

OLEAF4VALUE – Polyphenol-rich extracts from olive leaves modulate gut microbiota composition and metabolism in the in vitro MicroColon model, and show protective effects on the intestinal barrier function

Introduction

Olive production creates around 4.5 million tons of leaves each year. Although rich in bio-resources, only around 0.2% is currently used for extracts. The OLEAF4VALUE project aims to extract high value bioactive compounds (polyphenols, triterpenoids, essential oils, lipids, lignocellulose) and assess their health benefits. Polyphenols can act as prebiotics on the gut microbiota, and they are indicated to possess antioxidant activity and to suppress inflammation in intestinal cells.

Olive leaf extracts were screened for effects on gut microbiota composition and activity in the NIZO MicroColon technology, a high-throughput in vitro screening model. Experiments with and without spiking of the pathogen *Clostridioides difficile* into the healthy adult fecal inoculum were performed. Microbiota composition was analyzed by qPCR and 16S rRNA gene sequencing. Microbial metabolism was analyzed by short-chain fatty acid analysis (HPLC) and polyphenols metabolites analysis by UHPLC-MS/MS. Spent MicroColon supernatant, containing the fermentation derived metabolites was applied to a co-culture model that combines the human intestinal epithelial cell line Caco-2 with the human monocyte/macrophage cell line THP-1, to assess effects on barrier function and immune modulation.

Results

Results show that both microbial composition and metabolism can be modulated by olive leaf extracts. Oleuropein enriched extracts (20-40%) stimulated e.g. typical butyrate producers, which was accompanied by an increase in butyrate production. Notably, growth of the intestinal pathogen *C. difficile* was shown to be inhibited by several extracts. The oleuropein enriched (20%) extract showed the strongest effect on *C. difficile* inhibition and did not affect beneficial bifidobacteria abundance. Cell culture experiments with differentiated Caco-2 monolayers and THP-1 cells in co-culture, showed protective effects of some extracts/pure polyphenols and MicroColon spent supernatant on recovering intestinal barrier function after an inflammatory challenge.

Conclusion

In conclusion, olive leaf extracts tested show gut microbial modulatory properties, with potential beneficial effects on the host. More in-depth analyses such as shotgun metagenomics, metatranscriptomics analysis, and polyphenols metabolites analysis, are ongoing to better understand the mechanisms, and to substantiate the effects.