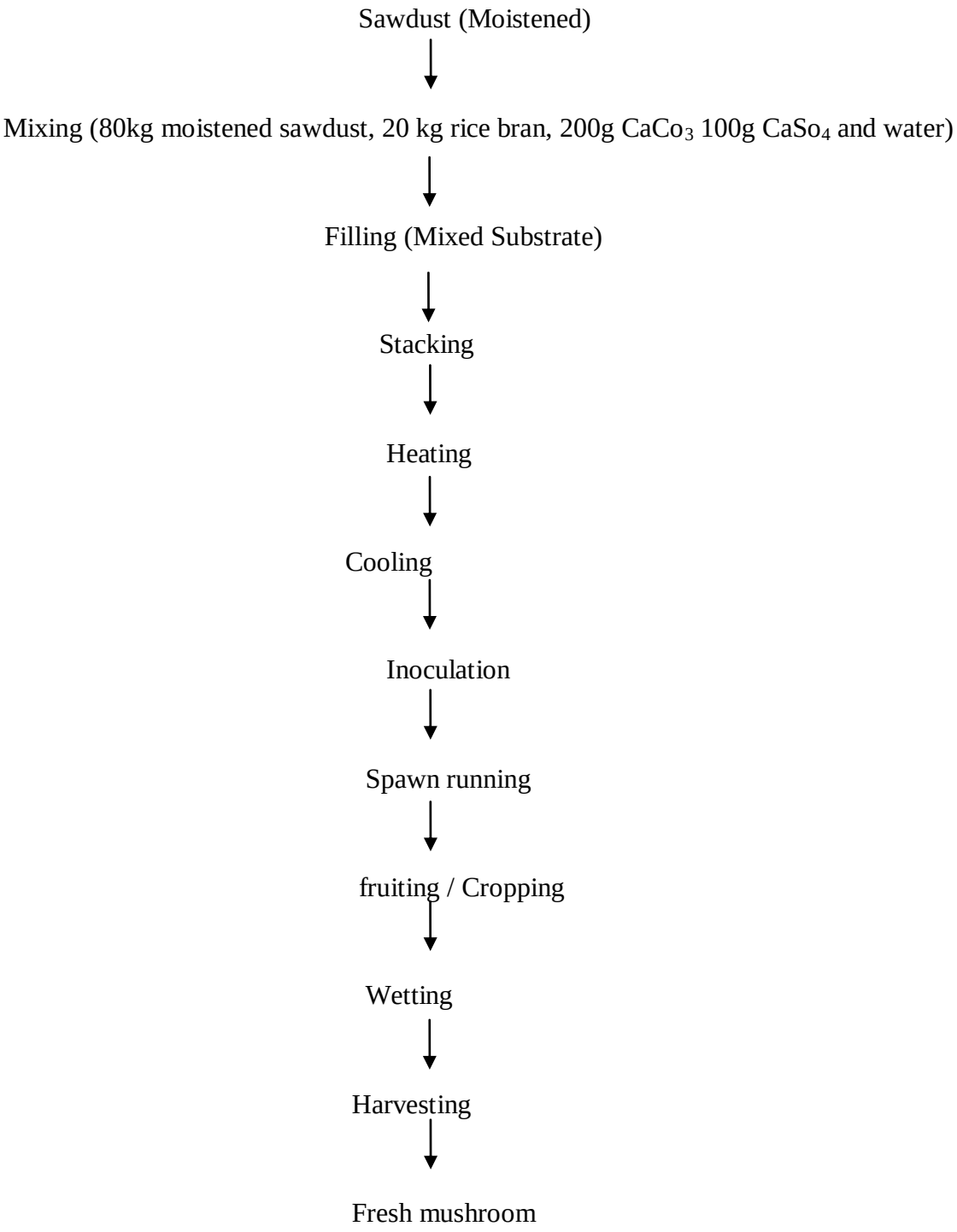

Fig. 2: Flow Chart of the substrate production/Cultivation of Oyster Mushroom Species *(Pleurotus oestreatus, Pleurotus florida* and *Pleurotus sajur caju)*

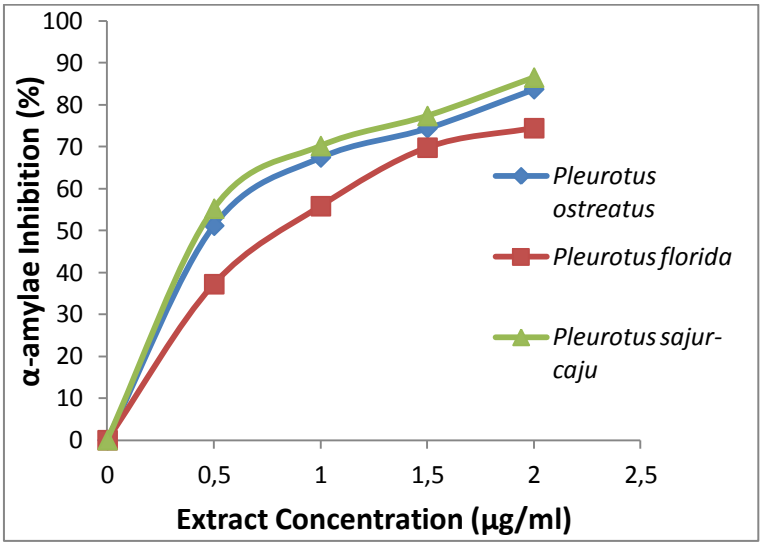

Fig. 3: α-amylase inhibition activities of aqueous extract of oyster mushroom species

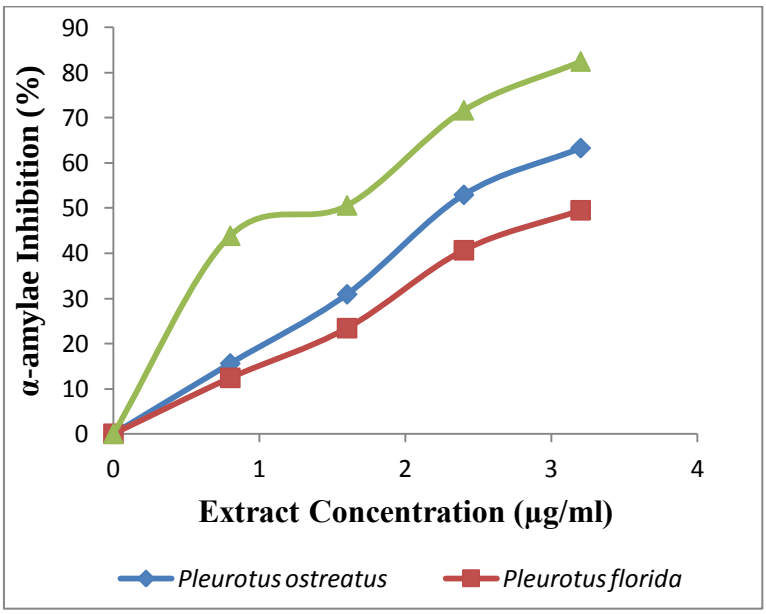

Fig. 4: α-glucosidase inhibition activities of aqueous extract of oyster mushroom species

4. CONCLUSION

In this study, the inhibition of saccharides hydrolysable enzymes by the three species of oyster mushrooms in a dose dependent manner. Hence, the antidiabetic effect of the oyster mushrooms might be attributed to the inhibitory activity of the hydrolysable enzymes thereby retarding the digestion of carbohydrate to delay rise in blood glucose.

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