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Antioxidant activity of *Agaricus bisporus* L. hexane and ethanol extracts obtained by Soxhlet and ultrasound-assisted extraction: the importance of the presence of ergosterol

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Mushrooms are well known for their richness in bioactive molecules such as antioxidants. Phenolic compounds, in particular phenolic acids, have been the most widely studied molecules regarding these effects [1]. Nevertheless, other molecules present in mushrooms, such as ergosterol, can also display bioactive properties [2]. Although being this high-value molecule more associated with hypocholesterolemic, antimicrobial and anti-inflammatory effects [2], it is also relevant to screen other bioactivities, such as antioxidant activity, either of the pure molecule or of mycosterols' rich extracts containing it. *Agaricus bisporus* L. is the most consumed mushroom worldwide, being ergosterol the most abundant mycosterol in its sterol fraction (represents almost 90%) [3]. Herein, *A. bisporus* ethanol and hexane extracts were prepared by Soxhlet and ultrasound-assisted extraction (amplitude: 75%;sonication time: 15 min), and further evaluated for their antioxidant activity. The *in vitro* assays used were: i) 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay, to evaluate the scavenging activity; and ii) ferricyanide Prussian blue assay, to evaluate the reducing power. The obtained extracts were characterized in terms of ergosterol content by HPLC-UV. The antioxidant activity of pure ergosterol was also assessed. The extracts obtained by Soxhlet showed higher antioxidant activity than the ones obtained by ultrasonication, which is in agreement with the higher levels of ergosterol found in the first extracts (677 and 186 mg/100 g dw for ethanol and hexane extracts, respectively). Ethanol extracts revealed higher antioxidant activity than the hexane extracts, which is also in agreement with the higher ergosterol content found in both samples (677 and 672 mg/100 g dw for Soxhlet and ultrasonication extracts, respectively). The pure ergosterol also showed antioxidant activity (e.g., DPPH EC₅₀ value = 0.46 mg/mL). Overall, the ethanol extract obtained by Soxhlet gave the highest DPPH scavenging activity (EC₅₀ = 2.2 mg/mL) and reducing power (EC₅₀ = 0.8 mg/mL), while the hexane extract obtained by ultrasonication revealed the lowest DPPH scavenging activity (EC₅₀ = 16.7 mg/mL) and reducing power (EC₅₀ = 2.2 mg/mL).

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