

Original Article

## ANTIDIABETIC POTENTIAL OF THE OYSTER MUSHROOM *PLEUROTUS FLORIDA* (MONT.) SINGER

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### ABSTRACT

**Objective:** The present investigation comprises, *in vitro* antidiabetic activity such as  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activities and *in vivo* antidiabetic activity of methanolic extract of *Pleurotus florida*.

**Methods:** The fruiting bodies of *Pleurotus florida* were obtained from Mushroom Unit, Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram, Dindigul, Tamil Nadu, India. Sample preparation, qualitative phytochemical analysis, *in vitro* antidiabetic activities namely  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity and *in vivo* antidiabetic activity namely evaluation of alloxan induced diabetic rats were carried out following the methods reported previously.

**Results:** *In vitro* and *in vivo* antidiabetic activity of *P. florida* exhibited significant results for its  $\alpha$ -amylase (94.93 $\pm$ 1.75 % at 1000  $\mu$ g/ml) and  $\alpha$ -glucosidase inhibitory activity (84.90 $\pm$ 0.42 % at 1000  $\mu$ g/ml) in a dose-dependent manner. The extract also showed significant antidiabetic activity on *in vivo* (p<0.05) at the tested dose level (200 mg/kg b. w) this was comparable to Glibenclamide, a standard antidiabetic drug.

**Conclusion:** The presence of phytochemicals namely phenols, flavonoids, saponins, tannins and terpenoids may be responsible for such antidiabetic activity. These results reveal that *P. florida* can be used as a potential antidiabetic agent.

**Keywords:** *Pleurotus florida*, Phenols, Flavonoids, Antidiabetic activity, Antidiabetic agent

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### INTRODUCTION

Diabetes mellitus is a metabolic disorder due to absolute or relative insulin deficiency [1]. According to World Health Organization [2] about 10 % of the total population and about one out of five persons above the age of 50 suffer from this disease and it is a major cause of morbidity and mortality. The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It is predicted to be doubled by the year 2030 to about 380 million where in India, China, and the United States will have the largest number of people with diabetes [2]. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision and weight loss [3]. The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with blindness, nephropathy that may be lead to renal failure or neuropathy with risk of foot ulcers, amputation, Charcot joints and sexual dysfunction [4]. Currently, available therapy for diabetes includes insulin and various oral antidiabetic agents such as sulfonylurea, biguanide, metformin,  $\alpha$ -glucosidase inhibitors, troglitazone but these are known to have a number of serious adverse effects in patients [5-7].

Plants and fungi are the main sources of natural compounds used for medicine. They have attracted considerable interest because of their wide variety of bioactive metabolites [8]. The mushrooms are being developed as nutraceutical and nutraceutical to garner the essence of mushrooms and to make consumption easy. Further, scientific validation of traditional knowledge bears evidence of the many positive effects of consuming mushrooms fresh or processed on human health. A wide variety of bioactive compounds isolated from many species of mushrooms have been identified [9].

*Pleurotus florida*, commonly known as the white oyster mushroom is one of the most economically important edible mushrooms that grow predominantly in tropical regions and it is considered as the valuable health food with high content of protein [10]. Moreover, this mushroom has been demonstrated to possess various valuable biological properties including antioxidant [11], antimicrobial

[12], anti-inflammatory [13], antitumor [14] as well as antidiabetic activities [15-17]. Till now most studies have been focused on *in vitro* antidiabetic activities of *P. florida* concerning  $\alpha$ -amylase and  $\alpha$ -glucosidase activities. Although a wide range of model systems are available for evaluation of antidiabetic activities, the choice mainly depends on the nature of the substances under investigation. There is evidence for discrepancies in antidiabetic activities of substances when tested *in vitro* and *in vivo*. Therefore, in the present study, the methanolic extract of *P. florida* was subjected to the qualitative analysis of phytochemicals and also for its antidiabetic activities using *in vitro* studies namely,  $\alpha$ -amylase,  $\alpha$ -glucosidase, and *in vivo* antidiabetic activity of alloxan induced diabetic rats were carried. The investigation of the local species may yield mycochemicals with novel medicinal properties that can be used for the development of therapeutic agents in diabetes and for other ailments.

### MATERIALS AND METHODS

The fruiting bodies of *Pleurotus florida* were obtained from Mushroom Unit, Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram, Dindigul, Tamil Nadu, India. Sample preparation [18], qualitative phytochemical analysis [19], *in vitro* antidiabetic activities namely  $\alpha$ -amylase [20] and  $\alpha$ -glucosidase [20] inhibitory activity and *in vivo* antidiabetic activity namely evaluation of alloxan induced diabetic rats were carried out following the methods reported previously [21].

### Animal studies

Animal experiments were carried out according to the guidelines of the Committee for the purpose of control of experiments on animals and approved by the Institutional Animal Ethics Committee (Reg. No.: CPCSEA/265).

### Statistical analysis

The results were expressed as mean values and standard deviation (SD). Linear regression analysis was used to calculate IC<sub>50</sub> value.

Data were analyzed using One-Way Analysis of Variance (ANOVA) followed by Turkey's multiple comparison post hoc tests using SPSS software 16.0 versions.

Values of  $p < 0.05$  were considered as statistically significant.

## RESULTS

### Qualitative phytochemical screening

Methanolic extract of *P. florida* was qualitatively analyzed and presented in table 1. Among the various phytochemicals assessed, the presence of phenols, flavonoids, saponins, tannins and terpenoids were detected.

### In vitro antidiabetic activity

The methanolic extract of *P. florida* showed significant inhibition of  $\alpha$ -amylase ( $94.93 \pm 1.75$  % at 1.0 mg/ml) and  $\alpha$ -glucosidase ( $84.90 \pm 0.42$  % at 1.0 mg/ml) inhibitory activity in a dose-dependent manner and the concentrations required for 50 % of the above inhibition ( $IC_{50}$ ) were  $35.96 \pm 0.35$   $\mu$ g/ml and  $202.02 \pm 3.84$   $\mu$ g/ml, respectively (table 2).

### In vivo antidiabetic activity

*In vivo* antidiabetic study revealed the significant reduction of blood glucose, serum cholesterol, serum triglyceride, LDL levels and significant increase of HDL level in *P. florida* treated diabetic rats in 14 d's trials (table 3).

**Table 1: Qualitative phytochemical screening of *Pleurotus florida***

S. No.	Test for extracts	Inference
1.	Phenols	Present
2.	Flavonoids	Present
3.	Saponins	Present
4.	Tannins	Present
5.	Terpenoids	Present

**Table 2: It shows *in vitro* antidiabetic activities of *Pleurotus florida* inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity**

Sample concentration ( $\mu$ g/ml)	$\alpha$ -amylase inhibition activity (%)	$IC_{50}$ value ( $\mu$ g/ml)	$\alpha$ -glucosidase inhibition activity (%)	$IC_{50}$ value ( $\mu$ g/ml)
200	$33.88 \pm 3.18$		$28.93 \pm 1.11$	
400	$53.80 \pm 4.98$		$38.53 \pm 1.79$	
600	$64.13 \pm 0.54$	$35.96 \pm 0.35$ $\mu$ g/ml	$50.40 \pm 0.32$	$202.02 \pm 3.84$ $\mu$ g/ml
800	$82.07 \pm 3.03$		$78.80 \pm 0.09$	
1000	$94.93 \pm 1.75$		$84.90 \pm 0.42$	

Data represent the mean  $\pm$  SEM ( $n = 3$ ) ( $p < 0.05$ )

**Table 3: It shows *in vivo* antidiabetic study blood glucose, serum cholesterol, serum triglyceride, LDL and HDL levels of *Pleurotus florida* treated diabetic rats in 14 d trials**

Experimental groups	Blood glucose (mg/dl)	Serum cholesterol (mg/dl)	Serum triglyceride (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Group-I	$81.40 \pm 3.22^{**}$	$34.00 \pm 1.74^{**}$	$33.40 \pm 3.45^{**}$	$22.00 \pm 2.10^{**}$	$24.60 \pm 1.47^{**}$
Group-II	$512.00 \pm 15.29$	$84.0 \pm 4.94$	$123.00 \pm 6.63$	$58.80 \pm 3.22$	$10.20 \pm 1.12$
Group-III	$298.20 \pm 12.20^{**}$	$72.60 \pm 3.43^{**}$	$94.60 \pm 4.81^*$	$34.20 \pm 1.61^{**}$	$15.20 \pm 2.44^{**}$
Group-IV	$124.40 \pm 7.84^{**}$	$32.20 \pm 2.49^{**}$	$38.20 \pm 1.90^{**}$	$23.60 \pm 1.90^{**}$	$25.80 \pm 0.98^{**}$

## DISCUSSION

*Pleurotus florida* is a good source of extractable phytochemicals with inhibitory potentials against key enzymes namely,  $\alpha$ -amylase and  $\alpha$ -glucosidase linked to Type 2 diabetes mellitus. *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. They might provide useful information on the mechanism of action of therapeutic agents [22-24]. 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,  $\alpha$ -amylase and  $\alpha$ -glucosidase, present in the small intestinal brush border that is responsible for the breakdown of oligosaccharides; disaccharides into monosaccharides suitable for absorption [25-28]. Inhibitors of these enzymes, like acarbose, delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise [22]. Jumepeang *et al.* [29] reported that the  $\alpha$ -amylase inhibitory activity was significantly higher as compared to acarbose drug currently administered for controlling glucose levels in diabetic patients. Natural products from plants have shown lower inhibitory activity against the  $\alpha$ -amylase activity and stronger inhibitory activity against noninsulin dependent diabetes mellitus

(NIDDM) with minimal side effects. This is a positive result since as explained earlier, the excessive inhibition of  $\alpha$ -amylase results in the abnormal bacterial fermentation of undigested carbohydrates in the colon, which in turn results in abdominal digestion, flatulence, meteorism and possibly diarrhoea [30].

Alpha-amylase inhibitors are copious in fungi, higher plants, and animals [31]. These living beings create a large number of diverse protein inhibitors of  $\alpha$ -amylases in order to regulate the activity of these enzymes. Like amylase inhibitors which are also well-known as starch blockers as they avoid dietary starches from being digested and absorbed by the body. This could be helpful for treating obesity and diabetes mellitus [32]. The presence of inhibition to  $\alpha$ -glucosidase activity in the extracts of *P. florida* could be caused by the presence of carbohydrate and protein (flavanols) which are suspected to be the competitive inhibitors for the  $\alpha$ -glucosidase enzyme. This is appropriate with the substrate of  $\alpha$ -glucosidase, which is a food starch and carbohydrate (glycogen) [33]. Alpha-glucosidase inhibitors focusing on reducing the digestion of carbohydrate are the most common and efficacious agents utilized for the treatment of Type 2 diabetes [34, 35]. Since  $\alpha$ -glucosidase inhibitors prevent the hybridization of carbohydrates into glucose, a lot of carbohydrates remain in the intestine. Therefore the bacteria will digest carbohydrates, which may cause gastrointestinal side effects such as flatulence and diarrhoea [36].

In the present study, *in vitro* antidiabetic studies revealed the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase activity. The percentage inhibition at 200, 400, 600, 800 and 1000  $\mu\text{g/ml}$  concentrations of *P. florida* on  $\alpha$ -amylase and  $\alpha$ -glucosidase showed a concentration dependent reduction in percentage inhibition. Therefore, the antidiabetic effect of *P. florida* might attribute to its inhibitory effect against  $\alpha$ -amylase and  $\alpha$ -glucosidase that delaying the digestion of carbohydrate to delay the postprandial rise in blood glucose. In the *in vivo* studies, blood glucose levels were assessed from 0 and 14<sup>th</sup> days in normal rats; diabetic-induced rats; mushroom extracts treated rats and also glibenclamide-treated rats. There is a significant reduction in all antidiabetic parameters on the 14<sup>th</sup> day in the rats treated with *P. florida* extracts. In the *in vivo* studies, alloxan-induced diabetic rats showed the significant increase in the levels of blood glucose than the diabetic rats ( $p < 0.05$ ). Blood glucose level was measured in normal and diabetic rats on day 0 and 14<sup>th</sup> day of drug treatment. After treatment with both species at 200 mg/kg b. w, the blood glucose levels on day 14<sup>th</sup> were significantly reduced compared to those on day 0 ( $p < 0.01$ ). The glibenclamide-treated rats also showed the significant reduction in serum glucose level ( $p < 0.05$ ). *P. florida* and glibenclamide administration attenuated hyperglycemia, while no significant changes were observed in normal and diabetic groups ( $p > 0.05$ ).

Mushrooms have been shown to be useful in supporting healthy cholesterol levels and have been shown to improve circulation; also they have been shown to help in maintaining blood sugar balance via blood sugar lowering effects, elevation of plasma insulin levels and enhanced liver metabolism of glucose and increase cellular insulin sensitivity [37]. Hyperglycemia caused by diabetes is known to be a cause of oxidative stress that leads mainly to the enhanced production of mitochondrial ROS. Oxidative stress induced by hyperglycemia leads to the activation of stress-sensitive signaling pathways, which worsen both insulin secretion; action and promote the development of Type 2 diabetic mellitus [38-41]. Fasting hyperglycemia is a hallmark of diabetic mellitus. It has been postulated but is still debated that the fasting hyperglycemia in noninsulin-dependent diabetic mellitus arises from the hepatic overproduction of glucose [42].

Wi et al. [43] suggested that the post-absorptive hyperglycemia in Streptozotocin-diabetic rats is largely due to decreasing peripheral glucose clearance, while increased hepatic glucose output might also be a contributing factor at a very high Streptozotocin dose. Krishna et al. [44] stated that polysaccharide extracted from *P. citrinopileatus* showed blood glucose lowering effect in rats. These findings suggest that mushrooms are promising antidiabetic nutraceuticals, but there is the lack of enough clinical evidence. Khan et al. [45] have reported that oral administration of *P. ostreatus* given to rat's leads to blood glucose lowering effect in both insulin-dependent and insulin-independent diabetic conditions. Prabu and Kumuthakalavalli [46] experimentally found that methanolic extract of *C. indica* exhibited antidiabetic activity by lowering the levels of blood glucose, serum cholesterol, serum triglyceride, LDL levels and significant increase of HDL level. Antidiabetic effects of ethanolic extract of *P. ostreatus* on alloxan induced diabetic rats was extensively studied and reported as an effective antidiabetic regimen [47].

## CONCLUSION

The methanolic extract of *P. florida* with its significant antidiabetic activity in rats, suggests its therapeutic potential for the prevention and control of diabetics; moreover, the mushroom species can be used as an easily accessible source of natural antidiabetic and as a possible food supplement or in the pharmaceutical industry. However, more intensive and extensive investigations are needed to exploit their valuable therapeutic potentials and the chemical characteristics of the antidiabetic components in the extracts should be further investigated.

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## CONFLICT OF INTERESTS

Declare none

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