Purification of antioxidant from cherry leaf by high-speed counter-current chromatography and on-line HPLC/DPPH radical scavenging assay

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Abstract

In this study, the identification and purification of antioxidant compound from cherry leaf was proposed by a novel strategy of high-speed countercurrent chromatographic (HSCCC) purification for the efficient and effective discovery of antioxidant from natural product based on on-line HPLC method with radical scavenging assay. To achieve a strategy for HSCCC purification, the antioxidants in cherry leaf extract are identified by on-line HPLC with DPPH radical scavenging assay. Then, the optimal condition of one targeted peak would be investigated for the two-phase solvent system (tert.-butyl methyl ether/acetonitrile/0.1% TFA in water, 2/2/3, V/V/V), and purified by HSCCC. The purification of this antioxidant (3.0 mg) from cherry leaf extract (30 mg) was performed by HSCCC with optimal two-phase solvent system. Using mass spectrometric and nuclear magnetic resonance analysis, this antioxidant was identified to 3-O-caffeoylquinic acid. Due to the advantages derived from on-line HPLC with DPPH radical scavenging assay and HSCCC technique, a rapid, efficient and effective strategy has been developed for the discovery of antioxidants from natural products.