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GC-MS analysis of bioactive components and evaluation of In-vitro pancreatic lipase inhibitory activity of aqueous extracts of *Pleurotus eryngii*

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Abstract
Present study was designed to conduct with main purpose to determine bioactive components and evaluation of aqueous extract of *Pleurotus eryngii* for *in-vitro* pancreatic lipase inhibitory activity. GC-MS analysis was carried out to determine the bioactive components and *in-vitro* pancreatic lipase inhibitory assay was carried out to determine IC_{50} values of aqueous extracts of *Pleurotus eryngii*. The results of the present study depicted that the aqueous extracts of *Pleurotus eryngii* possess *in-vitro* pancreatic lipase inhibitory activities at the concentration of 1-30 µg/ml and this could be attributed to the prevailing compounds identified in the GC-MS analysis *i.e.,* conhydrin, diethyl phthalate, phthalic acid-butyl hex-3-yl ester (alkaloids), ar-turmerone (sesquiterpenoid), palmitic acid, myristic acid, phenol and benzoic acid from ethanolic extract of *Pleurotus eryngii*. In conclusion, polyphenols, alkaloids terpenoids and Vitamin B class of secondary metabolites majorly identified in GC-MS analysis of aqueous extract of *Pleurotus eryngii* has been reported to possess the *in-vitro* pancreatic lipase inhibitory activities. Hence, further *in-vivo* studies in experimentally induced obese animal models could be recommended to access the safety and efficacy of aqueous extracts of *Pleurotus eryngii* to strongly recommend them as natural antiobesity agents in the formulations of natural antiobesity drugs.

Keywords: Anti-obesity, *Pleurotus eryngii*, Aqueous extracts, GC-MS, Pancreatic Lipase

Introduction
Today, with the improvement of better innovations and more noteworthy acknowledgment of their supplement esteems, mushrooms have involved a significant spot in food in a few pieces of the world [1]. Explores on the nutritive estimation of palatable mushrooms show that they might be viewed as sound nourishments, despite the fact that they are insufficient in calories and fat and comprise of about 90% water [2-4]. Mushrooms have been accounted for to be of remedial worth, valuable in forestalling illnesses like hypertension, hypercholesterolemia, malignant growth and furthermore having antibacterial and antiviral properties. These useful attributes are predominantly because of their substance organization [5-7]. Scientific experiments uncovered that of Shitake mushrooms, for example, *Lentinus edode*, *Grifola froundosa*, *Agaricus bisporus* and clam mushrooms fill in as characteristic vaults of Nutrient B like niacin, flavin and pyridoxine [8] and natural acids, for example, the glucons, monoterpenoids and diterpenoids, lipids, proteins, for example, hydrophobins and minor components, for example, selenium [9,10].
Obesity results from an unevenness including inordinate calorie utilization as well as deficient actual work. It is a perplexing medical problem including an assortment of components *viz.* digestion, conduct, climate, hereditary qualities and so on. The commonness of corpulence is developing at a frightful rate. The number of inhabitants in overall stoutness in 2011 has been dramatically increased when contrasted with the populace in 1980 [11]. Corpulence is viewed as a significant danger factor contributing an excessive number of persistent sicknesses, for example, type-2 diabetes, cardiovascular illnesses and certain malignancies. In this manner, compelling methods of forestalling and treating heftiness are required.
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 subsidiary of lipstatin got from *Streptomyces toxitricini*, is an intense inhibitor of gastric, pancreatic and carboxyl ester lipase and has end up being powerful for the therapy of human stoutness. Sibutramine (a monoamine reuptake inhibitor) and rimonabant (an endocannabinoid receptor blocker) are the other pancreatic lipase inhibitors utilized in the treatment of human heftiness [12]. Nonetheless, fat and uncommonly overweighed populace is hesitant to expect weight as a clinical issue and subsequently prior to going to a wellbeing proficient, begins his/her own treatment by utilizing extraordinary food sources, like diminished fat substance (light) items and healthful enhancements (counting natural concentrates) and all the more regularly, consumes less calories without scientific evidences.

Hence, food sources containing dynamic standards with clear metabolic targets and scientific evidence of their action may help in oneself battle against corpulence, coming to a higher number of people and in their very own previous phase of obesity. A wide scope of common items (counting unrefined concentrates) primarily got from plants have been accounted for as viable pancreatic lipase inhibitors. For example, berry polyphenols [13], triterpenes from *Sapindus sp* [14], monoterpenes from *Monarda punctata* [15], abietanes from *Salvia sp*. [16] and in excess of 70 plant separates [17] showed pancreatic lipase inhibitory action. The list of compounds and sources could be additionally stretched out with the discoveries of Birari and Bhutani, Slanc *et al*., Mahomoodally and Ramcharun, Irondi *et al*. [18-21], The last distribution pointed growths as a possible new wellspring of pancreatic lipase inhibitors since inside 60 consumables and non-eatable organisms species, pancreatic lipase inhibitory exercises were discovered going from 1% till 97% relying upon the species considered.

A couple of intriguing pancreatic lipase inhibitors were disengaged from consumable organisms, two of them were β-lactones with uncommon designs named percyquinnin (acquired from *Stereum complicatum*) and vibralactone (*Boreostereum vibrans*) with comparative IC_{50} (0.41 g/ml) [18,22]. For a couple of mushroom animal groups, the noticed exercises were additionally powerful *in-vivo* as indicated by the outcomes acquired with creature models. Ahn *et al*. revealed the counter weight impacts of Isaria sinclairii fruiting bodies [23] and Mizutani *et al*. exhibited the pancreatic lipase inhibitory action of water removes (polysaccharide-rich part) got from *Pleurotus eryngii* fruiting bodies [24]. Nonetheless, the vast majority of the previous outcomes were got from biochemical tests and no further examinations to assess them under gut conditions were completed. Positive scientific outcomes in impasse might be delude and produce abuse. For example, a published scientific proof of lipase inhibitory activity in some crude foodstuff or a spice doesn't imply that will have impact on fat retention yet can be deciphered that way and wrongly utilized for that reason.

Hence, in the present study the aqueous extract edible oyster mushrooms *viz. Pleurotus eryngii* was subjected GC-MS analysis for the determination of bioactive components and further evaluation for *in-vitro* pancreatic lipase inhibitory activities of aqueous extract of *Pleurotus eryngii* was carried out.

Materials and Methods

Plant material and extraction procedure

In-house eco-friendly cultivated edible oyster mushroom viz. *Pleurotus eryngii* was subjected aqueous extraction according to method described by Jose *et al*. 500 g of the harvested mushroom sample was washed to remove the surface pollutants, dried at 40 ºC until complete dry and powdered. These samples were subjected for the successive extraction with water. 25 g of powdered sample was filled in a Whatmann filter paper and kept inside tumble. 200 ml of the water 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 hot air over at 60 ºC for drying. The dried extracts thus obtained were used for analysis of GC-MS and *in-vitro* pancreatic lipase inhibitory activity [25].

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Sample preparation

Sample was grinded with GC grade methanol, centrifuged and the supernatant was collected and injected into the GC-MS system.

GC-MS instrument setup details

GC-MS analysis was performed using an Agilent make 5977B GC/MSD System. GC/MS system equipped with an TG 5MS silica Capillary column (30m×0.25mm ID) × MDF composed of 5% diphenyl/95% dimethyl polysiloxane with 0.25 µm film thickness. For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. The oven temperature was programmed from 80 ^{0}C with a hold of 2 mins and then 200 ^{0}C at 9 ^{0}C/min and a hold for 4 min and then to 300^{0}C at 10 ^{0}C/min and a hold for 5 min. Helium was used as carrier gas at flow at the flow rate of 1.5 ml/min. The injector temperature was 250 ^{0}C, injection size 1.0 µl needle with spitless mode. Injector temperature was 250 ^{0}C and ion source temperature was 230 ^{0}C. The interface and MS ion source were maintained at 300 ^{0}C and 230 ^{0}C, respectively. Mass spectra were taken at 70 eV; a scan interval of 0.2 seconds and fragments from with a mass scan range of 50-550 amu. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Data handling was done using Xcaliber software. The identification of compounds was based on comparison of their mass spectra with those of NIST Libraries. Software adopted to NIST 2014 (2.2.0.0) with AMDIS v.2.72 Version.

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 (SRL, Mumbai), Disodium hydrogen phosphate (SRL, Mumbai), Sodium Chloride (SRL, Mumbai), 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 0.1M buffer solution and stored at -20 °C in the dark until further analysis (100 µl).

Enzyme preparation

Porcine pancreatic lipase enzyme solution was prepared by dissolving 6 mg of the enzyme in 10 ml 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 12 ml of DMSO (dimethylsulphoxide). Test sample solutions were prepared as mentioned previously. Test sample solution or Standard (25 µl) was incubated with 50 µl of enzyme solution, 100 µl of buffer solution and 25 µl 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 and discussion

GC-MS analyses of the aqueous extract of *Pleurotus eryngii* led to the identification of 27 components (Figure 1). The 27 peaks identified account for 100% of the extract and listed along with respective retention time and the percentage of compound in the extract in Table 1.

Table 1: Chemical composition of aqueous extract of *Pleurotus eryngii*

Peak No.	RT	Area (%)	Name of Compound
1	7.37	0.06	Diacetone
2	7.65	1.14	Dimethyl Sulfoxide
3	11.58	0.16	Benzene 1,3,5-tris (methoxymethyl)
4	12.23	0.08	Dichlorobenzene
5	12.29	0.24	Phenol
6	13.27	93.98	N-Methyl-α-pyrrolidone
7	14.66	0.13	Creatine
8	15.60	0.51	Triacetonamine
9	16.91	0.26	Benzoic acid
10	19.32	0.35	Conhydrin
11	22.93	0.25	Ile-Val-Arg
12	23.18	0.13	Fluoroacetic acid, dodecyl ester
13	24.54	0.27	L-Glutamine
14	26.28	0.13	2,4-Di-tert-butylphenol
15	27.97	0.04	2-Ethylquinoline
16	28.15	0.07	9-Hexadecenol, E
17	28.31	0.06	Diethyl Phthalate
18	29.96	0.05	aR-Turmerone
19	31.93	0.08	Myristic acid
20	32.62	0.04	10-Heneicosene
21	35.73	0.08	Methtryptoline
22	35.78	0.10	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate
23	36.04	0.16	Palmitic acid
24	36.15	0.14	Phthalic acid, butyl hex-3-yl ester
25	36.99	0.13	Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro
26	39.27	0.26	Grape seed oil
27	45.38	1.25	Unknown

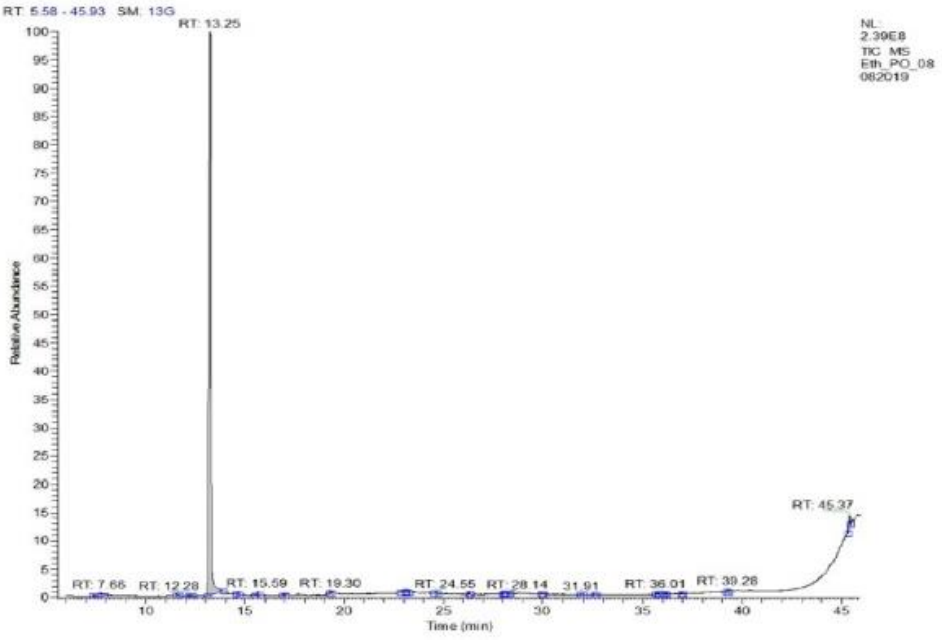

Fig 1: GC-MS spectra of aqueous extract of *Pleurotus eryngii*

The *in-vitro* pancreatic lipase inhibitory activity of aqueous extract of *Pleurotus eryngii* and Orlistat at the conc. of 1, 3, 10 and 30 µg/ml was found to be 36.40, 41.45, 53.08, & 69.06 and for Orlistat pancreatic lipase inhibitory activity was 37.18, 43.55, 66.08 & 74.53 respectively (Table 2). These findings depicted that aqueous extract of *Pleurotus eryngii* had the pancreatic lipase-inhibitory activities. The *in-vitro* pancreatic lipase inhibitory effects of aqueous extract of *Pleurotus eryngii* was comparable to that of standard lipase inhibitory drug *i.e.*, Orlistat at all the concentrations tested.

Table 2: In-vitro lipase inhibition activities of aqueous extract of *Pleurotus eryngii*

Aqueous Extracts	Concentration (µg/ml)	Pancreatic Lipase Activity Inhibition (%)
Pleurotus eryngii	1.00	36.40
	3.00	41.45
	10.00	53.08
	30.00	69.06
Orlistat	1.00	37.18
	3.00	43.55
	10.00	66.08
	30.00	74.53

The GC-MS examinations of the aqueous extract of *Pleurotus eryngii* prompted the distinguishing proof of 27 compounds. The 27 peaks recognized record for 100% of the extract. The predominant mixtures of aqueous extract of *Pleurotus eryngii* which have been accounted for to have pharmacological potentials *viz.* antiobesity, antimicrobial, antihyperglycemic, cancer prevention agent, calming and hostile to cancer-causing in the writing are conhydrin, diethyl phthalate, and phthalic corrosive butyl hex-3-yl ester (alkaloids) [26], ar-turmerone (sesquiterpenoid) [27], palmitic corrosive, myristic corrosive, phenol and benzoic corrosive [28].

In the current research investigation, findings of *in-vitro* pancreatic lipase inhibitory action of aqueous extract of *Pleurotus eryngii* uncovered that the aqueous extract of *Pleurotus eryngii* have the pancreatic lipase-inhibitory property. The aqueous extract of *Pleurotus eryngii* was found exceptionally compelling in repressing pancreatic lipase action *in-vitro* at a generally low concentration for example at a centralization of 1 µg/ml aqueous concentrate of *Pleurotus eryngii* inhibited 35.1% pancreatic lipase activity. Furthermore, the *in-vitro* lipase inhibition activity of *Pleurotus eryngii* was equivalent to that of standard Orlistat lipase inhibitory medication. These discoveries were comparable with recently revealed research examinations in the literature.

Namba *et al*. showed that aqueous extract of *Pleurotus eryngii* diminishes pancreatic lipids [29]. In another exploration study directed by Mizutani *et al*. reported that *Grifola frondosa* inhibits the pancreatic lipase by repressing hydrolysis of 4-methylumbelliferyl (4-MUO) and trioleoylglycerol emulsified with lecithin [24]. Besides, Mizutani *et al*. in another study investigated the mechanism underlying anti-lipase activity of *Pleurotus eryngii* extract *in-vitro* and its hypolipidemic property in fat-loaded mice. The results demonstrated that *Pleurotus eryngii* extract suppressed the elevations of plasma and chylomicron triacylglycerol levels and inhibited pancreatic lipase at concentrations of 50-300 µg/ml, indicating the hypolipidemic effect of *Pleurotus eryngii* extract was owed to pancreatic lipase inhibition resulting in low-absorption of fat. Hence, it was postulated that the possible mechanism of action that ameliorate obesity would be attributed to pancreatic lipase inhibition activities of *Pleurotus eryngii* extract [24]. In a research investigation detailed by Chen *et al*. the purified *Pleurotus eryngii* polysaccharide played out a solid capacity of inhibiting lipid aggregation in froth cells, bringing about just about 28.06% of lipid content left inside the cells when compared with 100% in the control [30]. Nonetheless, as opposed to our discoveries of lipase inhibitory activities of aqueous extract of and *Pleurotus eryngii*, Taniguchi *et al*. reported that shitake mushroom didn't show any impact on pancreatic lipase activity *in-vitro* [31].

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

Conclusion

Polyphenols, alkaloids terpenoids and Vitamin B class of secondary metabolites mainly recognized in GC-MS investigation of aqueous extract of *Pleurotus eryngii* has been accounted for to have the *in-vitro* pancreatic lipase inhibitory activities. Henceforth, further *in-vivo* research investigations in experimentally induced obese animal models could be recommended to evaluate the safety and efficacy of aqueous extracts of *Pleurotus eryngii* to strongly recommend them as natural anti-obesity agents in the formulations of natural antiobesity drugs.

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