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Research Article

IN VITRO STUDIES ON ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF *PLEUROTUS EOUS* MUSHROOM IN METHANOL AND AQUEOUS EXTRACT

SHOBA K[1], KRISHNAKUMARI S[2]*

[1]Department of Biochemistry, New Prince Shri Bhavani Arts and Science College, Chennai, Tamil Nadu, India. [2]Department of Biochemistry, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu, India

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ABSTRACT

Objective: The present study evaluates the antioxidant and antidiabetic activity of the mushroom.

Methods: Antioxidant activity was evaluated using hydroxyl radical, hydrogen peroxide, and antidiabetic activity using α-amylase and α-glucosidase.

Result: The antioxidant IC50 for the mushroom extracts methanol and aqueous(Hydroxyl radical) was found to be 290,440µg/ml (Hydrogen peroxide) 475,370 µg/ml and antidiabetic(α Amylase) IC50 was found to be 460,500 µg/ml and (α Glucosidase) 325,280 µg/ml respectively.

Conclusion: The result obtained in the *in vitro* methods suggests that *Pleurotus eous* mushroom can be administered for its antioxidant and antidiabetic activity.

Keywords: Antioxidant, Antidiabetic activity, α-amylase, α-glucosidase, Hydroxyl radical, Hydrogen peroxide.

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INTRODUCTION

Free radicals arising from metabolism or environmental sources interact continuously in biological systems, and their uncontrolled generation correlates directly with molecular level of many diseases [1]. The innate defense of human body is not enough for severe oxidative stress that further has been associated with cancer, aging, inflammation, neurodegenerative diseases, hypertension, arthrosclerosis, etc. Over production of various forms of activated species such as reactive oxygen species, reactive nitrogen species, and non-free radical species is considered to be the main contributor to oxidative stress [2]. Lots of research have clearly showed that free radicals would damage nearby structures including DNA, proteins, or lipids. Radical scavenging antioxidants are particularly important in antioxidative defense in protecting cells from the injury of free radical [3]. It is well known that free radicals are the major cause of various chronic and degenerative diseases, such as coronary heart disease, inflammation, stroke, diabetes mellitus, and cancer [4]. Studies have shown that mushrooms have hepatoprotective [5-7], anticancer [8-10], antimicrobial [11,12], and antidiabetic [13,14] activities. A study of ancient literature indicates that diabetes (Madhumeha/Prameha) was fairly well-known and well-conceived as an entity in India. Regulation of glucose level in the blood of the diabetic patient can prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species [15]. Diabetes mellitus is a metabolic disorder which can be controlled or prevented with lifestyle adaptations including exercise and appropriate diet [16]. An effective strategy for type 2 diabetes management is the strong inhibition of intestinal α-glucosidases and the mild inhibition of pancreatic α-amylase [17]. Mushrooms are known to contain compounds which help in proper functioning of the liver [18], pancreas, and other endocrinal glands, thereby promoting formation of insulin and related hormones which ensure healthy metabolic functioning [19-21]. Polysaccharides, such as beta glucans contained in mushrooms, have the ability to restore the function of pancreatic tissues by causing increased insulin output by β-cells, which leads to lowering of blood glucose levels [22].

The present study was carried out to investigate the methanol and aqueous extract of *Pleurotus eous* on the radical scavenging potential by employing hydroxyl radical, hydrogen peroxide, and antidiabetic of α-amylase and α-glucosidase activity.

METHODS

Sample collection

Fresh fruiting bodies of *P. eous* mushroom were cultivated in the mushroom units maintained at Kongunadu Arts and Science College, Coimbatore - 641 029, Tamil Nadu, India.

Extract preparation

Extract of mushroom was prepared using two different solvents (methanol and aqueous). Dried powered weighed carefully and used for methanol extract preparation through Soxhlet apparatus and aqueous extract boiled for 2 h and centrifuged. The supernatant collected is used for further use.

Antioxidant and antidiabetic activity of *P. eous* mushroom

Determination of hydroxyl radical scavenging activity

Deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium [23]. The reaction mixture containing FeCl3 (100 µM), ethylenediaminetetraacetic acid (EDTA) (104 µM), H_2O_2 (1 mM), and 2-deoxy- D-ribose (2.8 mM) in potassium phosphate buffer (20 mM, pH 7.4) was mixed with various concentrations of sample. Incubate for 1 h at 37°C. The mixture was heated at 95°C in water bath for 15 min followed by the addition of 1 mL each of trichloroacetic acid (2.8%) and thiobarbituric acid (TBA) (0.5% TBA in 0.025 M NaOH). Finally, the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 min. Absorbance of supernatant was measured at 532 nm. The hydroxyl radical scavenging activity of the mushroom extract was reported as percentage inhibition of deoxyribose degradation and was calculated using the following formula:

% Inhibition = (control OD-sample OD/control OD) ×100

Determination of hydrogen peroxide scavenging activity

This activity was determined according to the standard method with minor changes [24], take different concentrations of samples and standard, and add equal volume of H_2O_2 in test tubes. To this, add 10 µL of methanol and 900 µL of FOX reagent. Incubate 30 min at room temperature. Measure OD at 560 nm.

% Inhibition = (control OD-sample OD/control OD) ×100

Assay for α-amylase inhibition

The α-amylase inhibitory activity of extract and fractions was carried out according to the standard method with minor modification [25]. 100 µL of test samples and standard drug (20-100 µg/mL) were taken. Then, 250 µL of α- amylase (1 mg/mL) in 0.2 M sodium phosphate buffer (pH 6.9) was added to each tube and was incubated at 37°C for 20 min. Then, 250 µL of a 0.5% starch solution in 0.2 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 37°C for 15 min. The reaction was stopped with 1 mL of 3, 5 dinitrosalicylic acid. The test tubes were then incubated in a boiling water bath at 100°C for 10 min, cooled to room temperature. The reaction mixture was then diluted to 10 mL using distilled water, and absorbance was measured at 540 nm. The % α-amylase inhibitory activity is calculated by the following formula:

% Inhibition = (control OD-sample OD/control OD) ×100

Assay of alpha-glucosidase activity

The α-glucosidase inhibitory activity of extract and fractions was carried out according to the standard method with minor modification [26]. 50 µL of 0.2 M sodium phosphate buffer (pH 6.8) and 50 µL of 0.1 U glucosidase were taken in different tubes. To this, 50 µL of sample and standard of different concentrations was added (should not mix) and incubated at 37°C for 5 min. Then, 50 µL of p-nitrophenyl alpha-D-glucosidase was added, vortexed, and incubated at 37°C for 30 min. 50 µL of 0.1 M sodium carbonate was added. Absorbance was measured at 405 nm (Figs. 1 and 2).

% Inhibition = (control OD-sample OD/control OD) ×100

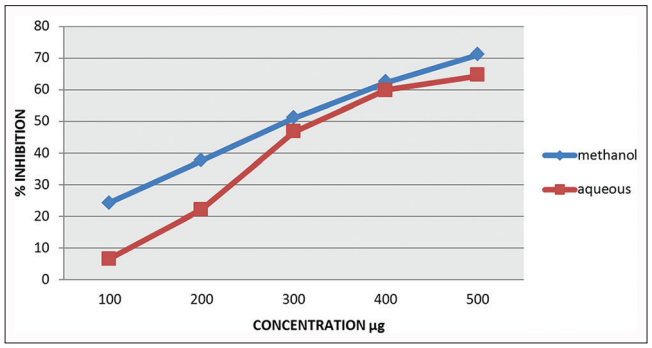

Fig. 1: Hydroxyl radical for methanol and aqueous extract

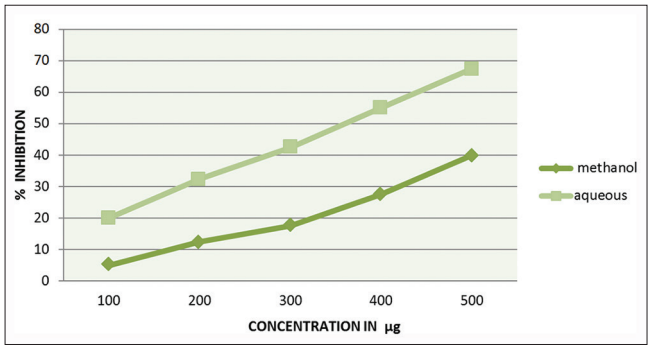

Fig. 2: Hydrogen peroxide for methanol and aqueous extract

RESULT

Different concentrations of the methanolic and aqueous extract of *P. eous* were tested for their antioxidant activity using different *in vitro* models. It was observed that free radicals were scavenged by the test compounds in a concentration-dependent manner in all the models. Inhibiting of α-amylase and α-glucosidase could be of importance in the management of diabetes mellitus as it also addresses some side effects associated with synthetic antidiabetic drugs. Hence, *pleurotus eous* mushroom ingestion will improve the quality of life of diabetic patients.

Statistical analysis

The results were expressed as mean values and standard deviation. Linear regression analysis was used to calculate IC_{50} value.

DISCUSSION

Hydroxyl radicals (OH) generated in the human body may play an important role in tissue injury at sites of inflammation in oxidative stress-originated diseases. Hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-2-ribose into fragments that formed a pink chromogen on heating with TBA at low pH. Ferric-EDTA was incubated with H_2O_2 and ascorbic acid at pH 7.4. While the addition of methanol extract to the reaction mixture found that they removed hydroxyl radical from the sugar and prevented their degradation. The methanol extract of *P. eous* mushroom showed potent hydroxyl radical scavenging activity. The antioxidant IC50 for the mushroom extracts methanol and aqueous(Hydroxyl radical) was found to be 290,440 µg/ml (Hydrogen peroxide) 475,370 µg/ml (Table 1), further the shown hydroxyl radical scavenging activity as dose dependent. *In vitro* tests can play a very important role in the evaluation of antidiabetic activity of drugs as initial screening tools, where the screening of a large number of potential therapeutic candidates may be necessary [27-29]. The therapeutic approach for treating Type 2 diabetes is to decrease the post-prandial glucose levels. This could be done by retarding the absorption of glucose through the inhibition of the carbohydrates hydrolyzing enzymes, α-amylase, and α-glucosidase, which is present in the small intestinal brush border is responsible for the breakdown of oligosaccharides; disaccharides into monosaccharides suitable for absorption [30-33]. Number of studies have been reported the alpha-amylase and alpha-glucosidase inhibitory activities in various plants and medicinal mushrooms. The similar activity was not investigated before in *P. eous* mushroom. In the present study, *in vitro* antidiabetic studies revealed the inhibition of alpha-amylase and alpha-glucosidase activity. The intestinal digestive enzymes alpha-amylase play a vital role in the carbohydrate digestion. One antidiabetic therapeutic approach reduces the post-prandial glucose level in blood by the inhibition of alpha-amylase enzyme. These can be an important strategy in the management of blood glucose [34]. The percentage inhibition at 100, 200, 300, 400, and 500 µg/mL concentrations of *P. eous* on á-amylase and á-glucosidase showed a concentration-dependent reduction in percentage inhibition (Figs. 3 and 4). Antidiabetic (α Amylase) IC50 was found to be 460,500 µg/ml and (α Glucosidase) 325,280 µg/ml respectively. (Table 2). Therefore, the antidiabetic effect of *P. eous* might attribute to its inhibitory effect against á-amylase and á-glucosidase that retarding the digestion of carbohydrate to delay the postprandial rise in blood glucose.

CONCLUSION

From the above results, it can be concluded that the methanolic extract of the mushroom *P. eous* showed more potent *in vitro* antioxidant activity, with higher percentage inhibition, than the aqueous extract. It may be concluded that mushrooms have immense potential and may be developed as effective and safe antidiabetic therapy.

CONFLICT OF INTERESTS

There is no conflict of interests regarding the publication of this paper.

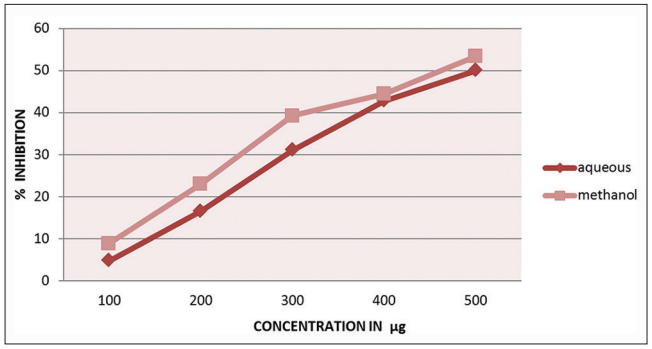

Fig. 3: Alpha-amylase in aqueous and methanol extract

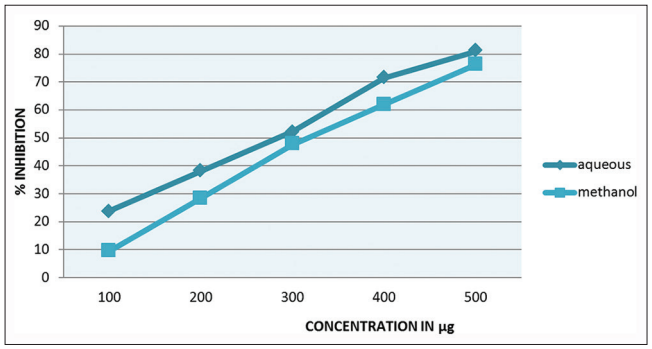

Fig. 4: Alpha-glucosidase in aqueous and methanol extract

Table 1: IC_{50} values of antioxidant extracts

Sample	Hydroxyl radical (µg)	Hydrogen peroxide (µg)
Methanol extract	290	475
Aqueous extract	440	370

Table 2: IC_{50} values of antidiabetic extracts

Sample	Alpha-amylase (µg)	Alpha-glucosidase (µg)
Methanol extract	460	325
Aqueous extract	500	280

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