



Spectroscopic and Chemometric Profiling of Propolis and Cerumen Across Species and Regions.

Key differences identified using rPLS and STOCSY.

Jonas Vind¹, Søren Balling Engelsen², Henrik Munch Jørgensen¹, Julie Christine Antvorskov³, Knud Josefsen¹, Violetta Aru².

Affiliations: ¹Department of Pathology, The Bartholin Institute, Copenhagen University Hospital, Copenhagen, Denmark, ²Department of Food Science, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg C, Denmark, ³Department of Clinical Research, Steno Diabetes Center Copenhagen, Translational Type 1 Diabetes Research, Herlev, Denmark

Background

Propolis and cerumen are complex mixtures of beeswax, plant resins, and secretions, with composition influenced by geography and bee species. Understanding these differences is key to identifying antioxidant compounds.

Aim

To compare the composition and antioxidant-related compounds in Scandinavian (SC) and Australian (AU) *Apis mellifera* propolis and Australian *Tetragonula carbonaria* cerumen using phase-resolved ¹H NMR and recursive weighting partial least squares regression (rPLS).

Experimental

Samples were extracted using a modified Folch method to yield hydrophilic and hydrophobic phases for ¹H NMR analysis. Composition was assessed by PCA and wax structure by signal ratios. RSA data were modeled using full PLS and rPLS, and key signals were validated by standard addition.

Results

Scandinavian propolis had more aromatic compounds; Australian propolis had more carbohydrates; cerumen was rich in terpenoids. Wax content was highest in Australian propolis, but only *T. carbonaria* wax differed structurally. rPLS identified ferulic acid and p-coumaric acid as RSA-linked, with higher levels in Scandinavian samples.

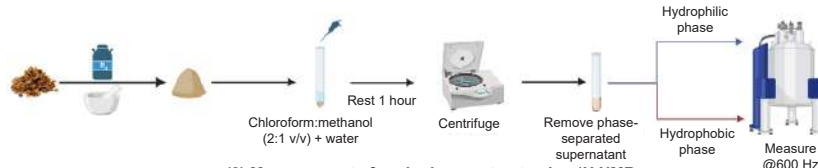
Conclusion

¹H NMR combined with rPLS effectively revealed species- and region-specific differences in propolis and cerumen. Ferulic acid was identified as a key antioxidant in the aqueous phase, reflecting both compositional and RSA variation.

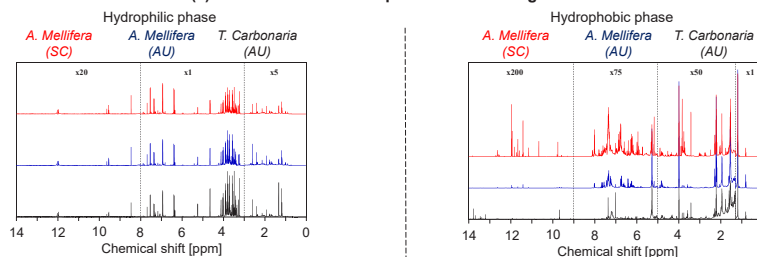
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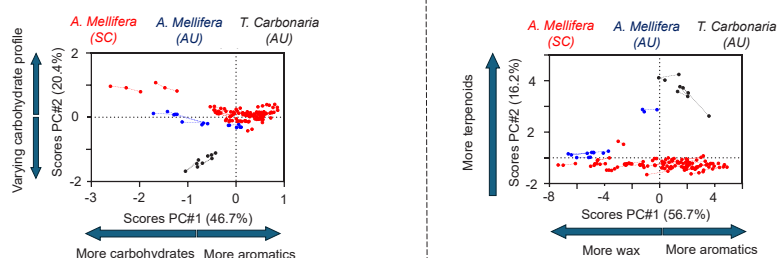
(1) Two phase (hydrophilic and hydrophobic) extraction protocol for propolis samples



(2) Measurement of each phase extract using ¹H NMR

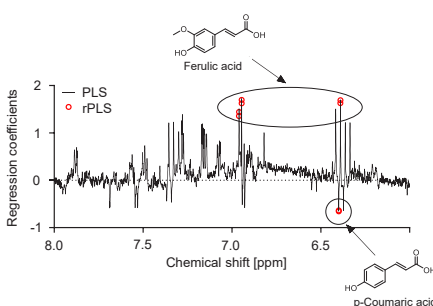


(3) Comparison of chemical composition using PCA

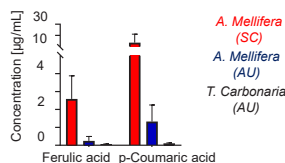


(4) Performance of rPLS to find most important anti-oxidative compounds in hydrophilic phase

A Full PLS and an rPLS model was created and compared. They performed similarly (PLS: RMSE_{cv} = 8.26%; rPLS: RMSE_{cv} = 7.99 %), and the selected signals in rPLS thus is indicative of important compounds for anti-oxidative effect of propolis.

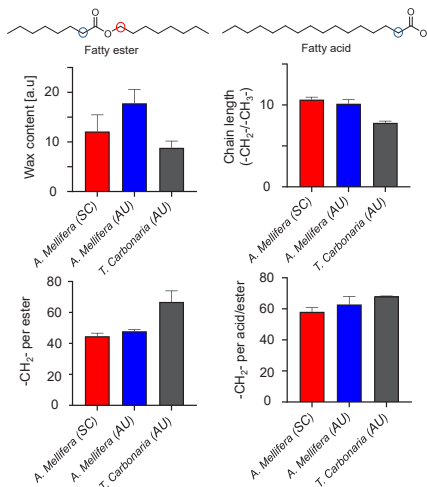


(5) Quantification of identified polyphenols



(4) Comparison of beeswax in propolis and cerumen samples

Wax analysis of propolis and cerumen samples. Wax content was defined by CH₂ signal intensity in ¹H NMR; chain length as the CH₂/CH₃ ratio; chain length per fatty ester as the CH₂ to red-circled protons ratio; and chain length per fatty ester and acid as the CH₂ to blue-circled protons ratio.



Jonas Pordel Vind¹, Violetta Aru², Henrik Munch Jørgensen¹, Julie Antvorskov³, Hasim Tekin¹, Knud Josefsen¹, Søren Balling Engelsen²

¹Bartholin Institute, Rigshospitalet Copenhagen Biocenter, Ole Maaløes Vej 5, Copenhagen N, Danmark

²Department of Food Science, University of Copenhagen, Rolighedsvej 2, 1958 Frederiksberg, Danmark

³Steno Diabetes Center, Herlev Hospital, Borgmester Ib Juuls Vej 1, Herlev, Denmark

Background & Purpose

Propolis, a resinous substance produced by bees, is valued for its antioxidant properties due to its high polyphenol content. However, its composition and biological activity vary by geographic origin. A rapid and reliable method for assessing its antioxidative properties would facilitate its application in health-related fields. This study aimed to identify the most effective spectroscopic technique for screening and predicting antioxidant activity.

Problem description

This study aimed to identify the most effective spectroscopic technique for screening and predicting antioxidant activity.

Methodology

Propolis samples were collected from Australia (N = 8), Denmark (N = 38), and Norway (N = 7). Four spectroscopic techniques were evaluated: infrared (IR), Raman, near-infrared (NIR), and proton nuclear magnetic resonance (¹H NMR) spectroscopy. IR, NIR, and Raman spectroscopy were performed on raw propolis, while ¹H NMR spectra were recorded from chloroform extracts. Antioxidant activity was assessed using the DPPH free radical scavenging assay, which measured radical scavenging activity (RSA). Spectral data were analyzed for correlation with RSA, and predictive models were developed for each technique.

Results & CONCLUSION: IR, Raman, and NIR spectroscopy revealed that Danish and Norwegian propolis contained higher polyphenol levels than Australian samples, which had the highest carbohydrate content. Consistently, Danish and Norwegian propolis exhibited greater RSA. The strongest correlations between spectral signals and RSA were found at 1,631 cm⁻¹ (Raman) and 1,666 nm (NIR). Among the tested techniques, NIR spectroscopy demonstrated the highest accuracy in predicting antioxidant activity (R² = 0.75).

Practical Implications for Beekeepers

A rapid and reliable method for estimating the antioxidant potential of propolis could help beekeepers identify and market high-quality samples, increasing their commercial value and applications in health and nutrition. Spectroscopic screening provides a fast, non-destructive alternative to conventional chemical analysis, enabling the differentiation of high-quality propolis. This approach could facilitate the development of high-value propolis-based products and expand their role in functional foods, nutraceuticals, and pharmaceuticals.