Bioactive Properties of *Clitocybe Alexandri*

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Introduction

Some mushrooms are known to have strong antioxidant capacity [1]. There is an accepted relationship between the physiopathology of several chronic diseases and oxidative stress. Therefore, the use of foods such as those mushrooms with antioxidant capacity, as phytochemical protectors, may be relevant for the prevention of oxidative stress related diseases such as cancer. Additionally, mushrooms have been described as a source of potential antitumour molecules, making them attractive candidates for drug discovery [2,3]. However, there are no such studies on the Portuguese wild mushroom *Clitocybe alexandri* .

Objective

The aim of the present work was to study extracts obtained from the wild mushroom *Clitocybe alexandri* for the *in vitro* antioxidant activity and growth inhibitory activity in human tumour cell lines.

Materials and methods

Clitocybe alexandri (Scop.: Fr.) Pat. (*Tricholomataceae*) was collected in Bragança (Northeast Portugal), in autumn 2008. Taxonomic identification was made according to different authors and representative voucher specimens were deposited at the herbarium of Escola Superior Agrária of Instituto Politécnico de Bragança. This is a saprotrophic and edible species. The samples were lyophilised and reduced to a fine dried powder.

The extracts studied were methanolic, ethanolic and polissacharidic.

For the antioxidant activity the following assays were used: evaluation of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity, reducing power and inhibition of lipid peroxidation (LPO) measured in liposome solutions [4].

For the analysis of extract-induced cell growth inhibition the SRB (sulforhodamine B) assay [5] was used, following treatment of four tumour cell lines (lung, breast, colon and gastric cancer) with the different extracts.

Results and discussion

The polysaccharidic extract presented the strongest antioxidant capacity (EC50 < 2.5 ± 0.0 mg/ml). Regarding the capacity to inhibit the growth of human tumour cell lines, the Ethanolic extract was the most effective, presenting the lowest GI50 values (GI50 < 17.95 ± 1.3 µg/ml).

Table 1. Antioxidant activity of *Clitocybe alexandri* extracts.

Extracts	η (%)	Phenolics (mg GAE/g)	DPPH scav. activity	Reducing power	LPO inhibition
Methanolic	47.7 ± 5.3 [a]	1.5 ± 0.1 [a]	28.7 ± 3.2 [a]	7.0 ± 0.4 [a]	4.5 ± 0.2[a]
Ethanolic	3.5 ± 0.2	6.3 ± 0.4	10.7 ± 0.8	2.3 ± 0.0	3.7 ± 0.1
Polysaccharidic	30.3 ± 2.8	-	2.5 ± 0.0	0.9 ± 0.0	1.2 ± 0.0

Results are expressed as EC_{50} (concentrations of extract in mg/ml that cause 50% of antioxidant activity, unless for reducing power that is 0.5 of absorbance), and show means ± SEM of 3 independent observations.

Table 2. Effects of *Clitocybe alexandri* extracts on the growth of human tumour cell lines.

Extracts	NCI-H460 (lung cancer)	MCF-7 (breast cancer)	HCT-15 (colon cancer)	AGS (gastric cancer)
Methanolic	34.85 ± 2.8	34.2 ± 1.4	36.9 ± 3.1	36.1 ± 2.3
Ethanolic	24.8 2.3	17.95 1.3	21.7 2.3	26.05 1.3
Polysaccharidic	24.55 1.8	46.8 1.6	59.1 0.7	51.75 0.9

Results are expressed as GI50 (concentrations of extract in µg/ml that cause 50% of growth inhibition of human tumour cell lines), and show means ± SEM of 3-6 independent observations performed in duplicate.g

Conclusions

In summary, polysaccharidic extract of *Clitocybe alexandri* was the most potent as antioxidant, while the ethanolic extract was the most potent as inhibitor of growth of human tumour cell lines. This interesting growth inhibitory activity proves that this mushroom, particularly the ethanolic extract is a promising source of bioactive compounds. As far as we know, there are no reports of growth inhibitory activity of the studied species against lung, colon and gastric human cancer cells. Future work will elucidate the mechanism of action of these extracts leading to the observed cell growth inhibition.

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