

# Evaluation of Açai and Maca Extracts for CYP3A4 Enzyme Induction

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The most abundant liver cytochrome P450 isoform is CYP3A4, an enzyme responsible for the metabolism of at least 60% of approved drugs. Notably, most anticancer agents are metabolized by CYP3A4. Increased consumption of botanical dietary supplements (BDS) has been observed to increase CYP induction and metabolism, and therefore decrease efficacy of drugs like anticancer agents. The purpose of this investigation was to establish the degree of CYP3A4 induction caused by two extracts commonly used in BDS by cancer patients and their potential for botanical-drug interactions.

Maca root (*Lepidium meyenii* Walp) has historically grown in the Andes Mountains and has been consumed as part of the local diet. Recent research suggests that maca root has anti-inflammatory and cancer cell growth inhibitory properties. Açai (*Euterpe oleracea* Mart) is a berry-producing palm grown across South America. Medicinal use of açai has gained popularity in North America due to its antioxidant activity. Current research has suggested that phenolic acid and other antioxidants in açai have antiproliferative activity on cancer cells. Both maca and açai were investigated for CYP3A4 induction potential and effects on the efficacy and toxicity of anticancer agents in the liver.

Methanol and acidic methanol extracts of maca and açai were prepared and subject to parallel artificial membrane permeability assay (PAMPA). PAMPA is an *in vitro* model for simulating the intestinal absorption of bioavailable compounds via passive diffusion. The donor site of its 96-well plate simulates intestinal content pre-absorption and non-passively diffused compounds post-absorption, while the acceptor site simulates the compounds that passively diffuse the intestinal membrane. PAMPA was used to separate the passively diffused CYP-inducing constituents of açai and maca in the acceptor site from non-passively diffused CYP3A4-inducing constituents in the donor site. These extracts were then subjected to investigation on human hepatocyte cells to determine the extent of CYP3A4 mRNA expression.

Initially, CYP-induction from açai and maca were added to the PAMPA donor site at concentrations of 1.5  $\mu\text{g}/\mu\text{L}$  and 0.75  $\mu\text{g}/\mu\text{L}$  in buffer and incubated at 37°C for five hours. Then, samples from the PAMPA donor and acceptor sites were applied to hepatocyte cell lines and incubated for 22 hours in the initial induction assay. Hepatocytes treated with both donor and acceptor site extract samples underwent RNA extraction and qPCR analysis to determine mRNA expression of CYP3A4. Only açai extracts significantly induced CYP3A4 compared to the DMSO control. Cytotoxicity was noticed in açai methanol and acidic methanol at 1.5  $\mu\text{g}/\mu\text{L}$  treatments after 20 hours' incubation.

When both extracts were subjected to PAMPA, passively diffused compounds in açai methanol extract at 1.5  $\mu\text{g}/\mu\text{L}$  showed significant CYP3A4 induction in hepatocytes after PAMPA assay, while maca extracts exhibited inhibitory activities. Finally, induction potential for non-passively diffused and passively diffused compounds in both açai extracts were compared. Higher hepatic CYP3A4 induction was found in liver cells treated with non-passively diffused constituents in the donor site. This finding suggests that other mechanisms such as ion channels and active transporters are involved in CYP3A4-induced intestinal transportation. In short, these results suggest that açai has the potential to produce botanical-anticancer drugs interactions by the mechanism of CYP3A4 induction.

For future investigation, elucidation of the chemical profile of the compounds that pass the PAMPA membrane and induce CYP3A4 is being considered. Specifically, identifying these compounds will require analysis via liquid chromatography-mass spectroscopy and Mass Profiler software. These data will be compared to data regarding CYP3A4 inhibition in human liver microsomes due to maca and açai extracts. The experiment may be repeated using manufactured maca and açai supplements to investigate the efficacy of these findings in mass produced botanical products available to the public consumer as compared to laboratory-prepared extracts.

**Statement of Research Advisor:**

Betty was trained on the CYP3A4 inhibition and induction assays to carry out the project and learned how to review and analyze papers from the literature about CYP3A4 inhibition and induction and about biological activity and chemistry of maca and açai. Her study was focused on the assessment of the interference of liver metabolic enzymes by two top botanicals in the U.S. market of dietary supplements and their potential to produce interactions with drugs.

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