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Note

In vitro α-amylase and α-glucosidase inhibitory potential of *Pleurotus ostreatus cv.* Florida extract

Deepak Agrawal, Ayushi Chourasia, Aditya Ganeshpurkar*, Abhishek Shrivastava & Nazneen Dubey

Shri Ram Institute of Technology- Pharmacy, Jabalpur, Madhya Pradesh, India

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Pleurotus ostreatus cv. Florida is one of the widely used edible mushroom. The polysaccharides from this mushrooms have been studied for antidiabetic potential; however, no efforts have been made to explore the potential of this mushroom to influence carbohydrate metabolizing enzymes viz. α-amylase and α-glucosidase. The present work was undertaken to investigate the inhibitory potential of *Pleurotus ostreatus cv.* Florida on enzymes α-amylase and α-glucosidase. Several concentrations of extracts were used to study inhibition of enzymatic activity of α-amylase and α-glucosidase. A dose dependent inhibitory effect on enzymes was observed. The current study, for the first time, uncovered α-amylase and α-glucosidase inhibitory potential of *Pleurotus ostreatus cv.* Florida. The study could be helpful to isolate and characterize compounds responsible for it.

Keywords: α-amylase, α-glucosidase, *Pleurotus ostreatus cv.* Florida, Oyster mushroom

Introduction

Diabetes mellitus is a metabolic disorder characterized by alteration in the metabolism of carbohydrates, proteins and fats[1]. This disorder of endocrine system is associated with hyperglycemia, weight loss and other secondary side effects[2]. The condition is observed by increase in the blood glucose levels due to lack or complete absence of insulin in the body[3]. Glycemic control is one of the promising strategies for management of diabetes mellitus owing to decreased risk of development of diabetes associated complications[4]. The general management principles for management of type II non-insulin dependent diabetes mellitus includes physical activities (exercise), dietary control and use of oral hypoglycemic drugs[5]. In the present time, one of the important strategy for the management of diabetes mellitus involves inhibiting carbohydrate-digesting enzymes (α-amylase and α-glucosidase) by bioactive compounds from natural sources. This leads to a decrement in postprandial blood glucose levels[6].

Pleurotus ostreatus cv. Florida is well recognized edible mushroom from ancient times. *Pleurotus* mushroom are abundant source of secondary metabolites such as steroids, terpenoids, alkaloids, phenols, lectins and flavonoids[7]. There are many scientific evidences which testify the role of *Pleurotus ostreatus cv.* Florida for antioxidant, antidiabetic[8], anthelmintic[9], antimicrobial and analgesic[10] effects. The aim of the present work was to determine α-amylase and α-glucosidase inhibitory effect of oyster mushroom *Pleurotus ostreatus* cv. Florida extract.

Material and Methods

Procurement and authentication of mushrooms

Mushrooms were purchased from Satpuda Mushroom Utpadak Sahkari Samiti Maryadit, Itarsi, India. Mushrooms were identified by Dr. R.K. Verma, Scientist F and Head, Forest Pathology Division, Tropical Forest Research Institute, Jabalpur, India. The voucher (Reference Number: 22Path/TFRI/JBP/607) was also deposited.

Chemicals

Dimethyl sulfoxide (DMSO), Tris-HCl buffer, and nitrophenyl glucopyranose were purchased from Central Drug House, India. All the other chemicals used in the work were of analytical grade.

Preparation of extract

About 50 g mushroom (powder) was defatted with petroleum ether for 24 hrs. The defatted mushroom was dried and then obtained powder was extracted with water: ethanol (1:1) for 48 h. The extract was filtered through What-man no. 4 filter paper and evaporated at 40°C. It was then stored at 4°C for later use.

Phytochemical screening

Phytochemical screening of extract was performed reported method[11]. Phytochemical tests for carbohydrates, saponins, steroids, phytosterols, tannins, flavonoids and alkaloids were performed.

Phyto-analytical studies

Determination of total phenolic compounds

Total soluble phenolic compounds in the extract were determined with Folin-Ciocalteu reagent[12].

*Correspondence:
E-mail: adityaganeshpurkar@gmail.com

Extract of mushroom was mixed with 2.9 mL of distill water then mixed with Folin-Ciocalteu followed by addition of 2 mL of 20% Na_2CO_3. The mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve of catechin from 2-20 µg/mL. All samples analyzed in triplicate

Assay for total flavonoids content

Total flavonoid content was determined using colorimetric assay[13]. Extract was mixed with 10% of 0.3 mL of $AlCl_3$ solution after 6 min the 5% solution of $NaNO_2$ of 0.3 mL was added. After 5 min, 0.5 mL of NaOH were added. The final volume was adjusted upto 10 mL with distill water. Absorbance was determined at 510 nm against a blank. The total flavanoid content is expressed in micrograms against the calibration curve of rutin from 2-20 µg/mL. All samples analyzed in triplicate.

Enzyme inhibition assay

Porcine pancreatic amylase inhibitory

PPA inhibitory assay was performed as per the standard method[14,15]. 2 mg of starch was suspended in each of the tubes containing 0.2 mL of 0.5 M Tris-Hcl buffer (pH 6.9 and 0.01 M $CaCl_2$). The tubes containing the substrate solution were boiled for 5 min and were then incubated at 37°C for 5 min. 0.2 mL of extracts was taken in each tube containing different concentrations (50, 100, 150, 200, and 250 µg/mL) of DMSO. PPA was dissolved in Tris-HCl buffer to form a concentration of 2 units/mL and 0.1 mL of this enzyme solutionwas added to each of the above-mentioned tubes. The reaction was carried out at 37°C for 10 min and was stopped by adding 0.5 mL of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4°C. The absorbance of the resulting supernatant was measured at 595 nm using a spectrophotometer (Shimadzu-1700). The α-amylase inhibitory activity was calculated as follows:

$$\alpha\text{ - Amylase inhibitory activity} = \frac{[(Ac+) - (Ac-)]}{[(As - Ab)/(Ac+) - (Ac-)]} \times 100$$

where Ac+, Ac-, As, and Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme activity), a test sample (with enzyme), and a blank (a test sample without enzyme), respectively.

α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity was determined using the standard method. The enzyme solution was prepared by dissolving 0.5 mg α-glucosidase in 10 mL phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin.It was diluted further to 1:10 with phosphate buffer just before use. Sample solutions were prepared by dissolving 4 mg sample extract in 400 µL DMSO. Five concentrations: 50, 100, 150, 200, and 250 µg/mL were prepared, and five µL each of the sample solutions or DMSO (sample blank) was then added to 250 µL of 20 mM p-nitrophenyl-α-D-glucopyranoside and 495 µl of 100 mM phosphate buffer (pH 7.0). It was pre-incubated at 37°C for 5 min and the reaction started by addition of 250 µL of the enzyme solution, after which it was incubated at 37°C for exactly 15 min. Phosphate buffer (250 µL) was added instead of the enzyme for blank. The reaction was then stopped by addition of 1000 µL of 200 mM Na_2CO_3 solution, and the amount of *p*-nitrophenol released was measured by reading the absorbance of the sample against a sample blank (containing DMSO with no sample) at 400 nm using UV-visible spectrophotometer.

Statistical analysis

The results are expressed as mean ± standard error of mean. Experiments were always performed in triplicates. Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test (**P* <0.001).

Results

Phytochemical screening and phytoanalytical studies

The extract was rich in amino acids glycosides, carbohydrates, andflavonoids. The total flavonoid content of the hydroethanolic extract of *Pleurotus ostreatus* cv. Florida, calculated from the calibration curve (R^2= 0.9998), was 1.20 rutin equivalents/g. The phenolic content (R^2=0.9971), was 0.1066 catechin equivalent/g.

Porcine pancreatic amylase inhibitory studies

The percentage inhibition displayed by each extract is shown in (Fig. 1A), which justifies the ability of mushroom extract in α-amylase inhibition. The percentage inhibition was 14.65 ± 1.94 to 62.55±1.07. Extract at concentration 200 µg/ mL showed 52.56.±1.40 % inhibition over enzyme α-amylase (Fig. 1).

α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity of mushroom extract is shown in (Fig. 1B). For all tested

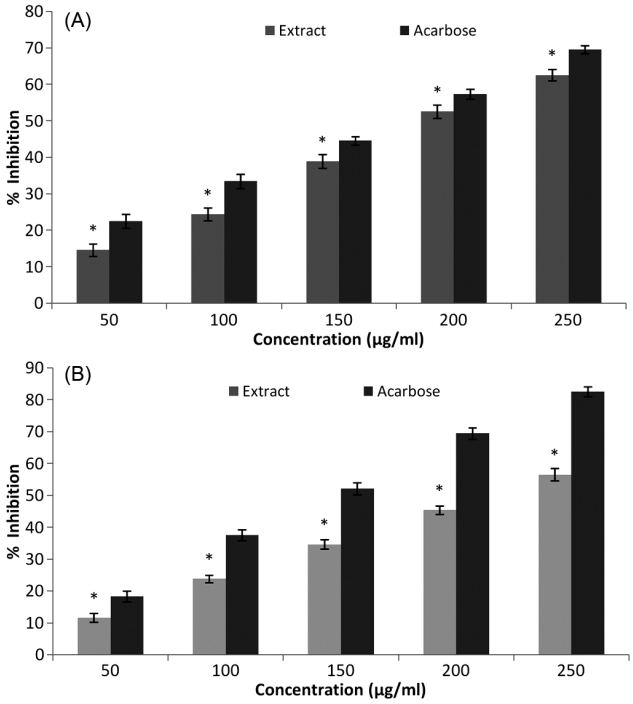

Fig. 1 — Inhibitory activity of *Pleurotus ostreatus cv.* Florida extract against (A) α-amylase; and (B) α-glucosidase. The results are expressed as mean ± standard error of mean. Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test (*P <0.001)

concentrations, percent α-glucosidase inhibition increased with increasing concentration of the extract.The percentage inhibition was 18.35 ± 1.70 to 82.54 ± 0.88. Extract at concentration 150 µg/ mL showed 55.42.±1.86 % inhibition over enzyme α-glucosidase.

Discussion

According to the International Diabetes federation (IDF), Type-2 diabetes affects 246 million people worldwide and is expected to rise to 380 million by 2025[16]. Insulin resistance is an important feature of type-2 diabetes, and is strongly associated with cardiovascular risk factors. In the present study, *Pleurotus ostreatus cv.* Florida extract was evaluated for possible α-amylase and α-glucosidase inhibition. The results revealed a strong association between the concentration of extract and inhibition of both enzymes. Extract was found to be rich in flavonoids and polyphenols. Flavonoids are known to form starch-flavonoids complex by hydrophobic interactions as well as by covalent bonds. This phenomenon retards breakdown of starch and a control over glycemic index is observed[17]. Flavonoids are also strong inhibitors of enzyme glucosidase[18]. The presence of 3-OH group on C-ring causes a significant inhibition of α-glucosidase. Similar effects are observed by polyphenols[19]. Glucosidase inhibitors like acarbose are accountable for decreasing post-prandial glucose levels[20] which seems to be beneficial in patients diagnosed with type 2 diabetes mellitus and aid in maintaining blood sugar levels in safe limits. A number of herbal remedies are used in management of metabolic disorders and inflammatory disorders[21-23].

Pleurotus ostreatus cv. Florida extract demonstrated a significant α-amylase and α-glucosidase inhibitory effect which can serve as a lead for isolation and identification of compounds responsible for this inhibition. However, more systematic studies are needed to confirm these results.

Conflict of interest

The author declares no conflict of interest.

References

1 Larsson SC, Type 1 and type 2 diabetes mellitus and incidence of seven cardiovascular diseases. *Int J Cardiol*, 262 (2018) 66.
2 American Diabetes Association, A. D. 8. Pharmacologic Approaches to Glycemic Treatment:Standards of Medical Care in Diabetes-2018. *Diabetes Care*, 41 (2018) S73.
3 Holst JJ, Insulin and glucagon: partners for life. *Endocrinology*, 158 (2017) 696.
4 DiMeglio LA, Acerini CL, Codner E, Craig ME, Hofer SE, Pillay K & Maahs DM, Glycemic control targets and glucose monitoring for children, adolescents, and young adults with diabetes. *Pediatr Diabetes*, 19 (2018) 105.
5 Chaudhury A, Clinical review of antidiabetic drugs: implications for type 2 diabetes mellitus management. *Front Endocrinol (Lausanne)*, 8 (2017) 6.
6 Reyes BAS, Selected Phyto and Marine Bioactive Compounds: Alternatives for the Treatment of Type 2 Diabetes. *Stud Nat Prod Chem*, 55 (2017) 1.
7 Arbaayah H & Kalsom UY, Antioxidant properties in the oyster mushrooms (Pleurotus spp.) and split gill mushroom (Schizophyllum commune) ethanolic extracts. *Mycosphere*, 4 (2013) 661.
8 Ganeshpurkar A, Kohli S & Rai G, Antidiabetic potential of polysaccharides from the white oyster culinary-medicinal mushroom Pleurotus florida (higher Basidiomycetes). *Int J Med Mushrooms*, 16 (2014) 207.
9 Rai G, Ganeshpurkar A, Bhadoriya S & Pardhi P, Investigation of anthelmintic potential of oyster mushroom Pleurotus florida. *Indian J Pharmacol,* 44 (2012) 539.
10 Ganeshpurkar A & Rai G, Experimental evaluation of analgesic and anti-inflammatory potential of Oyster mushroom Pleurotus florida. *Indian J Pharmacol*, 45 (2013) 541.
11 Harborne AJ, *Phytochemical methods a guide to modern techniques of plant analysis*, (Springer Science & Business Media) 1998.
12 Slinkard K. & Singleton V, Total phenol analysis: automation and comparison with manual methods. *Am J Enol Vitic*, 28 (1977) 49.

13 Dewanto V, Xianzhong W, Adom KK & Liu RH, Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Agric Food Chem*, 50 (2002) 3010.

14 Hansawasdi C, Kawabata J & Kasai T, α-Amylase inhibitors from roselle (*Hibiscus sabdariffa* Linn.) tea. *Biosci Biotechnol Biochem*, 64 (2000) 1041.

15 Ganeshpurkar A, Bhardwaj Y & Diwedi V, *In vitro* α - amylase and α-glucosidase inhibitory potential of *Trigonella foenum-graecum* leaves extract. *AYU*, 34 (2013) 109.

16 Iorio A, International Diabetes Federation. *Diabetes Res. Clin Pract*, (2018).

17 Takahama U & Hirota S. Interactions of flavonoids with α-amylase and starch slowing down its digestion. *Food and Function*, 9 (2018) 677.

18 Pereira DF, Cazarolli LH, Lavado C, Mengatto V, Figueiredo MS, Guedes A, Pizzolatti MG & Silva FR, Effects of flavonoids on α-glucosidase activity: Potential targets for glucose homeostasis. *Nutrition*, 27 (2011) 1161.

19 Nyambe-Silavwe H, Inhibition of human α-amylase by dietary polyphenols. *J Funct Foods*, 19 (2015) 723.

20 Sasaki M, Nishida N & Shimada MA beneficial role of rooibos in diabetes mellitus: A systematic review and meta-analysis. *Molecules*, 23 (2018).

21 Manju SL, A review on phyto-nanotechnology for therapy of alzheimer's disease. *Indian J Biochem Biophys*, 59 (2022) 867.

22 Dwarampudi LP, Spi D, Farha S & Gade R, Phytochemical evaluation and anti-psoriatic activity of the ethanolic extract of the leaves of *Momordica charanti*a. *Indian J Biochem Biophys*, 59 (2022) 751.

23 Ganeshpurkar A, Chaturvedi A, Shrivastava A, Dubey N Jain S, Saxena N, Gupta P & Mujariya R, *In silic*o interaction of Berberine with some immunomodulatory targets: A docking analysis. *Indian J Biochem Biophys*, 59 (2022) 848.