



A CHEMOMETRIC APPROACH: CHARACTERIZATION AND CLASSIFICATION OF COLOMBIAN PROPOLIS BASED ON INFRARED SPECTROSCOPY

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REMARKS

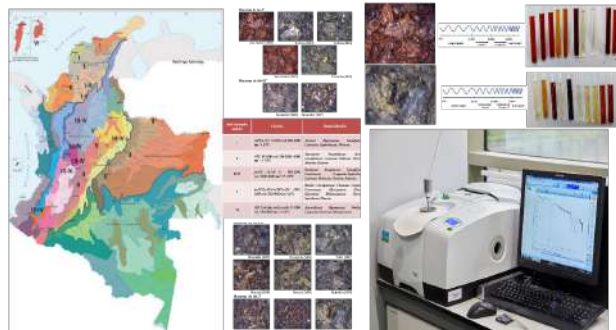
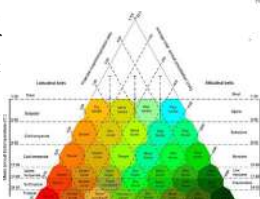
Propolis is a complex material of resinous consistency produced by bees which has a highly variable physical appearance, color, and consistency, depending on many factors such as geographic origin, types of vegetable sources, time of collection, and season of the year. Bees use propolis to seal openings in the hive to avoid the entrance of intruders, to maintain a constant inner temperature, to contribute to the attainment of an internal aseptic environment, and overall to protect the hive from widespread bacterial infection. Chemical composition is extremely complex and variable, showing the presence of beeswax, resin, essential oils, and pollen. Its composition is highly variable because of the richness and versatility of tropical flora. Therapeutic properties of propolis are well known, with antimicrobial, anti-inflammatory, anti-oxidative and cytotoxic qualities.



IR spectroscopy provides a global chemical "fingerprint" that captures information about all major classes of compounds. Also allows the study of the variability in the chemical composition, making it essential to use rapid, non-destructive, and reproducible techniques for its characterization. This approach provides molecular fingerprints that reveal the presence of functional groups associated with bioactive compounds, supporting authentication, quality control, and the correlation of chemical profiles with biological activities. The aim of this study is to apply FT-IR spectroscopy for the characterization of Colombian propolis through its ethanolic extracts, considering their relevance in tincture preparation.

MATERIALS AND METHODS

A total of 45 Colombian bee propolis were collected from different biogeographical zones of Holdridge's wild life: montan lower wet forest (*ml-WF*), premontane wet forest (*pm-WF*), Tropical rainforest (*t-RF*), Very humid low montane forest (*vh-LMF*), Premontane very humid forest (*pm-VHF*), Low montane dry forest (*lm-DF*) and Tropical dry forest (*t-DF*) in Caribbean, Andean and Inter-Andean Region.

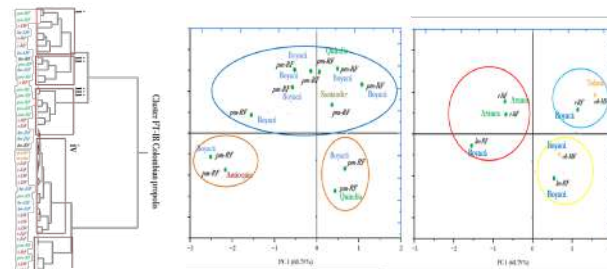
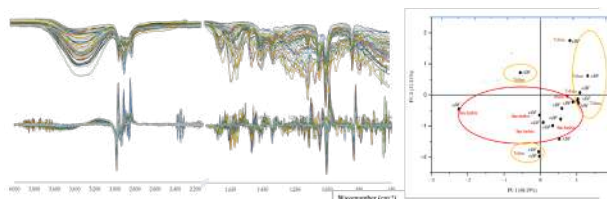
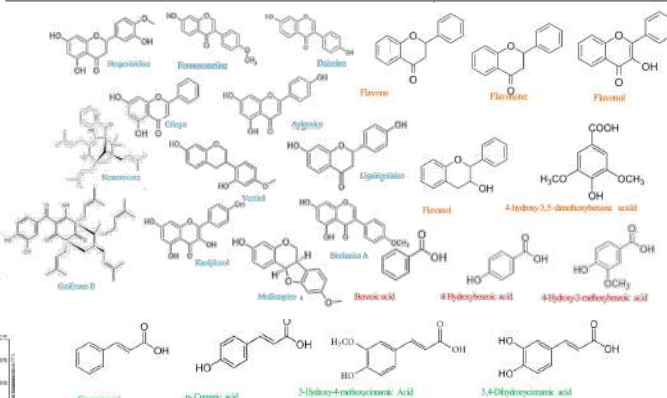


Ethanolic extract were obtained by cavitation process y mixing 5 g of raw propolis sample, with 25 mL of ethanol (96%/1 h/25°C). Fourier-transform infrared spectroscopy (FTIR) analyses were performed by using a Perkin Elmer Spectrum One spectrometer (Perkin Elmer, USA), in range from 4000 to 650 cm^{-1} / resolution of 4 cm^{-1} In each spectrum, 16 scans were co-added. The data were analyzed by FT-IR Spectrum software (Perkin Elmer). Once the spectra were registred, baseline correction treatment was applied to minimize the difference between the spectra during the baseline change, smoothed and normalized to reduce noise in spectral data and regularization. Chemometric evaluations were performed using OriginPro 2024™ and JMP Pro17 software

RESULTS AND DISCUSION

The FT-IR spectra of the ethanolic propolis extracts showed characteristic bands associated with major secondary metabolites, mainly phenols and polyphenols. The main signals included aliphatic $-\text{CH}_2$ and $-\text{HCH}_2$ vibrations (2930, 2876 cm^{-1}), carbonyl $\text{C}=\text{O}$ (1660–1720 cm^{-1}), hydroxyl $-\text{OH}$ (3200–3650 cm^{-1}), and substituted aromatic rings (1500–1600 cm^{-1}). Additional bands were observed at 3300–3600 cm^{-1} ($-\text{OH}$ groups of flavones and isoflavonoids), 1215 cm^{-1} ($\text{C}-\text{O}$ ester stretching), 1440–1231 cm^{-1} ($\text{C}=\text{C}$ aromatic vibrations), and 758 cm^{-1} (CH_2 stretching of aliphatic chains).

Band flavanones	cm^{-1}	Band flavonols	cm^{-1}	Band flavanols	cm^{-1}
$\nu(\text{C}-\text{H})$	3287-3060	$\nu(\text{C}-\text{H})$	3000-3070	$\nu(\text{C}-\text{H})$	3135-2910 cm^{-1}
$\nu(\text{C}=\text{O})$	1670-1625	$\nu(\text{C}=\text{O})$ de anillo B	1702-1654	$\nu(\text{AR}-\text{C}=\text{O})$	1670-1625 cm^{-1}
$\nu(\text{C}=\text{C})$	1620-1560	$\nu(\text{C}=\text{C})$ de anillo	1615-1611	$\nu(\text{C}=\text{C})$ de anillo B	1620-1560 cm^{-1}
$\nu(\text{C}=\text{C})$	1510-1480	$\delta(\text{C}-\text{H}) + \nu(\text{C}=\text{C})$ de anillo B	1520-1516	$\nu(\text{C}=\text{C})$ de anillo A	1530-1510 cm^{-1}
$\nu(\text{C}=\text{C}) + \nu(\text{C}=\text{O})$		$\nu(\text{C}=\text{C})$	1362-1354	$\delta(\text{HCC})$ de anillo C	
$\nu(\text{C}=\text{C}) + \nu(\text{C}=\text{O})$	1380-1350	$\nu(\text{C}=\text{O}) + \nu(\text{C}=\text{C})$	1293-1252	$\delta(\text{HCC})$ de anillo C	
$\nu(\text{C}=\text{C})$	1250-1200	$\nu(\text{C}=\text{C})$	1184-1180	$\nu(\text{C}=\text{C})$ de anillo A y B	1300-1235 cm^{-1}
$\nu(\text{C}=\text{C})$	740-700	$\nu(\text{C}=\text{O}) + \nu(\text{C}=\text{C})$	1046-1043	$\nu(\text{HCC})$ de anillo C	975-960 cm^{-1}
$\delta(\text{CCC})$ de anillo	635-610	$\nu(\text{C}=\text{C})$		$\nu(\text{HCC})$ de anillo B	880-845 cm^{-1}
				$\nu(\text{HCC})$ de anillo A	780-770 cm^{-1}
				$\delta(\text{CCC})$ de anillo A	545-515 cm^{-1}



Chemometric analysis using PCA and HCA successfully discriminated Colombian propolis ethanolic extracts. PCA showed that the main variance was explained by wavenumbers related to phenolic and flavonoid vibrations, while HCA grouped the samples into five consistent clusters. These results confirm that FT-IR spectroscopy, combined with multivariate tools, provides reliable spectral fingerprints for differentiating propolis according to biogeographical origin.

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Propolis is natural product produced by bees from resins collected from buds or plant exudates; it is used to seal cracks and reduce the risk of disease within the hive. The medicinal properties of propolis are well known, with antimicrobial, anti-inflammatory, anti-oxidative and cytotoxic qualities. Fourier transform infrared spectroscopy (FTIR) has become one of the most useful analytical tools because of its merits, including rapid detection, ease of operation and cost savings. It is based on changes in vibrational and rotational modes. The objective of the present work has been focused on the systematic and chemometric evaluation of ethanol extracts of Colombian propolis by means of attenuated total reflectance (ATR-FTIR) spectroscopy techniques. The evaluations proceeded by assessing the spectral signals of the extracts. All spectra were obtained in the range 4000 cm⁻¹ to 600 cm⁻¹. In the chemometric study, multivariate analysis techniques were applied, operating mainly in the spectral region of the fingerprint (1800 - 600 cm⁻¹) operating also with the zero order, first and second derivative registers. ATR-FTIR, sample analysis has revealed, spectral patterns of ethanolic extracts of Colombian propolis, thanks to the use of spectroscopy techniques, leading to the establishment of differences and similarities between samples, the use of these tools allows applications for routine analysis.