

GASTROINTESTINAL DIGESTION OF SAMBUCUS NIGRA L. FLOWER AND BERRY BIOACTIVE MOLECULES: IN VITRO AND IN VIVO EVALUATION

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Sambucus nigra L. is a flowering plant, which has been used for centuries as part of folk medicine, due to its bioactive properties. In this work the bioaccessibility of *S. nigra* (flower and berry) extracts after *in vitro* gastrointestinal digestion and their effect on toxicity and bioactive potential were studied. The extractions were performed with distilled water at 90 °C for 30 min. The obtained extracts were exposed to the INFOGEST harmonized *in vitro* digestion protocol. The chemical composition was analyzed before and after the digestion process, in terms of phenolics (TPC), flavonoids (TFC), and anthocyanins; additionally, phenolic compounds profile was determined by UPLC. The antioxidant activity was determined by FRAP, DPPH and ABTS methods. Cell viability and cellular reactive oxygen species (ROS) levels were assessed using normal cell line (L929) and cancerous cell lines (HeLa and HT29). The *in vivo* toxicity was measured by a simple model of *Artemia salina*. The chemical analysis revealed that berries evidence higher content of total extractives than the flower. When comparing the extracts before and after digestion there is an increase in the TPC on both extracts, while a significant decrease on the TFC. The compounds with the higher content were rutin, chlorogenic acid and rosmarinic acid in *S. nigra* flower extract, while in the *S. nigra* berry extract were rutin, resveratrol, ferulic and chlorogenic acid. The effect of the non-digested and digested extracts was significantly different on the different cell lines. The IC₅₀ of L929 cells was the highest, indicating low toxicity. The IC₅₀ of HeLa and HT29 cells was lower, particularly the extract obtained from the flower upon the digestion. These results indicate that these natural compounds can be used as adjuvants in anticancer therapy. In the presence of an oxidant agent tBHP, only the berry extract was able to significantly reduce the formation ROS in L929 cell line, while on the HeLa cells all the extracts were able to reduce it. The *in vivo* assay demonstrated a dose-dependent effect for all extracts. The berry digested aqueous extract induced the lowest mortality rate in all concentrations evaluated. The promising results obtained on the chemical and biological evaluation of the extracts indicate that the natural compounds isolated from *S. nigra* by-products can be used as potential ingredients for functional food formulations, and/or as bio-therapeutic agents.

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Palavras-chave : *Sambucus nigra*, Bioaccessibility, Toxicity, Gastrointestinal digestion, Phenolic compounds, Antioxidant activity