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ASSESSMENT OF *IN-VITRO* PANCREATIC LIPASE INHIBITORY ACTIVITY OF METHANOLIC EXTRACTS OF *PLEUROTUS ERYNGII*

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ABSTRACT

Current research investigation was conducted with the main purpose to evaluate for the in-vitro pancreatic lipase inhibitory activity of methanolic extract of Pleurotus eryngii. In-vitro pancreatic lipase inhibitory assay was carried out to determine IC_{50} values of methanolic extracts of Pleurotus eryngii. The results revealed that the methanol extract of Pleurotus eryngii possess in-vitro pancreatic lipase inhibitory activities at the concentration of 1-50 µg/ml. In conclusion, methanolic extract of Pleurotus eryngii has been reported to possess the in-vitro pancreatic lipase inhibitory activities. Hence, further in-vivo studies could be recommended to access the safety and efficacy of methanolic extract of Pleurotus eryngii to exploit it for biopharmaceutical and therapeutic purposes.

KEYWORDS: *Pancreatic Lipase, Pleurotus Eryngii, Methanol Extract, Antiobesity*

INTRODUCTION

Currently with the improvement of better innovations and more noteworthy acknowledgment of their supplement values, mushrooms have involved a significant spot in food in a few places of the world.[1] Investigates on the nutritive worth of consumable mushrooms demonstrate that they might be viewed as quality food sources, despite the fact that they are insufficient in calories and fat and comprise of around 90% water.[2] Mushrooms have been accounted for to be of remedial worth, helpful in forestalling infections like hypertension, hypercholesterolemia, malignant growth and furthermore having antibacterial and antiviral properties. These useful qualities are primarily because of their substance composition.[3,4] Research investigation uncovered that of Shitake mushrooms, for example, *Lentinus edode*, *Grifola froundosa*, *Agaricus bisporus* and shellfish mushrooms act as normal stores of Vitamin-B like niacin, flavin and pyridoxine,[5] and natural acids, for example, the glucons, monoterpenoids, and diterpenoids, lipids, proteins, for example, hydrophobins and minor components, for example, selenium.[6,7]

Obesity results from a lopsidedness including extreme calorie utilization or potentially deficient actual work. It is a perplexing medical problem including an assortment of elements *viz.* digestion, conduct, climate, hereditary qualities, and so forth... The pervasiveness of obesity is developing at an awful rate. The number of inhabitants in overall obesity in 2011 has been dramatically increased when contrasted with the populace in 1980.[8] Stoutness is viewed as a significant gamble factor contributing such a large number of ongoing illnesses, like type-2 diabetes, cardiovascular infections and certain malignant growths. Consequently, successful approaches to forestalling and it are expected to treat obesity.

Pancreatic lipase assumes a significant part in the processing of dietary fat. It hydrolyzes and changes over dietary triglycerols into monoglycerides and free unsaturated fats. Orlistat, a hydrogenated subordinate of lipstatin got from *Streptomyces toxitricini*, is a powerful inhibitor of gastric, pancreatic and carboxyl ester lipase and has ended up being compelling for the treatment of human obesity. Sibutramine (a monoamine reuptake inhibitor) and rimonabant (an endocannabinoid receptor blocker) are the other pancreatic lipase inhibitors utilized in the therapy of human obesity.[9] Nonetheless, obese and specially overweighed populace is hesitant to accept weight as a clinical issue, and in this manner prior to going to a health professionals, begins his ⁄ her own treatment by utilizing extraordinary food varieties, like diminished fat substance (light) items and nutritional supplements (including herbal extracts) and more often, diets without scientific evidence.

Accordingly, food sources containing dynamic standards with clear metabolic targets and scientific proof of their action might help in oneself battle against weight, coming to a larger number of people and in their very own previous phase corpulence. A wide scope of normal items (including crude concentrates) principally acquired from plants have been accounted for as viable pancreatic lipase inhibitors. For example, berry polyphenols,[10] triterpenes from Sapindus sp.[11], monoterpenes from Monarda punctata[12], abietanes from Salvia sp.[13] and in excess of 70 plant extracts[14] showed pancreatic lipase inhibitory action. The list of mixtures and sources could be additionally reached out with the discoveries of Birari and Bhutani, Slanc et al, Mahomoodally and Ramcharun, Irondi et al.[15-18]

A couple of fascinating pancreatic lipase inhibitors were secluded from edible fungi, two of them were β-lactones with strange arrangements named percyquinnin (acquired from *Stereum complicatum*) and vibralactone (*Boreostereum vibrans*) with comparative IC_{50} (0.4lg mL^{-1}).[15,19] For a couple of mushroom species, the noticed exercises were additionally compelling *in-vivo* as indicated by the outcomes acquired with animal models. Ahn et al announced the counter corpulence impacts of *Isaria sinclairii* fruiting bodies,[20] and Mizutani et al exhibited the pancreatic lipase inhibitory activity of water separates (polysaccharide-rich part) acquired from *Pleurotus eryngii* fruiting bodies.[21] Notwithstanding, the majority of the previous outcomes were gotten from biochemical tests and no further examinations to assess them under gut conditions were done. Positive scientific outcomes in impasse might be deceive and create abuse. For example, a published scientific evidence of lipase inhibitory activity in some crude food item or a spice doesn't imply that will affect fat ingestion yet can be deciphered that way, and wrongly utilized for that reason.

Hence, in the present study the methanolic extractedible oyster mushroom*viz. Hypsizygus Pleurotus eryngii*was subjected to analysis of *in-vitro* pancreatic lipase inhibitory activities in order to exploit it for biopharmaceutical and therapeutic purposes.

MATERIALS AND METHODS

Plant Material and Sample Preparation

The edible oyster mushroom *viz. Pleurotus eryngii* was purchased from local market and500 g of the harvested mushroom sample was washed to remove the surface pollutants, dried at 40ºC until complete dry and powdered. These samples were subjected for the successive extraction with methanol.

Extraction Procedure

The mushroom sample was subjected to methanolic extraction. 25 g of powdered sample was filled in a Whatmann filter paper and kept inside tumble. 200 ml of the solvent was added in tumble. The tumble was fit into a round bottom flask containing 700 ml of the solvent and run for 6-8 hours at the temperature based on the boiling point of the respective solvent using soxhlet apparatus. Later the extract was subjected for the distillation for 2-3 hours. These extracts were kept in water bath at 40ºC for drying. The dried extracts thus obtained were used for GC-MS analysis.[23]

IN-VITRO PANCREATIC LIPASE INHIBITORY ASSAY

Chemical and Reagents

The chemical and reagents used were PNPB (para-nitrophenylbutyrate), porcine pancreatic lipase (Advanced Enzymes, Mumbai), Sodium dihydrogen phosphate (SD Fine Chemicals, Mumbai), Disodium hydrogen phosphate (SD Fine Chemicals), Sodium Chloride (SD Fine Chemicals), Triton-X-100 (Sigma Aldrich, USA), acetonitrile (Sigma Aldrich, USA), Orlistat (Biocon, Bangalore, India). All the chemical and reagents used were of analytical grade (AR).

Sample Preparation

Sample solutions were prepared by dissolving the dried extracts with 0.1 M buffer solution and stored at -20°C in the dark until further analysis (100µl).

Enzyme Preparation

Porcine pancreatic lipase enzyme solution was prepared by dissolving 6 mg of the enzyme in 10ml of buffer solution by gentle vertexing. It was prepared immediately before use.

Assay Procedure

Total assay volume was 200 µl. Substrate used was p-Nitrophenylbutyrate (PNPB). PNPB working solution was prepared with 8.403 µl of PNPB stock solution in a vial and volume was made up to 10 ml by acetonitrile solution. Solution of the standard drug was prepared by dissolving one capsule content of Orlistat in 12ml of DMSO (dimethylsulphoxide). Test sample solutions were prepared as mentioned previously. Test sample solution or Standard (25µl) was incubated with 50µl of enzyme solution, 100µl of buffer solution and 25µl of PNPB solution for 30 minutes at 37˚C. Lipase activity was determined by measuring the hydrolysis of PNPB to p-nitrophenol at 400 nm using an ELISA plate reader (Bioteck).

% Inhibitory activity was calculated using the following formula: % Inhibition= Absorbance of blank- Absorbance of test/ Absorbance of blank × 100

RESULTS

The results of *in-vitro* pancreatic lipase inhibitory activity of methanol extract of *Pleurotus eryngii* and orlistat was represented in Table 1. These findings depicted that that methanol extract of *Pleurotus eryngii* had the pancreatic lipase-inhibitory activities and it was dose dependent manner. Furthermore, results revealed that the *in-vitro* pancreatic lipase inhibitory effects of methanol extract of *Pleurotus eryngii* was comparable to that of standard lipase inhibitory drug *i.e.* orlistat at all the concentrations tested.

Table 1: In-Vitro Lipase Inhibition Activities of Methanol Extract of *Pleurotus Eryngii*

Methanol Extracts	Concentration (µg/ml)	Pancreatic Lipase Activity Inhibition (%)
Pleurotus eryngii	1.00	40.10
	5.00	45.30
	10.00	56.80
	50.00	72.60
Orlistat	1.00	41.80
	5.00	47.50
	10.00	70.10
	50.00	78.50

DISCUSSION

The principal mixtures of methanolic concentrate of *Pleurotus eryngii* which have been accounted for to have pharmacological possibilities *viz.* antiobesity, antimicrobial, antihyperglycemic, antioxidant, anti-inflammatory and anti-carcinogenic in the literature are conhydrin, diethyl phthalate, and phthalic corrosive butyl hex-3-yl ester (alkaloids),23 ar-turmerone (sesquiterpenoid)[24], palmitic corrosive, myristic corrosive, phenol, and benzoic acid.[25] In the current study, results of *in-vitro* pancreatic lipase inhibitory activity of methanol extricate *Pleurotus eryngii* uncovered that the methanol concentrate of *Pleurotus eryngii* have the pancreatic lipase-inhibitory property.

In an research work did by Namba et al showed that water concentrate of *Pleurotus eryngii* diminishes pancreatic lipids.[26] In one more research study led by Mizutani et al announced that *Grifola frondosa* restrains pancreatic lipase by repressing hydrolysis of 4-methylumbelliferyl (4-MUO) and trioleoylglycerol emulsified with lecithin.[21] Besides, Mizutani et al in another investigation researched the mechanism of action of lipase activity of *Pleurotus eryngii* extract *in-vitro* and its hypolipidemic property in fat-loaded mice. The outcomes portrayed that *Pleurotus eryngii* extract stifled the rises of plasma and chylomicron triacylglycerol levels and hindered pancreatic lipase at concentration of 50-300 µg/mL, showing the hypolipidemic impact of *Pleurotus eryngii* extricate was owed to pancreatic lipase hindrance bringing about low-retention of fat. Subsequently, it was hypothesized that the conceivable mechanism of action that enhance obesity would be ascribed to pancreatic lipase hindrance activity of *Pleurotus eryngii* extract.[21] In a research study revealed by Chen et al the refined *Pleurotus eryngii* polysaccharide played out serious areas of strength for an of restraining lipid aggregation in froth cells, coming about in just around 28.06% of lipid content left inside the cell contrasted with 100 percent in the control.[27]

The *in-vitro* pancreatic lipase inhibitory activity of methanolic extract of *Pleurotus eryngii* could be attributed to the prevailing compounds *i.e.*conhydrin, diethyl phthalate, phthalic acid-butyl hex-3-yl ester (alkaloids), ar-turmerone (sesquiterpenoid), palmitic acid, myristic acid, phenol, and benzoic acid.

CONCLUSION

In conclusion, methanolic extract of *Pleurotus eryngii* reported to possess *in-vitro* pancreatic lipase inhibitory activities. Hence, further *in-vivo* studies could be recommended to access the safety and efficacy of methanolic extract of *Pleurotus eryngii* to exploit it for biopharmaceutical and therapeutic purposes.

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