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## Biotransformation of tangeretin mediated by human liver cytochrome P450 enzymes

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Tangeretin is a flavonoid found in citrus plants. Structurally, it presents an O-polymethoxylated derivative of flavone. It has been extensively studied due to the possible beneficial effects on human health that include neuroprotective, anti-inflammatory, antiasthmatic, antidiabetic, anticancer and other properties. Although metabolism and pharmacokinetics of tangeretin has been studied, due to numerous metabolic products, not all metabolic reactions of tangeretin have been well characterized.

Thus, the objective of this work was to characterize tangeretin metabolism mediated by cytochromes P450. For this purpose, human liver microsomes (HLM) and recombinant cytochrome P450 enzymes were used. Metabolism was monitored by liquid chromatography coupled with mass spectrometry (electron spray ionization, time of flight detection) for metabolites determination and diode array detector for quantification.

Tangeretin generated more than 10 metabolites in the incubations with HLM. The largest number of detected metabolites refers to single and double demethylated tangeretin derivatives in various combinations and at different positions on the rings A and B. Two major metabolites, most commonly found in tangeretin incubations with HLM, were indirectly identified based on data obtained by LC-MS/MS analysis. MS/MS spectra showed a loss of -14.01564 Da on the ring B with the retention time of 9.52 min. Since only one methoxy group at position 4' is present in the tangeretin ring structure B, this metabolite was characterized as a 4'-demethylated tangeretin derivative, i.e., 4'-hydroxy-5,6,7,8-tetramethoxyflavone. Similarly, the structure of the second most commonly found metabolite detected at 7.83 min showed a mass increase of 1.9799 Da on the ring B, indicating demethylation at the 4' position and hydroxylation at the positions 3' and the formation of 3',4'-dihydroxy-5,6,7,8-tetramethoxyflavone.

Tangeretin metabolism was mediated by CYP1A2, CYP2D6, and CYP3A4 enzymes. Based on the amount of metabolite produced, it can be concluded that the most important enzymes are CYP1A2 and CYP3A4.

**Keywords:** Tangeretin, cytochrome P450 enzymes, metabolism

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## Binding of berberine and sanguinarine with G-quadruplex and duplex DNA revealed by surface-enhanced Raman spectroscopy

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Surface-enhanced Raman scattering (SERS) spectroscopy was applied to study binding of natural alkaloid molecules, berberine and sanguinarine, with G-quadruplex (G4) DNA structure. The antiparallel "basket" type G4 structure was obtained upon annealing the human telomeric sequence $d[TTAGGG]_4$ (Tel24) in presence of sodium ions. In addition, the alkaloids selectivity for four-stranded over double-stranded DNA was investigated using calf thymus (ct) DNA. The SERS spectra of the alkaloid ligands, the DNA structures and their complexes in the [ligand]/[DNA] molar ratio of 1/1, 1/3 and 1/6 were acquired upon NIR excitation in a citrate-reduced silver colloid aggregated with sodium sulfate.

The same slight decrease in the SERS intensity of berberine bands, regardless of the [ligand]/[ct-DNA] molar ratio, implied very weak interactions with ct-DNA, associated with structurally nonspecific binding of the berberine molecules along the phosphate helix. Moreover, the SERS spectra of sanguinarine with ct-DNA resembled the spectrum of the ligand alone, not indicating any interactions of the sanguinarine molecules with the nucleic acid. On the other hand, notable spectral changes were observed for the complexes of the alkaloid molecules with G-quadruplex, particularly pronounced for the complexes of the [ligand]/[G4] molar ratio 1/3. While significant diminution in the overall SERS intensity indicated stacking of the berberine molecules onto the G4 structure, the upward shift of the guanine bands implied interactions of the sanguinarine molecules with the G4 grooves. The observed SERS spectra clearly pointed to different binding modes of berberine and sanguinarine with antiparallel G-quadruplex as well as to higher affinity of both alkaloid molecules for G-quadruplex over duplex DNA.

**Keywords:** SERS, DNA, G-quadruplex