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Sustainable valorization of phenolic compounds from *Castanea sativa* shells for pharmaceutical application

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Chestnut (*Castanea sativa*) fruits have an enormous global ecological and economic impact, despite the large generation of by-products during its industrialization process, namely shells,¹. On the last years, the valorisation of chestnut shells for nutraceutical purposes arises as a challenge due to its richness in bioactive compounds, particularly phenolics. Subcritical Water Extraction (SWE) is an eco-friendly promising method to extract bioactive compounds using water as solvent, with fast processing time and high yield². Therefore, the aim of this work was to produce a bioactive extract with antioxidant, antiradical scavenging power and antimicrobial activity, as well as low cytotoxic effect on buccal epithelial cell lines (HSC3 and TR146), by optimizing the extraction temperature (110 °C – 180 °C) of *C. sativa* shells through SWE. The optimal temperature of extraction was 110 °C, revealing the highest phenolic and flavonoid contents (239.53 mg of gallic acid equivalents (GAE)/g dry weight (dw) and IC₅₀=148.68 µg/ml, respectively) as well as the highest antioxidant activity (4240.38 µmol of ferrous sulphate (FeS)/g). Additionally, the 110 °C extract showed the higher antiradical activity (IC₅₀=426.88 g/ml for DPPH assay) and a good capacity to scavenge reactive oxygen species, namely HOCl and ROO· (IC₅₀=4.47 g/ml and 0.73 mol of Trolox equivalents/mg dw, respectively). Also, high concentrations of phenolic acids, such as gallic and protocatechuic acids and flavonoids (catechin, epicatechin and rutin), composed the phenolic profile. All extracts obtained demonstrated antimicrobial activity against different microorganisms present in the oral cavity during oral mucositis state, such as *Porphyromonas gingivalis*, *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*. The MTT assay revealed that the lowest IC₅₀ was achieved for the 110 °C extract in the HSC3 and TR146 cell lines (IC₅₀=1325.03 and 468.15 µg/ml, respectively). This work demonstrated the potentialities of SWE to valorize *C. sativa* shells as a valuable source of compounds that may be suited for the potential application for oral mucositis treatment.

Keywords: bioactive compounds, chestnut shells, oral mucositis, sustainability.

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