IN VITRO HYPOLIPIDEMIC AND HYPOGLYCAEMIC PROPERTIES OF MUSHROOM EXTRACTS

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INTRODUCTION

Mushrooms are considered as a valuable food due to their unique taste, nutritional properties, and biological effects [1]. They are source of several classes of phytochemicals, including phenols, terpenoids, steroids, and polysaccharides that demonstrated a wide range of biological activities [2]. Obesity is a metabolic disorder, which results from excessive accumulation of body fat, associated with several comorbidities, including cardiovascular diseases, hypertension, various types of cancer, and type 2 diabetes mellitus [3]. Several natural compounds possess the ability to reduce body weight and to prevent diet-induced obesity by inhibiting enzymes that interfere with the hydrolysis and absorption of dietary carbohydrates and lipids, such as α-amylase, α-glucosidase, and pancreatic lipase [4,5].

This study was planned to investigate the hypoglycaemic and hypolipidemic activity of basidiomata belonging to two edible mycorrhizal fungal species in the family *Boletaceae*: *Leccinum duriusculum* (Fig. 1) and *Lanmaoa fragrans* (=*Boletus fragrans*) (Fig. 2), growing the former in poplar tree forests, the latter in association with oaks, and collected in Calabria (southern Italy).


Fig. 1


Fig. 2

Both fungi produce robust mushrooms, showing greyish-brown or reddish-brown pilei, respectively, club-shaped and tapering stipes, which are whitish-to-greyish and covered by fine dense brownish woolly scales in the former, yellowish above, reddish-brown below, and fibrillose-dotted in the latter. Tubes and pores are greyish, but tending to pinkish then to brown tones with age, in *L. duriusculum*, bright yellow in *L. fragrans* [6].

In this study, the hypoglycaemic and hypolipidemic activity of *Leccinum duriusculum* and *Lanmaoa fragrans* from Calabria (southern Italy), was investigated. *L. duriusculum* and *L. fragrans* are two symbiotic edible mushrooms belonging to the Boletaceae family, growing the former in poplar tree forests, the latter in a mycorrhizal association with oaks.

MATERIALS AND METHODS

The basidiomata of *L. duriusculum* and *L. fragrans* were dried and exhaustively subjected to chemical extraction at room temperature by maceration with *n*-hexane, dichloromethane, and methanol as solvents. The obtained extracts were investigated for their potential inhibitory activity against α-amylase, α-glucosidase, and pancreatic lipase. In the α-amylase inhibitory assay, the enzyme solution was prepared by adding 0.0253 g of enzyme in 100 mL of cold water, and the starch solution was prepared by stirring 0.125 g of potato starch in 25 mL of sodium phosphate buffer (20 mM) and sodium chloride (6.7 mM) [7]. Samples (at concentrations in the range 1000-25 μg/mL) were added to starch solution, and left to react with the enzyme at room temperature for 5 min. The absorbance was read at 540 nm. In the α-glucosidase inhibitory activity test, a mixture of sample (at concentrations in the range 25-1000 μg/mL), maltose solution, and the enzyme was left to incubate at 37 °C for 30 min [7]. Then, perchloric acid was added, and the mixture was centrifuged. The supernatant was collected and mixed with *o*-dianisidine and peroxidase/glucose oxidase and left to incubate at 37 °C for 30 min. The absorbance was read at 500 nm. Acarbose was used as a positive control in both tests. Lipase inhibitory activity was determined as previously reported [7]. Samples (2.5-40 mg/mL) were added to 4-nitrophenyl octanoate (NPC), porcine pancreatic lipase and Tris-HCl buffer (pH 8.5). The mixture was incubated at 37 °C for 30 min. The absorbance was measured at 405 nm. Orlistat was used as a positive control.

RESULTS AND DISCUSSION

Both mushrooms have been dried and exhaustively extracted at room temperature with *n*-hexane, dichloromethane, and methanol as solvents. The results showed that the used solvents took an important role in the extraction yield. Methanol was the most effective solvent, resulting in the highest extraction yield for both mushrooms (25.76 and 21.44% for *L. duriusculum* and *L. fragrans*, respectively), followed by dichloromethane (3.01 and 2.56% for *L. duriusculum* and *L. fragrans*, respectively), and *n*-hexane (1.33 and 1.13% for *L. duriusculum* and *L. fragrans*, respectively). The abilities of mushrooms extracts to inhibit α-amylase, α-glucosidase and lipase are presented in Table 1 and Figure 1. The methanol extracts of both mushroom species exhibited the most promising results in inhibiting lipase with IC_{50} values of 35.02 and 22.40 μg/mL, for *L. duriusculum* and *L. fragrans*, respectively, that are of interest if compared with the positive control orlistat (IC_{50} of 37.63 μg/mL). Pancreatic lipase inhibition is one of the most largely studied mechanisms to counter obesity. The inhibition of this enzyme delays the digestion of triglycerides to absorbable free fatty acids with reduction of post-prandial hyper-triacylglycerolemia.

Table 1. Hypoglycaemic and hypolipidemic activity (IC_{50}, μg/mL) of mushroom extracts.

	α-Amylase	α-Glucosidase	Lipase
Leccinum duriusculum			
Methanol	335.21 ± 2.36****	41.91 ± 1.32*	35.02 ± 0.59^{ns}
Dichloromethane	184.55 ± 2.85****	182.80 ± 2.75****	44.85 ± 1.83**
n-Hexane	187.87 ± 1.58****	341.34 ± 3.22****	101.47 ± 3.54****
Lanmaoa fragrans			
Methanol	875.62 ± 7.35****	162.66 ± 2.15****	22.40 ± 1.83****
Dichloromethane	398.27 ± 4.19****	247.62 ± 2.62****	42.98 ± 1.56*
n-Hexane	924.67 ± 9.35****	315.94 ± 0.96****	64.69 ± 2.08****
Positive control			
Acarbose	50.01 ± 1.42	35.50 ± 1.12	
Orlistat			37.63 ± 1.01

Data are expressed as means ± S.D. (n= 3). Differences within and between groups were evaluated by one-way ANOVA followed by a multicomparison Dunnett's test (α= 0.05): ns not significant, ****p< 0.0001, **p < 0.01, *p< 0.1 compared with the positive controls.

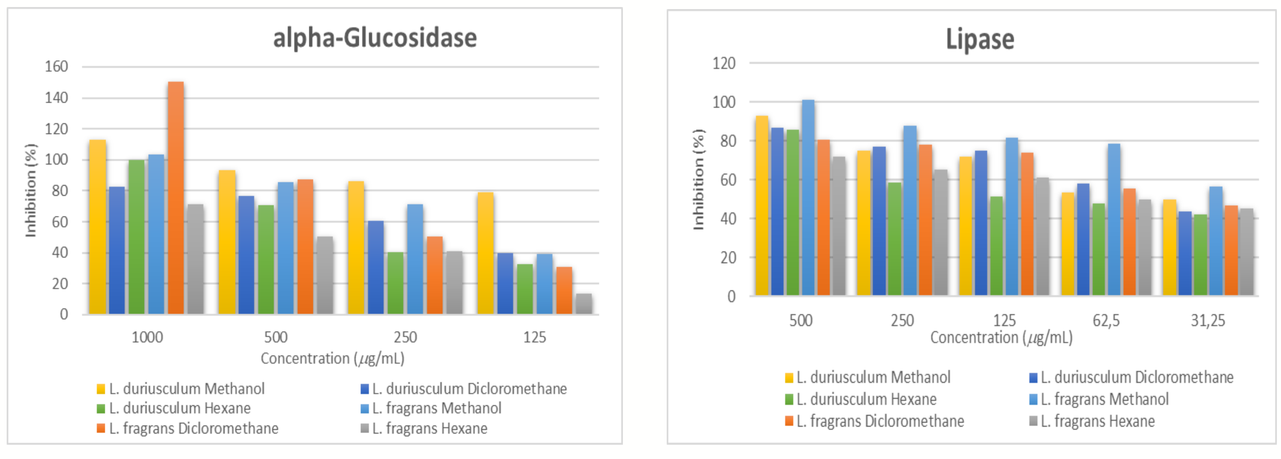

Figure 1. Lipase and α-glucosidase inhibitory activity of mushroom extracts.

All investigated samples inhibited both α-amylase, α-glucosidase enzymes in a concentration-dependent manner, but the most promising activity was found against α-glucosidase. In particular, the methanol extract of *L. duriusculum* exhibited the highest inhibitory activity with an IC_{50} value of 41.91 μg/mL. The other extracts inhibited the enzyme with IC_{50} values in the range 162.66-341.34 μg/mL.

Mushrooms are rich source of fiber, water, and natural insulin-like enzymes, helpful in breakdown of glucose in foods and reduce insulin resistance. They also contain vitamins, minerals and amino acids and different bioactive compounds including polysaccharides, flavonoids, terpenoids, lectins, that promote good functioning of liver and pancreas, thus being helpful in insulin production and release, and confirming healthy metabolic functions.

CONCLUSION

Data obtained from this work provided evidence that *L. duriusculum* and *L. fragrans* mushrooms are able to inhibit key enzymes that interfere with the hydrolysis and absorption of dietary carbohydrates and lipids, suggesting their potential use for the development of new potential agents for the management of obesity and type 2 diabetes mellitus. However, further research is required to confirm these effects *in vivo.*

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