Antioxidant activities of different by-products of two Sicilian *Citrus x limon* (L.) Osbeck varieties and in vivo toxicity on zebrafish embryos

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Citrus x limon (L.) Osbeck is a widely cultivated[1] and used species for its nutritional value and beneficial properties of its fruits. It is harvested in southern Italy, particularly in Sicily, where there are two most representative IGP varieties: "Femminello siracusano" (FS) and "Femminello zagara bianca" (FZB). The agro-industrial production of lemon juice usually generates large quantities of by-products. These can still contain interesting metabolites that are abundant in this species, such as polyphenols, with anti-inflammatory and antioxidant properties, effective in the prevention of various diseases[2,3]. The aim of this work was therefore to compare the phenolic profile of the by-products (skin, albedo, seeds, and pulp) of the two Sicilian cultivars at the ripe and unripe stages, to identify the by-product and ripening stage with the highest phenolic fraction and biological activity.The by-products were extracted by sonication with 50% EtOH and the total polyphenol content (TPC) and relative radical scavenging capacity were determined using spectrophotometric assays. In addition, detailed UHPLC-HRMS characterization revealed the presence of phenolics, coumarins, and 3-hydroxy-3-methylglutaryl flavonoids (HMG-flavonoids) as major metabolites of peels, seeds, pulp, and albedo. The results obtained after multivariate analysis (PCA and PLS) identified the peels of the early ripening stage of FZB as the most phenolically active. A semi-quantitative analysis of the most representative secondary metabolites of the extract was then carried out using UHPLC-UV. Finally, the FZB extract was subjected to an in vivo toxicity test on zebrafish (Danio rerio) embryos to gain a better understanding of the primary chemical bioactive of *Citrus x limon* and their safety in vivo.

References

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