

Identification of Potent Anti-Inflammatory Compounds in Propolis by Chemometric Analysis

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Abstract

Introduction: Propolis is a resinous substance produced by honeybees and traditionally used for its anti-bacterial and anti-inflammatory properties. However, its complex and variable chemical composition has limited the development of standardized therapeutic formulations. This study aimed to identify the most potent anti-inflammatory compounds of propolis by combining chemical profiling and functional immune assays.

Methods: A total of 52 ethanolic extracts of propolis from Australia, Denmark, and Norway were analyzed using high-performance liquid chromatography (HPLC). Eight polyphenols with reported anti-inflammatory properties were used as reference standards. Human peripheral blood mononuclear cells (PBMCs) were stimulated with lipopolysaccharide (LPS) or phytohemagglutinin-L (PHA), and the effect of propolis extracts and individual polyphenols on cytokine secretion was assessed using ELISA and MesoScale multiplex assays. Partial least squares (PLS) regression was used to relate HPLC signals to IL-1 β concentrations, predicting the most potent anti-inflammatory compounds. These predictions were subsequently confirmed *in vitro*.

Results: Propolis samples from Australia, Denmark, and Norway reduced IL-1 β secretion by 13, 41, and 37%, respectively. PLS analysis identified four signals most strongly associated with IL-1 β inhibition. Among these, model selection based on adjusted R² and the corrected Akaike Information Criterion (AICc) highlighted caffeic acid and chrysin as the most potent contributors. The anti-inflammatory potency of these two compounds were confirmed *in vitro*. Additional cytokine profiling revealed that propolis decreased IL-1 β , IL-10, and IFN- γ while increasing IL-4, IL-6, and TNF- α , indicating a complex immunomodulatory.