

**Antioxidants Discovery for Differentiation of Monofloral Stingless Bee Honeys
Using Ambient Mass Spectrometry and Metabolomics Approaches
education**

ABSTRACT

Stingless bee honey (SBH) is a natural, sweet product produced by stingless bees (Meliponini tribe) that has been used as a traditional medicine to treat various illnesses. It has been shown that SBH has high nutritional value and health-promoting properties due to the presence of plant bioactive compounds from different botanical flora of the foraged nectar. In this study, the antioxidant activities of seven monofloral honeys from acacia, agarwood, coconut, dwarf mountain pine (DMP), Mexican creeper (MC), rubber, and starfruit botanical origins were investigated. The antioxidant properties of SBH studied had a range from 19.7 to 31.4 mM TE/mg for DPPH assays, 16.1 to 29.9 mM TE/mg for ABTS assays, 69.0 to 167.6 mM TE/mg for ORAC assays, and 45.5 to 89.3 mM Fe²⁺/mg for FRAP assays. Acacia honey showed the highest level of antioxidant properties. The models built from mass spectral fingerprints from direct ambient mass spectrometry showed distinct clusters of SBH by botanical origin and correlated with the antioxidant properties. An untargeted liquid chromatography-mass spectrometry (LC-MS) metabolomics approach was undertaken to identify the antioxidant compounds that could explain the unique antioxidant and compositional profiles of the monofloral SBH by its botanical origin. The antioxidants that were identified predominantly consisted of alkaloids and flavonoids. Flavonoid derivatives, which are potent antioxidants, were found to be key markers of acacia honey. This work provides the fundamental basis for the identification of potential antioxidant markers in SBH associated with the botanical origin of the foraged nectar.