

In vitro growth inhibitory activity of the Portuguese wild mushroom *Clitocybe alexandri* in human tumour cell lines

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INTRODUCTION

Some mushrooms are a powerful source of bioactive compounds. Indeed, many pre-clinical studies have been conducted in human tumour cell lines and in some cases a number of compounds extracted from mushrooms have entered clinical trials [1].

Our previous results showed that phenolic (methanolic and ethanolic) and polysaccharidic extracts from *Clitocybe alexandri* inhibited the growth of four human cell lines (lung, breast, colon and gastric cancer) (Table 1) [2].

Table 1. 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)	
Phenolic (methanolic)	34.85 ± 2.8		34.2 ± 1.4		36.9 ± 3.1		36.1 ± 2.3	
Phenolic (ethanolic)	24.8	2.3	17.95	1.3	21.7	2.3	26.05	1.3
Polysaccharidic (boiled water)	24.55	1.8	46.8	1.6	59.1	0.7	51.75	0.9

Results are expressed as GI_{50} (concentrations of extract in $\mu\text{g/ml}$ used that cause 50% of growth inhibition of human tumour cell lines), and show means \pm SEM of 3-6 independent observations performed in duplicate.

The aim of the present work was to:

- further elucidate the mechanism of action of the extracts that leads to the observed cell growth inhibition, by analysing the cell cycle profile of the NCI-H460 cells treated with each extract
- identify and quantify the chemical compounds present in the phenolic and polysaccharidic extracts.

MATERIAL AND METHODS

Samples of *Clitocybe alexandri* (Gillet) Konrad (Tricholomataceae) were collected in Bragança (Northeast Portugal), in Autumn 2008, lyophilised and reduced to a fine dried powder. Methanolic, ethanolic and boiling water extracts were obtained.

The effect of the extracts on tumour cell growth inhibition was verified with the SRB assay and the GI_{50} of each extract was determined for each of the cell lines studied (NCI-H460, MCF-7, AGS and HCT-15) (Table 1) [2]. NCI-H460 cells were treated with the GI_{50} or twice the GI_{50} concentration of the three extracts and changes to the normal cell cycle distribution were analysed by flow cytometry.

The chemical compounds present in the phenolic or polysaccharidic extracts were identified and quantified by different approaches: the phenolic compounds (in the phenolic extracts) by HPLC-DAD and the monosaccharides and oligosaccharides (in the polysaccharidic extracts) by HPLC-RI.

REFERENCES

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RESULTS AND DISCUSSION

The phenolic (methanolic and ethanolic) and polysaccharidic extracts from *Clitocybe alexandri* showed influence on the cell cycle distribution of the NCI-H460 cells. The reason for using both these concentrations (GI_{50} or twice the GI_{50}) was to increase the possibility of detecting the effect on the cell cycle, given the fact that the GI_{50} was determined with an assay that detects alterations in protein content, not in cellular viability. All the extracts induced an S phase cell cycle arrest, particularly evident at the highest concentration tested.

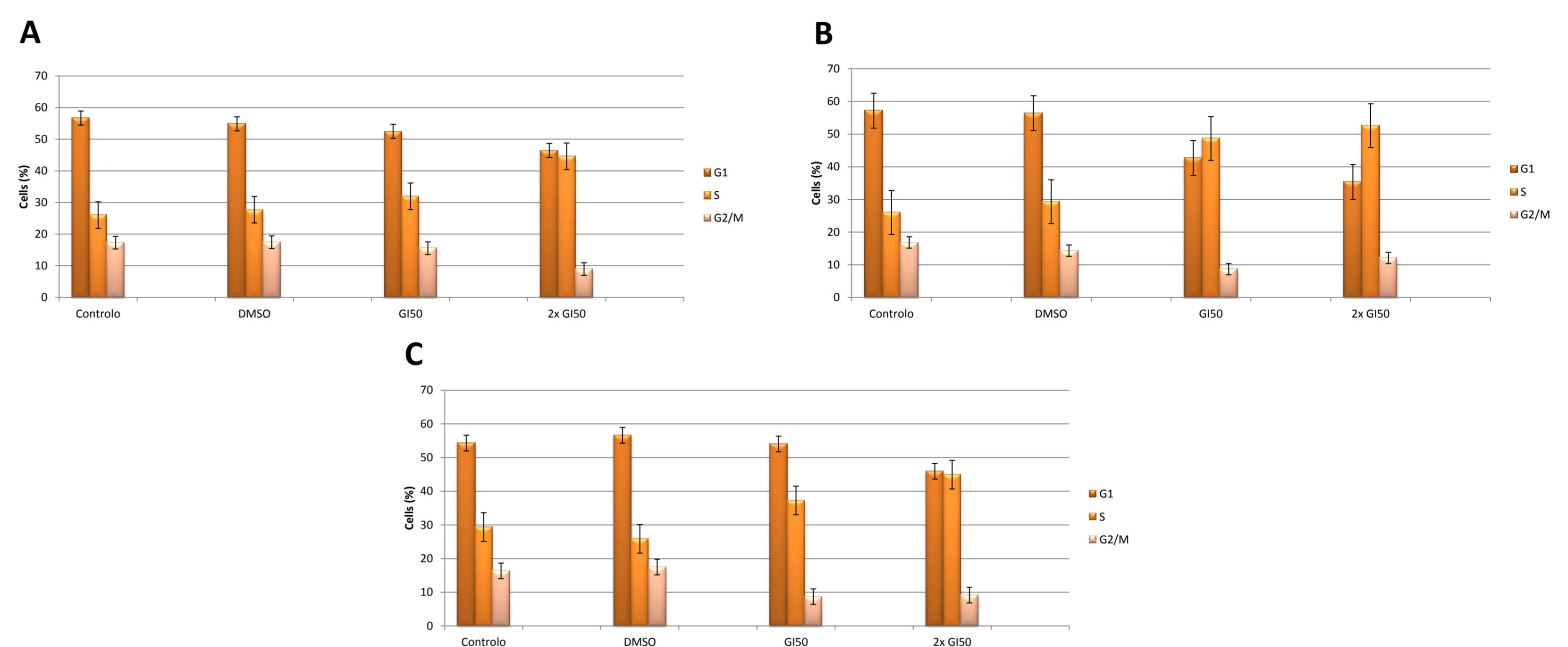


Figure 1. Cell cycle analysis of NCI-H460 cells treated for 48h with phenolic (ethanolic - A and methanolic - B) and polysaccharidic (C) extracts from *Clitocybe alexandri* at their GI_{50} or $2 \times GI_{50}$ concentrations. Untreated cells were as the control and a solvent control was also included (DMSO). Results are the mean \pm SD of three independent experiments performed in duplicate.

The main compounds isolated from the phenolic extract were protocatechuic acid (16.42 ± 2.5 mg/Kg, dw), p-hydroxybenzoic acid (8.34 ± 0.40 mg/Kg) and cinnamic acid (6.38 ± 0.29 mg/Kg). Regarding the polysaccharidic extract the main compounds isolated and identified were manitol (monosaccharide derivative) and trehalose (disaccharide).

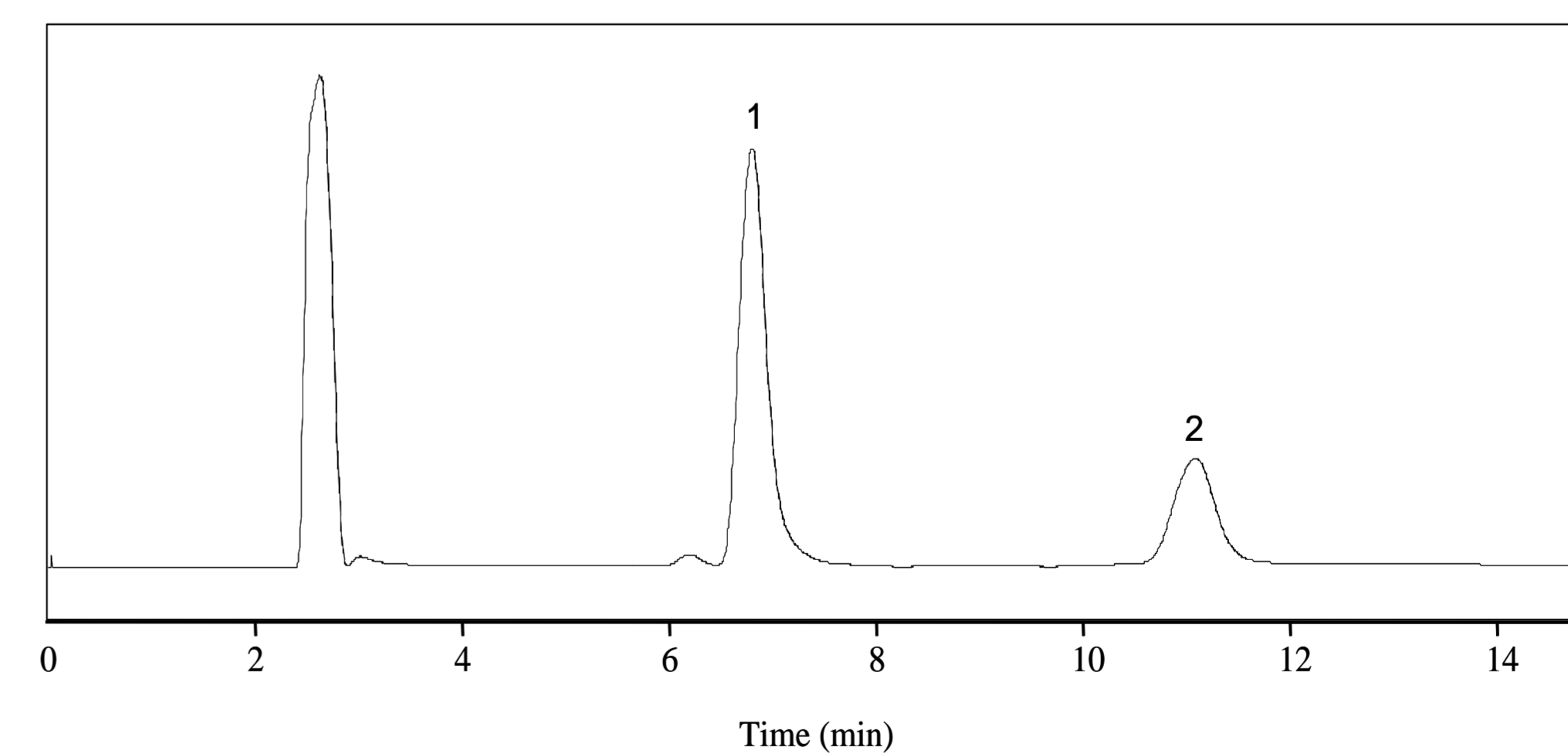


Figure 2. Individual sugar chromatogram of *Clitocybe alexandri*. 1- Mannitol; 2- Trehalose.

In an attempt to identify the compounds responsible for the cell growth inhibitory activity of this mushroom, the activity of the purified compounds was assessed using the SRB assay. None of the so far isolated and identified compounds presented GI_{50} values below $150 \mu\text{M}$ (data not shown), which suggests that there may be other, not yet identified, compounds in the extracts or that a combination of the compounds is responsible for the biological activity found in the extracts

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