

CHEMICAL MARKERS IN ITALIAN PROPOLIS: CHRYSIN, GALANGIN AND CAPE AS INDICATORS OF GEOGRAPHIC ORIGIN

Allodi M¹, Cappellucci G², Baini G², Miraldi E², Costantino G¹, Spaggiari C¹, Biagi M^{1,2}
 1) Department of Food and Drug, University of Parma, Parco Area delle Scienze, 43124 Parma, Italy;
 2) Department of Physical Sciences, Earth and Environment, University of Siena, Via Laterina, 8, 53100 Siena, Italy.

METHODS

Bee propolis, a natural resinous material, is widely known for its medicinal and biological benefits. Despite variations in its botanical and geographical origin, propolis consistently shows antioxidant, immunomodulatory, and antimicrobial properties [1]. However, these general properties are insufficient for its rational application in modern medicine, highlighting the need for further research.

A comprehensive research is essential to explore the chemical and pharmacological properties of propolis, focusing on its mechanisms of action, specific targets, and the unique phytocomplexes that vary with its geographical origin.

The aim of this work, focused on Italian propolis (generally poplar-type), is to better investigate and to clarify the chemical composition of samples collected in different geographical areas, providing an update and deeper insight into the current literature.

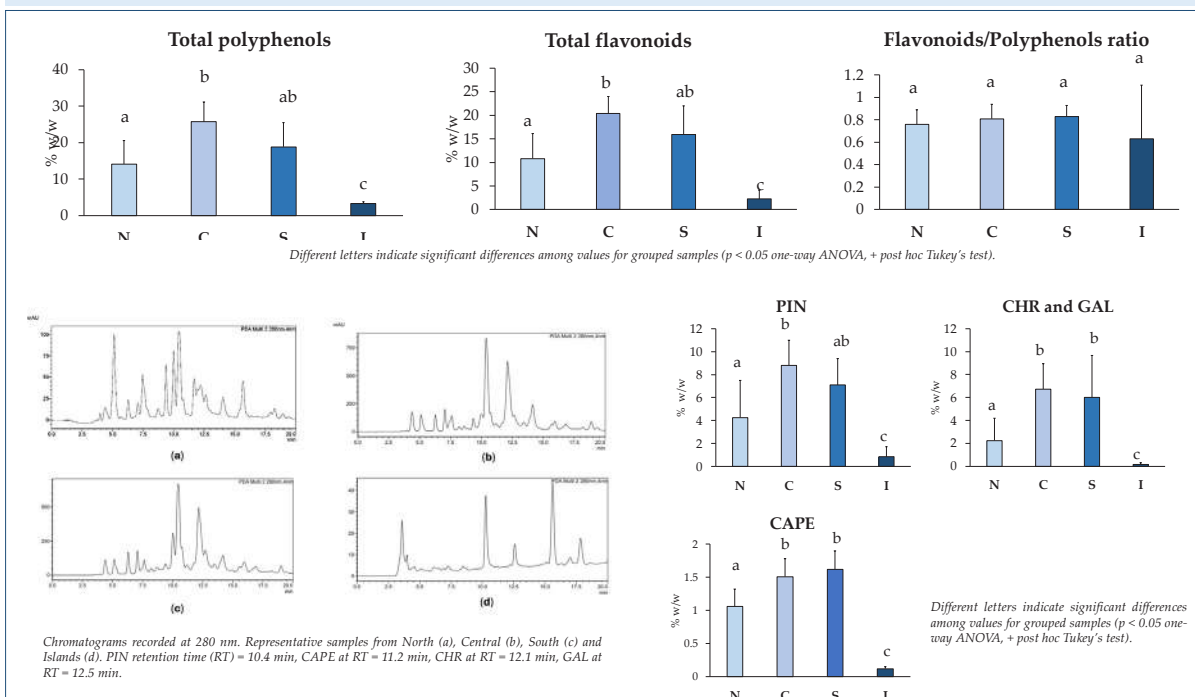
Twenty-seven propolis samples were collected in the same year (2020) throughout Italy and were grouped by geographical areas: Northern (N), Central (C), Southern regions (S) and Islands (I). The cleaned and dewaxed samples were solubilized in EtOH 75% (v/v) at a concentration of 10 mg/mL.

TPs analysis was performed following the Folin-Ciocalteu assay, measuring absorbance at 700 nm. Gallic acid was used as standard and results were expressed as % w/w gallic acid equivalent.

For **TFs analysis**, samples were diluted 1:200 in 75% ethanol and absorbance was recorded at 353 nm. Galangin was used as the standard and results expressed as % w/w galangin equivalent [2;3].

For the **HPLC analysis**, a gradient elution mode was employed using water and methanol, both acidified with 0.1% FA, as mobile phases. Chromatographic detection was performed at 280 nm. Samples were diluted at a 1:10 ratio, filtered (0.44 µm) and injected (10 µL). Peak identification was carried out by comparing retention times and UV spectra with reference standards: pinocembrin (PIN), chrysin and galangin (CHR and GAL), caffeic acid phenethyl ester (CAPE).

RESULTS



CONCLUSION

This study demonstrates that simple and rapid UV methods, such as total polyphenol (TPs) and total flavonoid (TFs) analysis, can effectively distinguish propolis from different geographical regions. Poplar-type propolis is primarily characterized by a high polyphenol content, particularly flavonoids, with significant regional variations. Despite these differences, flavonoids consistently represented the main phenolic subclass, maintaining a similar TF/TPs ratio across samples grouped by geographical area. Furthermore, HPLC-DAD analysis of key markers enabled a more precise determination of propolis origin.

In conclusion, while this research provides a solid foundation for developing more advanced methods to assess and authenticate the geographical origin of propolis, it remains a preliminary study. Further investigations are required to explore specific subregions and refine the analysis beyond the major constituents.

Marika Allodi¹, Giorgio Cappellucci², Giulia Baini², Elisabetta Miraldi², Gabriele Costantino¹, Chiara Spaggiari¹, Marco Biagi¹

¹Department of Food and Drug, University of Parma, Parma, Italy

²Department of Physical Sciences, Earth and Environment, University of Siena, Siena, Italy

Bee propolis, composed of terpenes, wax and phenolic compounds, despite variations in its botanical and geographical origin, is widely known for its antioxidant, immunomodulatory and antimicrobial properties. However, these general properties are insufficient for its rational application in modern medicine and comprehensive research is essential to explore the chemical and pharmacological properties of propolis, focusing on its mechanisms of action, targets, and the unique phytocomplexes that vary with its geographical origin. The aim of this work, focused on Italian propolis, is to better investigate and to clarify the chemical composition of samples collected in different geographical areas, providing an insight into the current literature. It also aims to develop methods for the identification of chemical markers in Italian propolis and to determine their geographic origin to ensure the quality of propolis product. Analyzing 27 samples collected in the same year from various regions (Northern, Central, Southern Italy, Capraia Island and Sicily), the total polyphenol (TP) and total flavonoid (TF) content, alongside the quantification of pinocembrin, chrysin, galangin, and caffeic acid phenethyl ester (CAPE) were investigated. Additionally, DPPH assays were conducted to evaluate the antiradical activity of propolis samples. Our results revealed that TP and TF vary significantly in samples from different regions and that pinocembrin is almost always present in Italian propolis, although at different levels. However, a more precise differentiation of the geographical origin was achieved through the quantification of the sum of chrysin and galangin, and CAPE. Conversely, the DPPH assay showed largely uniform results lacking statistical significance.

This study contributes to the growing research on propolis by providing a more structured approach to characterizing its chemical composition by geographic origin. The results offer valuable insights into the development of more refined methods for distinguishing the origin of propolis, which may be useful for quality control, regulatory frameworks, and commercial evaluation. Building on this preliminary study, future research should aim to expand the scope by comparing sub-areas such as river regions, Apennine and Alpine propolis, as well as samples from inland hives. Additionally, significant efforts should focus on enhancing the analytical depth beyond the examination of major constituents.