

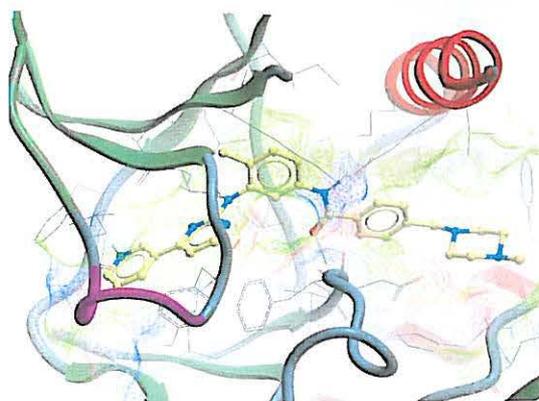
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28 a 30 Novembro 2010  
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**LIVRO DE RESUMOS**  
**ABSTRACT BOOK**



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## IN VITRO GROWTH INHIBITORY ACTIVITY OF THE PORTUGUESE WILD MUSHROOM *CLITOCYBE ALEXANDRI* IN HUMAN TUMOUR CELL LINES

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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) [2]. The aim of the present work was to: i) further elucidate the mechanism of action of the ethanolic extract that leads to the observed cell growth inhibition, by analysing the cell cycle profile of the NCI-H460 cells treated with this extract and ii) identify and quantify the chemical compounds present in the phenolic and polysaccharidic extracts.

Wild mushrooms were collected from the North of Portugal and classified as *Clitocybe alexandri*. Methanolic, ethanolic and polysaccharidic extracts were prepared. The effect of the extracts on tumour cell growth inhibition was verified with the SRB assay and the GI50 of each extract was determined for each of the cell lines studied (NCI-H460, MCF-7, AGS and HCT-15) [2]. NCI-H460 cells were treated with the GI50 or twice the GI50 concentration of the ethanolic extract and changes to the normal cell cycle distribution analysed by flow cytometry. The chemical compounds present in the phenolic or polysaccharidic extracts were purified, 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.

All the extracts were capable of causing cell growth inhibition and provided GI50 concentrations below 60 µg/ml in all the cell lines [2]. Results from the cell cycle profile demonstrated that the ethanolic extract induced an S phase cell cycle arrest, particularly evident at the higher concentration tested. Analysis of the cell cycle profile of the other extracts is currently being carried out. 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).

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 GI50 values below 150 µM, 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.

[1] Ferreira ICFR, Vaz JA, Vasconcelos MH, Martins A. Anti-cancer Agents in Medicinal Chemistry, 2010, 10, 424-436. [2] Vaz JA, Heleno SA, Martins A, Almeida GM, Vasconcelos MH, Ferreira ICFR. Food and Chemical Toxicology, 2010, 48, 2881-2884.

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