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PP-351 [Apitherapy]

Rigshospitalet

UNIVERSITY OF COPENHAGEN

Uncovering the Most Active Anti-Inflammatory Compound **Propolis.** A Combined Chemical and Immunological Approac

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Background

Propolis is a resinous bee product traditionally used for its anti-inflammatory effects. Its complex and variable composition, however, poses challenges for developing standardized therapies.

Aim

To identify the most potent anti-inflammatory compounds in propolis by combining HPLC-based chemical profiling with functional immune assays.

Experimental

Ethanolic extracts of 52 propolis samples from Australia, Denmark, and Norway were analyzed by HPLC. 8 polyphenol standards were used as benchmark compounds. PBMCs were stimulated with LPS, and IL-1β levels were measured by ELISA. PLS regression linked HPLC signals to IL-1β concentrations, followed by in vitro confirmation of predicted compounds.

Results

Scandinavian propolis reduced IL-1β more effectively than Australian samples. PLS analysis highlighted four signals linked to IL-1β inhibition, with caffeic acid and chrysin emerging as the strongest contributors. In vitro tests confirmed their effects.

Conclusion

Caffeic acid and chrysin are key anti-inflammatory compounds in propolis. Integrating chemical and functional data enables the identification of bioactive constituents and supports propolis as a source of immunomodulatory agents.

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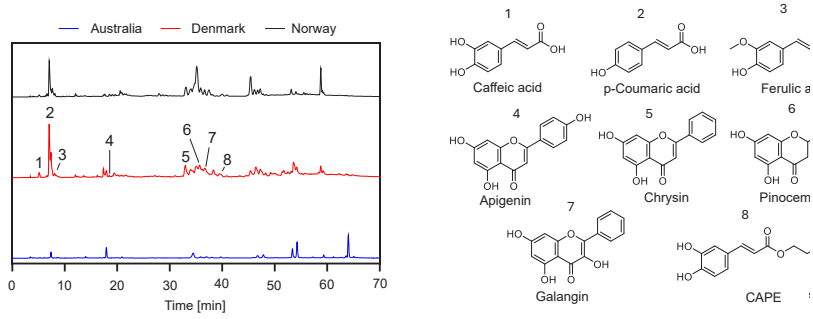
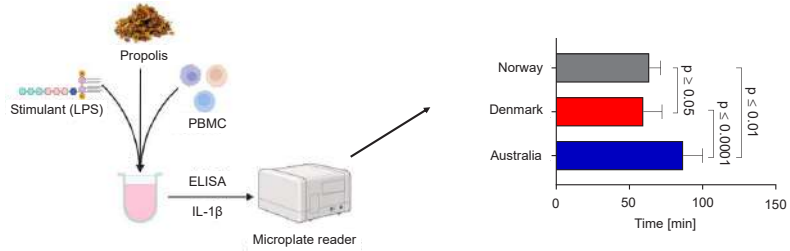
(1) HPLC chromatograms of propolis samples and identified polyphenols

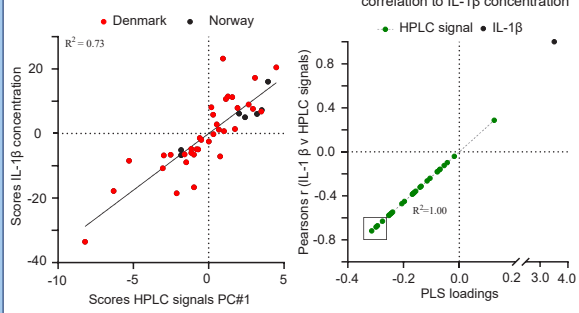


(2) Testing of propolis samples anti-inflammatory activity in human immune cells (PBMC)

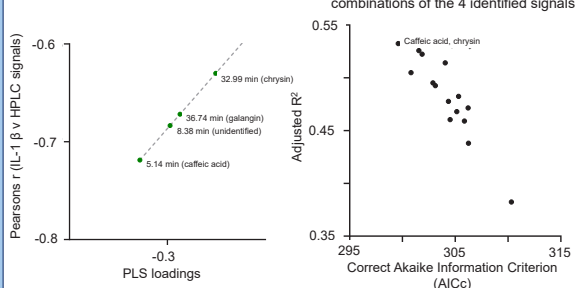


(3) Correlate HPLC data with anti-inflammatory activity

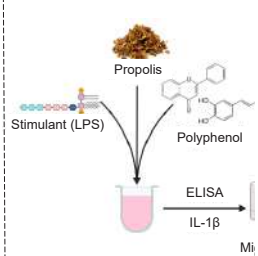
Step 1: HPLC data (X) v IL-1β concentration (Y) **Step 2:** Loadings plot and each loadings correlation to IL-1β concentration

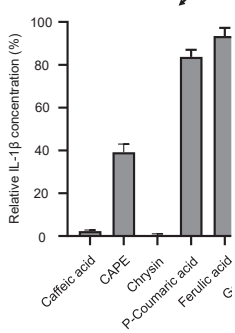


Step 3: Zoomed in loadings/correlations plot **Step 4:** Comparison of models using combinations of the 4 identified signals



(4) Test polyphenols anti-infla effect to confirm caffeic acid a are most potent of all comp





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Discovery of Novel Anti-Inflammatory Compounds in Propolis

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Background
Immunosenescence increases immune activity with age, possibly contributing to diseases such asrheumatic disorders and cancer. These conditions are typically managed with NSAIDs, corticosteroids, and cytostatic agents. However, side effects often limit long-term use, highlighting the need for safer alternatives. Propolis, a resinous substance produced by bees, has well-documented anti-inflammatory properties and represents a potential alternative.

Problem description
This study investigated whether propolis contains novel compounds with anti-inflammatory activity.

Methodology
The immunosuppressive effects of ethanol-extracted propolis were evaluated by measuring its ability to inhibit IL-1 release from human peripheral blood mononuclear cells (PBMCs) stimulated with LPS or PHA. The composition of propolis was analyzed using reverse-phase HPLC. Multivariate data analysis was applied to HPLC signals and anti-inflammatory data. Principal Component Analysis (PCA) was used to analyze the full HPLC chromatograms from all samples. The relative anti-inflammatory effect of each compound was estimated based on HPLC peak concentration and potency.

Results & CONCLUSION: Propolis completely inhibited IL-1 release, whereas dexamethasone achieved a 70% reduction. Propolis samples from within each region showed slight variations, whereas samples from different regions varied more significantly. Danish and Norwegian samples exhibited greater activity (~26%) than Australian samples (~5 %), likely due to higher polyphenol content. Reverse-phase HPLC identified bioactive compounds, including p-coumaric acid and pinocembrin, which inhibited immune activity by 19.9% and 16.0%, respectively. Mass spectrometry is currently being used to analyze three unidentified compounds, which are estimated to contribute 8.3%, 7.7%, and 7.2% to the inhibitory activity of propolis.

Practical Implications for Beekeepers
Identifying novel anti-inflammatory compounds in propolis could enhance its medical applications and commercial potential. Beekeepers can leverage this knowledge to promote high-quality propolis with potent bioactive properties, increasing its value in nutraceutical and pharmaceutical markets. Recognizing regional variations in propolis composition may enable producers to market distinct varieties for targeted health applications, further strengthening its commercial and therapeutic potential.