Antioxidant activity and growth inhibitory activity of Portuguese wild mushrooms

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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 mushrooms *Lepista inversa* and *Clitocybe alexandri*.

Objective

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

Materials and methods

Samples of *Clitocybe alexandri* (Gillet) Konrad *(Tricholomataceae)* and *Lepista inversa* (Scop.: Fr.) Pat. *(Tricholomataceae)* were 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. Both species are saprotrophic and edible. 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.

Acknowledgements

The authors are grateful to Fundação para a Ciência e a Tecnologia (FCT, I&D 4040/2007), FEDER and POCI for financial support. J.A. Vaz also thanks FCT for the BD/43653/2008 PhD grant.

GMA is supported by FCT, Portugal, and the European Social Fund.

Results and discussion

All extracts presented antioxidant activity, the most potent being the polysaccharidic extract of *Lepista inversa* (EC50 < 1.8 ± 0.1 mg/ml). Regarding the growth inhibitory activity in human tumour cell lines, the ethanolic extract of *Clitocybe alexandri* was the most potent (GI50 < 17.95 ± 1.3 µg/ml in all cell lines).

Table 1. Antioxidant activity of mushrooms extracts.

Species	Extracts	η (%)	Phenolics (mg GAEs/g)	DPPH scavenging activity	Reducing power	β-carotene bleaching inhibition
Clitocybe alexandri	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
	Polysaccharides	30.3 ± 2.8	-	2.5 ± 0.0	0.9 ± 0.0	1.2 ± 0.0
Lepista inversa	Methanolic	39.0 ± 1.9 [a]	3.6 ± 0.1 [a]	10.6 ± 1.1 [a]	2.9 ± 0.1 [a]	1.1 ± 0.1 [a]
	Ethanolic	4.6 ± 0.5	10.8 ± 0.7	9.3 ± 0.5	1.4 ± 0.1	1.5 ± 1.1
	Polysaccharides	32.2 ± 3.1	-	1.8 ± 0.1	0.7 ± 0.0	0.9 ± 0.1

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 mushrooms extracts on the growth of human tumour cell lines.

Species	Extracts	NCI-H460 (lung cancer)	MCF-7 (breast cancer)	HCT-15 (colon cancer)	AGS (gastric cancer)
Clitocybe alexandri	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
	Polysaccharides	24.55 ± 1.8	46.8 ± 1.6	59.1 ± 0.7	51.75 ± 0.9
Lepista inversa	Methanolic	36.3 ± 5.1	45.2 ± 3.1	39.7± 4.6	67.4 ± 5.5
	Ethanolic	118.3± 2.5	79.1 ± 11.8	42.3 ± 4.5	58.5 ± 3.3
	Polysaccharides	155.0± 3.5	137.4 ± 1.3	77.4 ± 5.5	99.9 ± 7.8

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 *Lepista inversa* was the most potent as antioxidant, while the ethanolic extract of *Clitocybe alexandri* 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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