

# Identification of Phase I & Phase II Metabolites of Maca Constituents

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Maca (*Lepidium meyenii* Walpers) is a plant cultivated in central Peru, and its root is a common active ingredient in the botanical dietary supplements for its effects on a variety of health issues such as sexual dysfunction, anemia, and cancer. The plant contains compounds that have cytotoxic and antioxidant activities, but information on the metabolism of the bioactive compounds is limited. The goal of this study was to identify Phase I and Phase II metabolites of the bioactive constituents of maca extracts, such as induction and inhibition of CYP3A4 and their interactions with anticancer drugs, for further study.

To predict the interactions of the maca compounds and their metabolites with anticancer drugs, Phase I and Phase II reactions of maca constituents were conducted, and the resulting metabolites were monitored and assessed for their increase in polarity and decrease in toxicity as well as possible inhibition of CYP3A4. Before performing the metabolic experiments, the permeability of the components was determined by incubating three different extracts in dichloromethane, methanol, and acidic methanol with buffer in a parallel artificial membrane permeability assay (PAMPA), which mimics the role of intestinal membrane. Then, the compounds were categorized into two groups: those that passively diffused across the membrane and those that did not. Both groups were incubated with human liver microsomes, phosphoric buffer at pH of 7.4, and nicotinamide adenine dinucleotide phosphate (NADPH) and/or uridine 5'-diphosphoglucuronic acid (UDPGA) to initiate Phase I and Phase II reactions and to obtain corresponding metabolites. Furthermore, for the Phase II experiment, alamethicin was added to the sample to activate UDPGA sequestered in the hepatocytes, and saccharolactone was added to inhibit glucuronidase, which reverses the Phase II reaction. The obtained metabolites of maca were analyzed with rapid resolution liquid chromatography, and their identity was confirmed via various layers by comparing obtained liquid chromatography (LC/MS) and mass spectrometry (MS/MS) data to previously published literature or database and elucidating the structure of a compound with ACD/Spectra software.

The permeability test illustrated that among twenty-seven known chemical constituents of maca, six chemical constituents showed high permeability—these compounds have passively diffused through the membrane from donor site to acceptor site—while other twenty-one constituents displayed low permeability. The second part of the experiment suggested the presence of twenty-three metabolites of maca chemical constituents, and further analysis with MS/MS, database, literature, and chemical software identified and confirmed eight metabolites produced via Phase I and Phase II reactions of maca extract. Among these eight metabolites, five metabolites were produced through hydrolysis and oxidation while three metabolites were produced through glucuronidation. While NMR analysis will provide further structural confirmation, a method to discern the compounds should be organized prior to the analysis, since botanical extract contains a wide range of compounds that are not necessarily bioactive or of interest. After finalizing the confirmation of produced metabolites, the samples will be tested for its possible inhibiting effect against CYP3A4 and will be incubated with and without anticancer drugs to observe the inhibition and interaction of the metabolites. The study provides a confirmed chemical profile of maca that may offer insight into active compounds that are known to be effective against cancer.

## Statement of Research Advisor:

Da has developed an approach to assess the formation of Phase I and Phase II metabolites of intestinally permeable maca constituents and to elucidate the corresponding structure with mass spectrometry. The maca metabolites identified in this study have not been reported in the primary literature. The established approach will help to in the prediction of any potential maca-anticancer drug interactions.

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