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Assessment of nutraceutical potential of a chestnut by-product towards circular economy – *In-vitro versus in-vivo* studies †

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Abstract: The increasing demand for nutraceuticals has pressured the industry to seek new pro-healthy compounds. Agro-industrial residues generated across the food supply chain have been explored as sources of added-value molecules, reducing their environmental impacts, and creating additional economic revenue towards the implementation of circular economy [1]. Chestnut (*Castanea sativa*) shells (CS) are an exceptional source of antioxidants [2,3]. Although the European legislation on nutraceuticals' validation remains vague, an in-depth assessment of *in-vitro* and *in-vivo* bioactivity should be accomplished, with metabolomics arising as a valuable tool. This study explores the nutraceutical potential of CS extract prepared by Subcritical Water Extraction (SWE) after *in-vitro* simulated digestion and *in-vivo* bioavailability assays on rats (50 and 100 mg/kg body weight, *per os* administered once daily for 7 days) using metabolomic techniques by LC-ESI-LTQ-Orbitrap-MS and LC/DAD-ESI-MS. Antioxidant activity was evaluated by spectrophotometric assays, while hypoglycemic and neuroprotective properties were assessed using commercial kits. The extract optimization and safety on intestinal cells were attested in our previous work [2]. The relationship between metabolomic fingerprinting and potential oxidative stress biomarkers was ascertained by multivariate analysis. The results unveiled higher phenolic concentrations retained after intestinal digestion, reaching 40% of bioaccessibility. The metabolomic profiling sustained the antioxidant, hypoglycemic, and neuroprotective effects observed before and after *in-vitro* digestion probably ascribed to phenolic acids and hydrolyzable tannins metabolites. Considering animal studies, metabolites from phase I and II reactions were identified in rat tissues, mainly derived from phenolic acids, flavonoids, and lignans. The multivariate analysis predicted the outstanding contribution of phenolic metabolites to the *in-vivo* antioxidant responses. This study supports the use of CS extract as potential anti-aging ingredient for prevention of oxidative stress-triggered diseases in nutraceuticals.

Keywords: *Castanea sativa*; phenolic compounds; *in-vitro* digestion; animal studies; metabolomic.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data used to support the findings of this study can be made available by the corresponding author upon request.

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