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Lentinus squarrosulus (Mont.) mushroom: Molecular identification, *in vitro* anti-diabetic, anti-obesity and cytotoxicity assessment

SHORT TITLE: Inhibitory potential of Lentinus squarrosulus

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Abstract
Introduction: Mushrooms are fungi with nutritional and health benefits. *Lentinus squarrosulus* Mont. an edible fungus has traditional utilization and relevance in local therapy for the management of metabolic diseases. In that view, the present study evaluated the *in vitro* anti-obesity, anti-diabetic and cytotoxic potential of the chloroform/methanol extract (CME) and aqueous extract (AE) of the mushroom.
Methods: *Lentinus squarrosulus* was identified using molecular biology tools. The CME and AE obtained sequentially were subjected to alpha-amylase, alpha-glucosidase and lipase inhibitory enzyme assays, as well as total phenolic content (TPC) and total flavonoid content (TFC) estimations. The cytotoxic potential of *L. squarrosulus* extract fractions were assessed using the brine shrimp lethality assay.
Results: The molecular identification of the mushroom showed that the internal transcribed spacer (ITS) sequence was an equivalent match to that of *L. squarrosulus* with a high percentage similarity, and thus assigned a unique accession number (KT120043.1). The CME of *L. squarrosulus* had higher TPC, TFC and alpha-glucosidase inhibitory activity than AE. Furthermore, AE of the mushroom showed a higher lipase inhibitory potential with IC_{50} value of 22.28 ± 0.65 µg/mL than the CME, while that of the standard, orlistat was 2.28 ± 0.34 µg/mL. However, these extracts exhibited very low or no alpha-amylase inhibitory and cytotoxic activity at the tested concentrations.

Conclusion: This study reveals that CME extract of *L. squarrosulus*, rich in polyphenols and flavonoids possess considerable alpha-glucosidase and lipase inhibitory activities.

Keywords: *Lentinus squarrosulus*, lipase, alpha-glucosidase, alpha-amylase, cytotoxicity

1. INTRODUCTION

Diabetes mellitus (DM) is a health disease that results from an impairment in the secretion of insulin and some unavoidable degree of resistance to insulin in the periphery leading to constant hyperglycemia. The chronic form of this hyperglycemia can disrupt protein, lipid and carbohydrate metabolism leading to serious health concern. Affecting about 90% of diabetics globally is the Type-2 form of the disease (T2DM) making it the most common.[1] A steady rise in cases as well as diabetes prevalence, has been recorded over the past few decades. More than 422 million people are diabetic around the world as diabetes causes the death of about 1.6 million people every year. [2] Alpha-amylase and alpha-glucosidase are complex sugar hydrolyzing enzymes that are mainly secreted from the pancreas and the intestinal chorionic epithelium respectively. Inhibiting these enzymes is one of the approaches for T2DM therapy as well as reducing postprandial glucose level, since it can prevent excess glucose absorption by decreasing the rate of carbohydrate breakdown.[3] This metabolic disease has also been found to have a close relationship with obesity. [4] Obesity defines an abnormal or excessive accumulation of massive body storage fats which may be caused by a mismatch between the rate of intake and expenditure of energy.[5] The number of obesity and overweight cases are increasing and if this trends continue, it is estimated that 2.7 billion adults will be overweight, over 1 billion affected by obesity, and 177 million adults severely affected by obesity by 2025.[2] Furthermore, an increase in the storage of lipids in the pancreas can instigate the abnormal functioning of the insulin-producing pancreatic β cell which may result to T2DM.[6] The major enzyme in lipid digestion is the pancreatic lipase. Its role is to facilitate the absorption of dietary fats by catalyzing the hydrolysis of triacylglycerol into free fatty acids and monoacylglycerol in the lumen of the intestine.[7] Research interest on inhibitors of pancreatic lipase activity has received much attention, possibly due to their anti-obesity activity by delaying the lipid breakdown process.[8]

Mushrooms are widespread in nature. They are regarded as fungi that are visible with definitive basidiocarps (fruiting bodies). Edible mushrooms have served as food supplements and source of bioceuticals because they possess myriads of compounds that elicit biological activities and play vital roles in human nutrition and health. *Lentinus squarrosulus* mushroom has been reported to be commonly eaten with medicinal properties. *Lentinus squarrosulus* is edible and belongs to the Polyporaceae family. The fruiting body known as the basidiocarp is morphologically characterized by a whitish-greyish surface with conspicuous squamules.[9] *Lentinus squarrosulus* usually exists on old or fallen tree trunks, and buried or exposed roots of decaying deciduous trees in some tropical rain forest regions of Africa (Nigeria) and Asia. It usually grows in caespitose clusters of up to three to six basidiocarps.[10] In Nigeria, *L. squarrosulus* is popularly known as "*Ero atakata"* in South-Eastern part [11] and "*Olu-awo, erirokiro or osun awo*" in South-Western part.[12] In traditional medicine, it is used in ulcer treatment,[13] alleviating anaemic symptoms, decrease the chances of infertility in both men and women [14] and to lower the risk of metabolic diseases.[15,16] Previous studies indicated that *L. squarrosulus* contain phenolics, tannins, saponins, flavonoids, alkaloids, terpenes, quinolones and anthraquinones.[17] It has also

been reported to possess antimicrobial,[18] immunomodulatory,[19] antioxidant, anticancer and antihyperglycemic activities.[13,20]

Currently, synthetic drugs are available for α-amylase and alpha-glucosidase inhibitors as well as treatment for obesity but the associated undesirable side effects such as bloating, abdominal discomfort, flatulence, and emesis, insomnia, myocardial infarction and constipation have rendered them less attractive as therapeutic agents.[21] A natural remedy that will be effective, less expensive and relatively safe is desirable.

In order to overcome these short-comings combined with the purported therapeutic effects of the mushroom in traditional medicine, the investigation became imperative. Hence, this study sought to assess anti-diabetic, anti-obesity and cytotoxicity potentials of *L. squarrosulus* as well as identifying the mushroom using molecular techniques.

2. MATERIALS AND METHODS

2.1 Reagents and chemicals

Sodium hydroxide (NaOH) solution (2 M), 1% w/v starch, 3, 5-Dintrosalicylic acid (DNSA), potassium sodium tartrate tetrahydrate, 3% methanol, acarbose, phosphate buffer (20 mM, pH 6.9), 3, 5-dinitrosalicylic acid (DNSA) solution (96 mM), maltose standard solution 0.2% (w/v), alpha-amylase (*Aspergillus niger*) sodium bicarbonate (Na_2CO_3), para-nitro phenyl glucopyranoside (pNPG), p-nitrophenol, alpha-glucosidase enzyme (*Saccharomyces cerevisiae*), orlistat, porcine pancreatic lipase enzyme (PPL), para-nitro phenyl butyrate (pNPB), dimethyl formamide (DMF), sodium chloride (NaCl), ethanol, Isolation buffer (Tris-EDTA), RNAse, polyvinylpyrrolidone (PVP), ITS1 and ITS4 primers, Taq DNA polymerase, Taq buffer, DNA Template, sterile water, dNTPs-mix and ethidium-stained agarose gel. All other chemicals and reagents used were of quality analytical grade procured from commercially available sources.

2.2 Mushroom sample collection and morphological identification

The mushroom sample collection was carried out on the basis of the reported morphological features and characteristics of the mushroom (*L. squarrosulus*) as described in mycological treaties.[22,9,10,23] The fruiting bodies of the mushroom were harvested from the University of Ibadan.

2.3 Molecular identification of the mushroom sample (*L. squarrosulus*)

Extraction of the genomic DNA

The total DNA of the genome was extracted from the fruiting body of the mushroom using Plant/ Fungi DNA Isolation Kit (Norgen Biotek Corporation, Thorold, ON, Canada) strictly following the manufacturer's instructions for use. The DNA after extraction was then stored at -20 ^{0}C until when required.

PCR amplification of the genomic DNA

Amplification of the extracted genomic DNA of the mushroom sample was performed by polymerase chain reaction (PCR) using ITS1 (5'~TCCGTAGGTGAACCTGCGG~3') and ITS4 (5'~TCCTCCGCTTA TTGATATGC~3') primers. The reaction involved the use of Taq polymerase 'Ready to Go' mixture (Pharmacia, Sweden), the primers and DNA template solution. A GenAmp PCR System 2400, Perkin-Elmer, USA was used to achieve 30 cycles of

denaturation at 95 ˚C for 30 seconds; primer hybridization/annealing at 50 ˚C for 1 minute; and primer extension at 72 ˚C for another 1 minute. The products after amplification were subjected to gel purification and electrophoresis on ethidium-stained agarose gel (0.7%). The products thereafter were directly sequenced and aligned using Clustal W.[24] The data obtained from the aligned sequences were used to plot a dendrogram tree using Molecular Evolutionary Genetic Analysis (MEGA) 4 software.

2.4 Extraction of *L. squarrosulus* mushroom sample

The collected mushroom was freeze-dried and grounded to coarse powder with the use of an electric blender. Two hundred grams (200 g) of the powdered mushroom was weighed and macerated in chloroform/methanol (1:1) at 70˚C for 24 hours in a sonicator. It was thereafter, filtered and the filtrate collected and concentrated to obtain the chloroform/methanol extract. The residue was dried and macerated in distilled water at 70˚C for 24 hours in a sonicator. The extract from the residue was filtered and lyophilized to obtain the aqueous extract. [25,26] The weight of the chloroform/methanol and aqueous extracts were recorded and the different percentage yield (%) calculated.

2.5 *In vitro* anti-diabetic activity

2.5.1 Evaluation of alpha-amylase inhibition activity of *L. squarrosulus* extracts

A reported method [25] was used to determine the inhibition of alpha amylase activity of the chloroform/methanol and aqueous extracts of *L. squarrosulus*. Five graded concentrations of the extracts and acarbose (104.19 - 1667 µg/ mL) were obtained through two-fold serial dilution in phosphate buffer (20 mM, pH 6.9). Five hundred microliter (500 µL) of the extracts/acarbose was dispensed into well-labeled test tubes, and 500 µL of 2 units of α- amylase solution was added. The resulting mixture was pre-incubated for 15 min at a temperature of 35˚C followed by the addition of 500 µL starch solution (1%) to initiate the reaction. The reaction mixture was further incubated for 5 min at a temperature 25 ˚C. Finally, 500 µL of the colour reagent (96 mM 3, 5-dinitrosalicylic acid and 5.31 M sodium potassium tartrate in 2 M sodium hydroxide) was added to terminate the reaction and the tubes incubated inside the water bath at 80 ºC for 15 minutes. The test tubes were removed and made to cool on ice, thereafter 4.5 mL of distilled water was added in order to dilute the reaction mixture. Two hundred microliter (200 µL) each of the content in the test tubes was measured into a 96-well microtitre plate and the absorbance (α-amylase activity) read at 540 nm. The concentration of maltose formed was extrapolated from a maltose standard curve. Positive control (containing serially diluted acarbose) and negative control tubes (phosphate buffer) were also included.

2.5.2 Evaluation of alpha-glucosidase inhibition activity of *L. squarrosulus* extracts

A previously reported method was used.[25] The mushroom extracts and acarbose were prepared in phosphate buffer (pH 6.9). Five graded concentrations of the mushroom extracts and acarbose (78.13 - 1250 µg/mL) were determined by two-fold serial dilution in test tubes. One hundred (100 μL) of α-glucosidase enzyme (1 U/mL) was pre-incubated with 50 μL of the varying concentrations of the mushroom extracts for 10 minutes at room temperature. Then, the reaction was started by the addition of 50 μL of 3 mM para-nitrophenyl glucopyranoside (pNPG). The reaction mixture was brought to a stop by the addition of 2.5 mL of 0.1M Na_2CO_3 solution after incubating at room temperature for 20 minutes. Two hundred microliter (200 µL) each of the content in the test tubes was dispensed into a 96-well microtitre plate and the enzymatic activity

of α-glucosidase was thereafter determined by measuring the amount of the yellow para-nitrophenol released from the pNPG at 405 nm on the SPECTRAmax Gemini XS microplate reader. Positive control (containing serially diluted, acarbose) and negative control tubes (phosphate buffer) were also included.

2.6 *In vitro* anti-obesity activity

Evaluation of anti-lipase activity of *L. squarrosulus* extracts

Pancreatic lipase inhibition activity of the mushroom extracts was determined using para-nitrophenyl butyrate (pNPB) as the substrate. The effect of the mushroom extracts on lipase activity was determined using a modified method described.[27] The mushroom extracts and orlistat were prepared in phosphate buffer (pH 6.9). Two-fold serial dilution in test tubes were used to obtain five graded concentrations of the mushroom extracts and orlistat (78.13 - 1667 µg/mL). The mushroom extracts and orlistat (50 µL) were then, pre-incubated with 50 µL of pancreatic lipase for 1 hour in the reaction buffer at room temperature. 1 µL of pNPB was added to initiate the reaction followed by further incubation of the reaction mixture for 5 minutes at the room temperature (29^0C). The amount of p-nitrophenol released in the mixture was estimated at 405 nm using UV-Vis spectrophotometer (SPECTRAmax Gemini XS, Molecular Devices, USA). Positive control (containing serially diluted orlistat) and negative control tubes (phosphate buffer) were also included.

2.7 Cytotoxicity assessment

The cytotoxicity assessment of *L. squarrosulus* mushroom extracts were carried out using the brine shrimp lethality assay. The eggs of *Artemia salina* (brine shrimp) were obtained from an Aquarium shop, UK. The brine shrimp eggs were hatched in natural seawater (from Bar Beach, Lagos) contained in a small reservoir tank, under adequate illumination for 48 hours. The hatched nauplii (larvae) were attracted to the illuminated side of the tank and collected with a Pasteur pipette. Ten of the brine shrimps (nauplii) were transferred into each extract at selected varying concentrations (five dilutions, 1.6-5.0 µg/mL) in tubes. Cyclophosphamide was used as the positive control. After 24 h, the number of surviving nauplii were counted. The percentage mortality of each concentration and the LC_{50} of were calculated and compared with the control. Data were carried out in triplicate. Crude extracts were considered toxic when their LC_{50} values are less than 100 µg/mL.[28]

2.8 Phytochemical composition of *L. squarrosulus* mushroom extracts

Determination of total phenolic content (TPC)

The TPC of the chloroform/methanol and aqueous extract of *L. squarrosulus* were assessed using the Folin-Ciocalteau reagent and a previously reported method.[29] Briefly, 0.1 mL of mushroom extract (1 mg/mL) was mixed with 0.1 mL of Folin-Ciocalteau's reagent (1:1 v/v) and incubated for 5 minutes. 1 mL of 7% sodium carbonate (Na_2CO_3) solution was added to the mixture and distilled water was used to make up the volume of the mixture to 2.5 mL. This was thoroughly mixed and kept in the dark for 90 minutes at room temperature. Absorbance was measured using UV-Vis spectrophotometer at 750 nm against the reagent blank. The TPC was expressed as gallic acid equivalent (GAE)/g of dry matter based on the standard gallic curve.

Determination of total flavonoid content (TFC)

The TFC of the mushroom extracts was determined using the aluminum chloride colorimetric method.[30] The mushroom extracts solution, 0.3 mL of 1 mg/mL, was added to a mixture containing 3.4 mL of 30% methanol, 0.15 mL of 0.3 M sodium nitrite ($NaNO_2$), and 0.15 mL of 0.3 M aluminum chloride ($AlCl_3.6H_2O$). The resulting mixture was incubated for 5 minutes at room temperature. Then, 1 mL of 1M NaOH was added to the mixture. The absorbance of the reaction mixture was measured against the blank at 506 nm using UV-Vis Spectrometer. The total flavonoid content, expressed as mg rutin equivalent (RE)/g of the dry matter was calculated from the calibration curve.

2.9 Statistical analysis

The IC_{50} /LC_{50} values are expressed as mean ± standard error of mean (SEM) of the three independent values. The values were compared using the Mann-Whitney U test. P-values of < 0.05 were considered as a statistically significant difference.

3. RESULTS

3.1 Molecular identification of *L. squarrosulus* mushroom

The Internal transcribed spacer (ITS) region sequence of the rDNA was used to identify the mushroom. The BLAST analytic result from Genbank revealed that the ITS sequence of the mushroom sample matched that of *Lentinus squarrosulus* Mont. with the unique accession number (KT120043.1). The dendrogram tree for *L. squarrosulus* is represented in Fig. 2.

3.2 Extract yield of *L. squarrosulus* mushroom

The percentage yield of CME and AE of *L. squarrosulus* mushroom were 3.72 and 9.10 % respectively (Table 1).

3.3 Phytochemical analysis of *L. squarrosulus* extracts

Total phenolic content (TPC) and total flavonoid content (TFC)

The results of the TPC and TFC of the CME and AE of *L. squarrosulus* are presented in Table 1. From the table, the CME of *L. squarrosulus* (239.92 ± 0.65 mg GAE/g sample) showed higher TPC than the AE (220.75 ± 0.34 mg GAE/g sample). Similarly, the TFC of AE of *L. squarrosulus* (217.43 ± 0.85 mg RE/g sample) was less than that of the CME (348.86 ± 0.32 mg RE/g sample). However, both extracts of *L. squarrosulus* possess high TPC and TFC.

3.4 Inhibition of alpha- amylase, -glucosidase and lipase activities of *L. squarrosulus*

Both extracts (CME and AE) of *L. squarrosulus* exhibited very low alpha-amylase inhibitory activity at the tested concentrations with IC_{50} values of >1670 µg/mL when compared to that of the standard, acarbose with an IC_{50} value of 726.49 ± 1.66 µg/mL (Table 2). The CME of *L. squarrosulus* showed better inhibitory activity with an IC_{50} value of 451.13 ± 2.14 µg/mL than the AE extract (IC_{50} > 1250 µg/mL) against α-glucosidase (table 2). Although, it is not as active as acarbose with an IC_{50} value of 235.51 ± 1.34 µg/mL against α-glucosidase (Table 2). Furthermore, CME and AE exhibited lipase inhibitory activity with IC_{50} values of 28.11 ± 1.37 and 22.28 ± 0.65 µg/mL respectively. However, the orlistat had the best lipase inhibitor activity (IC_{50} =2.28 ± 0.34 µg/mL).

3.5 Cytotoxicity assessment

The result of the cytotoxicity assessment using brine shrimp lethality assay showed that the CME and AE of *L. squarrosulus* had LC_{50} of 257.1 ± 1.33 µg/mL and 251.1 ± 3.59 µg/mL respectively, while cyclophosphamide had LC_{50} value of 15.19 ± 2.00 µg/mL.

4. DISCUSSION

To harness the numerous potentials that an organism offers, accurate and reliable taxonomy of such organism is very important in order to confirm the biological specie for various prospective utilization. Molecular techniques which employs the use of DNA barcoding using the ITS region sequencing, eliminates the challenge of irregular morphology and possible indiscrimination among macro-fungal species, which are often associated and remains a setback with morphological taxonomy. Molecular technique was used to identify *L. squarrosulus* in this study.[31] The ITS rDNA region sequence as a proposition is considered as one of the most essential tools for identifying fungal species that are isolated from environmental and biological sources.[32] The present study revealed that the ITS sequences obtained for the mushroom sample when compared to those in the database using NCBI-BLAST showed significant sequences similar to the query sequences used to identify the organism with a high percentage similarity. Hence, the BLAST result identified the mushroom sample as *L. squarrosulus* with a phylogenetic tree generated to further reveal and confirm the identity of the mushroom specie.

In this study, it was observed that the extraction yield of the mushroom in aqueous is more than that of the chloroform/methanol. Water or an aqueous solution is more polar than chloroform/methanol which suggest that the extraction yield increases with increase in polarity. Similar findings were reported[23] that supports the result of the present study. Thus, the yield of extraction may possibly depend on one or more of the solvents with different polarity, time of extraction, temperature, pH, and composition of the sample.

Edible and medicinal mushrooms hold definite promises as functional food and nutritional supplements to manage diabetes mellitus and obesity due to their rich bioactive components.[33,34] These natural active substances or their primary metabolites consumed in traditional medicine or as nutraceuticals contain antioxidants, fibres and other phytochemicals that are known to favourably demonstrate both anti-obesity and antidiabetic activities through the modulation and regulation of diverse cellular and physiological pathways. These effects include appetite regulation, modulation of lipid absorption and metabolism, enhancement of insulin sensitivity, thermogenesis and changes in the gut microbiota.[34]

Disease conditions such as obesity, hyperlipidemia and T2DM are likely to occur when there is an imbalance between energy intake and energy expenditure.[35,36] In order to treat these complications and obesity, the enzymatic inhibition of pancreatic lipase is a very essential path. Dietary fats consisting of triglycerides are hydrolysed to release fatty acids and glycerol, which are absorbed by the mucosa of the small intestine. In response to food intake, the enzyme, lipase is therefore secreted by the pancreas into the small intestine to catalyze the hydrolysis step. The drug orlistat which is used to treat obesity inhibits the activity of human pancreatic lipase by forming a bond that is covalently linked with the enzyme at its active site.[34,36] Many of phytochemically active components such as flavonoids, saponins, polyphenols, and caffeine have shown to inhibit pancreatic lipase activity *in vitro* and was comparable to orlistat.[36] In the present study, both extracts of *L. squarrosulus* inhibited pancreatic lipase enzyme activity. Mushrooms

have been well documented to show anti-obesity effects through various possible mechanism. A study evidenced that *Lentinus edodes* and *Cordyceps militaris* reduced triglycerides, Total cholesterol, plasma glucose and hypertension in diabetic rat.[37] *Lentinus strigosus* another specie of Lentinus was reported to possess antiobesity activity by affecting the food intake and locomotion of *Caenorhabditis elegans*.[38] In this study, *Lentinus squarrosulus* exhibits anti-obesity effect through inhibition of pancreatic lipase activity. *Pleurotus sajor-caju* and *Adiantum capillus-veneris* have also shown similar anti-obesity activity through the same mechanism in addition to lowering total cholesterol, triglycerides and atherogenic index.[5,39] These observed activities are attributed to the presence of bioactive components such as saponins, flavonoids and polyphenols.

Diabetes mellitus is a consequence of unequilibrated insulin production and/or insensitivity to the effect of this hormone in signal transduction of cellular receptors. Most of the T2DM complications are due to hyperglycemia as their main cause.[4] One of the effective strategies for T2DM management is the inhibition of complex polysaccharide hydrolysis by pancreatic α-amylase and absorption limitation of glucose by inhibiting intestinal alpha-glucosidase enzyme. Mushrooms have been reported to have anti-diabetic properties with different mechanism of action. The β-glucans and their enzymatically hydrolyzed oligosaccharides from the mushroom *Agaricus brasiliensis* show antiarteriosclerotic and antihyperglycemic activities, indicating antidiabetic activity in diabetic rats which corroborates with enhanced insulin in insulin secretion from pancreatic islets as well as proliferation of islets in diabetic or normal rats.[36] The results of our study revealed that the chloroform methanol extract of *L. squarrosulus* had the highest alpha-glucosidase inhibitory activity while the extracts of *L. squarrosulus* did not show any inhibitory effect on alpha-amylase enzyme at the concentrations tested. Stonkjovic et al. in 2017 reported the antidiabetic activity of methanol extract of *M. conica* mushroom that did not inhibit alpha-amylase activity but had inhibitory potential on alpha-glucosidase activity.[37] Moreso, a previous study revealed terpenoids as inhibitors of alpha-glucosidase.[39] *Lentinus squarrosulus* has been reported to contain bioactive components such as contain terpenoids, saponins and polyphenols amongst others.[40] Hence, the antidiabetic activity of *L. squarrosulus* through inhibition of α-glucosidase activity leading to delayed process of glucose uptake may be attributable to the presence of terpenoids and other bioactive components.

The need to know the toxic potential of edible and medicinal mushrooms is paramount for safe consumption and utilization. The brine shrimp test is a rapid and simple bioassay for testing extract lethality as a means of ascertaining their cytotoxic properties. The test system has proven to be convenient for monitoring the biological activities of products of natural origin.[41] *Lentinus squarrosulus* mushroom extracts appeared less toxic than cyclophosphamide, a standard toxic drug. Hence, it is relatively safe at the tested concentration.

5. CONCLUSION

In conclusion, obesity and T2DM are however complex disease conditions, and to prevent, treat and manage these and its complications requires a holistic approach which involves combination of factors such as regular exercise, diet modifications and pharmacotherapy which requires further advancements. Mushrooms due to their numerous bioactive components and reported therapeutic advantages appear promising in the search for the treatment of obesity and T2DM.

Therefore, the outcome of the present study indicates that chloroform methanol extract of *L. squarrosulus* mushroom, rich in polyphenols and flavonoids possess considerable alpha-glucosidase and pancreatic lipase inhibitory activities and also appears less toxic. Thus, it might be explored or combined with existing treatment to reduce the prevalence of diabetes, obesity and management of their complications.

CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

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Fig. 1: Harvested fruiting bodies of *Lentinus squarrosulus* Mont. Mushroom

Table 1: Percentage yield, total phenolic content (TPC) and total flavonoid content (TFC) of *Lentinus squarrosulus* mushroom extracts

Mushroom extracts	Percentage yield (%)	TPC (GAE/g sample)	TFC (mg RE/g sample)
CME	3.72	239.92 ± 0.65	348.86 ± 0.32
AE	9.10	220.75 ± 0.34	217.43 ± 0.85

GAE - Gallic acid equivalent, RE - Rutin equivalent

Table 2: Cytotoxicity assessment, α-amylase, α-glucosidase and pancreatic lipase inhibitory activities of *Lentinus squarrosulus* mushroom extracts

	IC_{50} / LC_{50} (Mean ± SEM) µg/mL		
	***Lentinus squarrosulus* extracts**		**Standard drugs**
***In vitro* Assays**	**CME**	**AE**	
Alpha-amylase inhibition	> 1670	> 1670	*726.49 ± 1.66
Alpha-glucosidase inhibition	451.13 ± 2.14	> 1250	*235.51 ± 1.34
Pancreatic lipase inhibition	28.11 ± 1.37	22.28 ± 0.65	#2.28 ± 0.34
Cytotoxicity assessment	257.10 ± 1.33	251.10 ± 3.59	+15.19 ± 2.00

*Acarbose, #Orlistat, +Cyclophosphamide

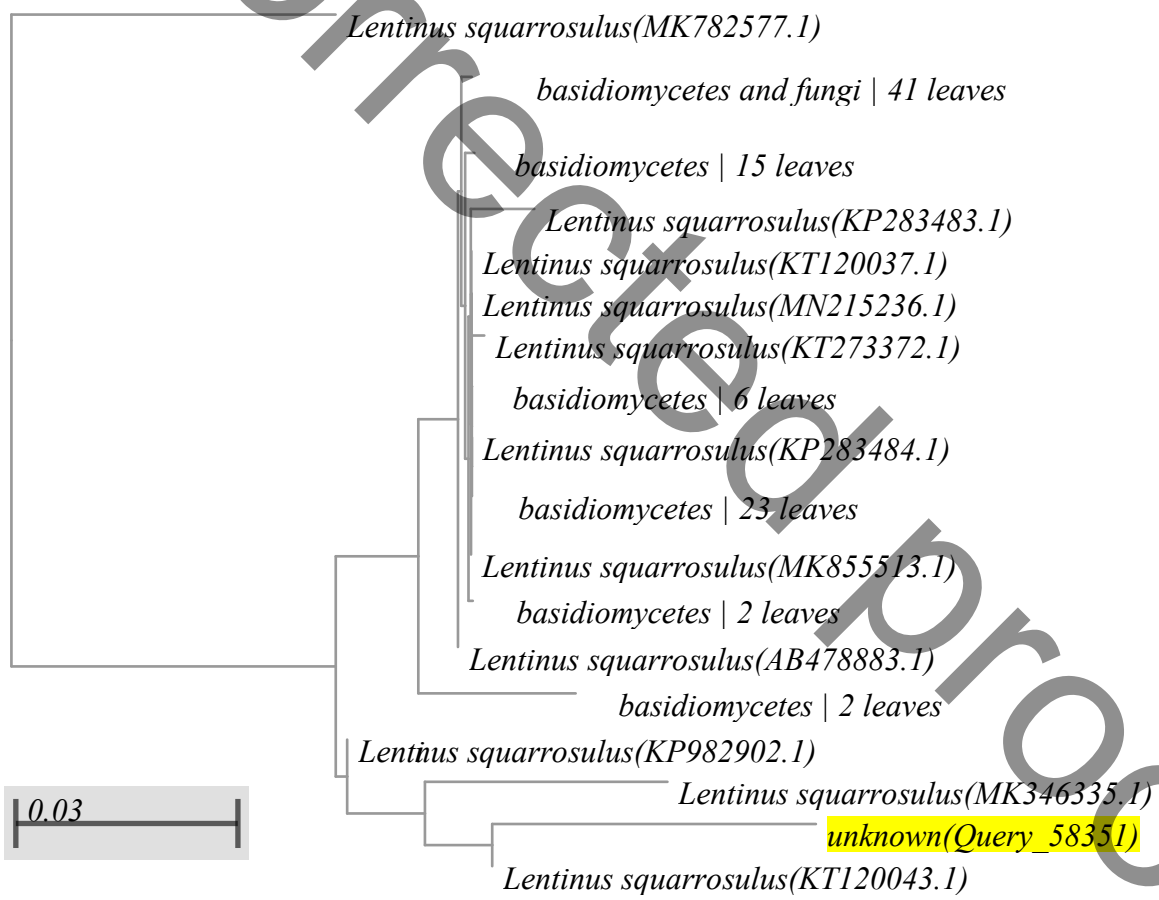

Fig. 2: Dendrogram tree of *Lentinus squarrosulus* Mont. from phylogenetic analysis

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