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Determination of Bioactive Compounds in Edible Mushroom *Pleurotus eryngii*

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ABSTRACT
The current study designed to conduct with the main purpose to determine bioactive components of methanolic extract of edible oyster mushroom *viz. Pleurotus eryngii* using GC-MS technique. *P. eryngii* samples was purchased from local market, and subjected to methanolic extraction using Soxhlet extraction method. The results of GC-MS analysis delineated that the following prevailing bioactive compounds identified in the methanolic extract of *P. eryngii* i.e., conhydrin, diethyl phthalate, phthalic acid-butyl hex-3-yl ester (alkaloids), ar-turmerone (sesquiterpenoid), palmitic acid, myristic acid, phenol, and benzoic acid. In conclusion, polyphenols, alkaloids, terpenoids and Vitamin B class of secondary metabolites are the majorly identified in GC-MS analysis of methanolic extracts of *P. eryngii*. Therefore, methanolic extract of *P. eryngii* could be exploited for biopharmaceutical and therapeutic applications.
Keywords: Methanolic extracts. *P. eryngii*, Therapeutic, Biopharmaceutical applications

1. Introduction

Due to recent advancements in technologies and greater realization of mushroom nutrient values, mushrooms have occupied an important place in food in several parts of the world [1]. Researches on the nutritive value of edible mushrooms indicate that they may be regarded as healthy foods, even though they are deficient in calories & fat and consist of about 90% water [2-4]. Mushrooms have been reported to be of therapeutic value, useful in preventing diseases such as hypertension, hypercholesterolemia, cancer and also having antibacterial and antiviral properties. These functional characteristics are mainly due to their chemical composition.[5-7] Scientific experiments revealed that of Shitake mushrooms such as *Lentinus edode, Grifola froundosa, Agaricus bisporus* and oyster mushrooms serve as natural repositories of Vitamin-B such as niacin, flavin and pyridoxine,[8] and organic acids such as the glucons, monoterpenoids, and diterpenoids, lipids, proteins such as hydrophobins and trace elements such as selenium [9,10].

Obesity results from an imbalance involving excessive calorie consumption and/or inadequate physical activity. It is a complex health issue involving a variety of factors *viz.* metabolism, behavior, environment, genetics, etc... The prevalence of obesity is growing at a dreadful rate. The population of worldwide obesity in 2011 has been more than doubled as compared to the population in 1980 [11]. Obesity is considered to be a major risk factor contributing too many chronic diseases, such as type-2 diabetes, cardiovascular diseases and certain cancers. Thus, effective ways of preventing and treating obesity are required.

Pancreatic lipase plays an important role in the digestion of dietary fat. It hydrolyzes and converts dietary triglycerols into monoglycerides and free fatty acids. Orlistat, a hydrogenated derivative of lipstatin derived from *Streptomyces toxitricini*, is a potent inhibitor of gastric, pancreatic and carboxyl ester lipase and has proved to be effective for the treatment of human obesity. Sibutramine (a monoamine reuptake inhibitor) and rimonabant (an endocannabinoid receptor blocker) are the other pancreatic lipase inhibitors used in the treatment of human obesity [12]. However, obese and specially overweighed population is reluctant to assume obesity as a medical problem, and therefore before turning to a health professional, starts his/her own therapy by using special foods, such as reduced fat content (light) products and nutritional supplements (including herbal extracts) and more often, diets without scientific evidence. Therefore, foods containing active principles with clear metabolic targets and scientific evidence of their activity may help in the self-fight against obesity, reaching to

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a higher number of individuals and in an earlier stage of their own obesity. A wide range of natural products (including crude extracts) mainly obtained from plants have been reported as effective pancreatic lipase inhibitors [13-21].

Only a few interesting pancreatic lipase inhibitors were isolated from edible fungi, two of them were β-lactones with unusual configurations named percyquinnin (obtained from *Stereum complicatum*) and vibralactone (*Boreostereum vibrans*) with similar IC_{50} (0.4 lg mL^{-1}) [18,22]. For a few mushroom species, the observed activities were also effective *in-vivo* according to the results obtained with animal models. Ahn et al reported the anti-obesity effects of *Isaria sinclairii* fruiting bodies [23], and Mizutani et al demonstrated the pancreatic lipase inhibitory activity of water extracts (polysaccharide-rich fraction) obtained from *Pleurotus eryngii* fruiting bodies [24]. Hence, in the present study the methanolic extracts edible oyster mushroom *viz. Pleurotus eryngii* was subjected to GC-MS analysis for the determination of bioactive components.

2. Materials and Methods

2.1 Plant Material and sample preparation

The edible oyster mushroom *viz. Pleurotus eryngii* was purchased from local market and 500 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.

2.2 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 [25].

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

2.3.1 Sample preparation

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

2.3.2 GC-MS instrument setup details

GC-MS analysis was performed using a Shimadzu GC – MS - QP 2010 gas chromatograph system. Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) 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 80ºC with a hold of 2 mins and then 200ºC at 9ºC/min and a hold for 4 min and then to 300ºC at 10 ºC/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 250ºC, injection size 1.0 µL neat, with spitless mode. Injector temperature was 250ºC and ion source temperature was 230ºC. The interface and MS ion source were maintained at 300ºC and 230ºC, 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.

3. Results

GC-MS analyses of the methanolic extract of *Pleurotus eryngii* led to the identification of 27 components (Figure 2). The 20 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.

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TABLE 1: Chemical composition of methanolic extract of *Pleurotus eryngii*

Peak No.	RT	Area (%)	Name of Compound
1	7.99	1.36	Dimethyl Sulfoxide
2	11.9	0.35	Benzene, 1,3,5-tris(methoxymethyl)
3	12.61	0.43	Phenol
4	13.58	94.18	N-Methyl-α-pyrrolidone
5	15.92	0.71	Triacetonamine
6	17.26	0.46	Benzoic acid
7	19.63	0.54	Conhydrin
8	23.24	0.47	Ile-Val-Arg
9	23.52	0.35	Fluoroacetic acid, dodecyl ester
10	24.88	0.5	L-Glutamine
11	26.62	0.33	2,4-Di-tert-butylphenol
12	28.63	0.26	Diethyl Phthalate
13	30.28	0.25	aR-Turmerone
14	32.24	0.28	Myristic acid
15	32.93	0.24	10-Heneicosene (c,t)
16	36.04	0.28	Methtryptoline
17	36.1	0.3	Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate
18	36.34	0.38	Palmitic acid
19	36.47	0.34	Phthalic acid, butyl hex-3-yl ester
20	37.31	0.33	Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro

4. Discussion

The GC-MS results of our study revealed that ethanolic extract of *Pleurotus eryngii* led to the identification of 20 components, and it account for 100% of the extract. The prevailing compounds of ethanolic extract of *Pleurotus eryngii* which have been reported to possess pharmacological potentials *viz.* antiobesity, antimicrobial, antihyperglycemic, antioxidant, anti-inflammatory and anti-carcinogenic in the literature are conhydrin, diethyl phthalate, and phthalic acid-butyl hex-3-yl ester (alkaloids) [26], ar-turmerone (sesquiterpenoid) [27], palmitic acid, myristic acid, phenol, and benzoic acid [28].

Namba et al demonstrated that water extract of *Pleurotus eryngii* reduces pancreatic lipids [29]. In another research study conducted by Mizutani et al reported that *Grifola frondosa* inhibits pancreatic lipase by inhibiting hydrolysis of 4-methylumbelliferyl (4-MUO) and trioleoylglycerol emulsified with lecithin [24]. Furthermore, 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 study reported by Chen et al the purified *Pleurotus eryngii* polysaccharide performed a strong ability of inhibiting lipid accumulation in foam cells, resulting in only about 28.06% of lipid content left inside the cell compared to 100% in the control [30].

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The *in-vitro* pancreatic lipase inhibitory activity of methanolic extracts of *Pleurotus eryngii* 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 methanol extract of *Pleurotus eryngii*.

CONCLUSION

Polyphenols, alkaloids terpenoids, and Vitamin B class of secondary metabolites majorly identified in GC-MS analysis of methanolic extract of *Pleurotus eryngii* have been reported to possess the *in-vitro* pancreatic lipase inhibitory activities in the literature. Hence, *in-vivo* studies could be recommended to evaluate pancreatic lipase of activities of methanolic extract of *Pleurotus eryngii* could exploited for biopharmaceutical and therapeutic applications.

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