



CHARACTERIZATION OF COLOMBIAN PROPOLIS: FINGERPRINTING BY ELECTROSPRAY IONIZATION MASS SPECTROMETRY (ESI-MS)

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REMARKS

Propolis is recognized worldwide and has been registered in the Chemical Abstracts Service (CAS) 9009-62-5, as a natural product elaborated by different genera of bees, from mucilaginous exudations, gums, lipophilic materials, latex and resinous plant material, which they bring to their hives and mix with glandular secretions from the hypopharynx and wax glands.

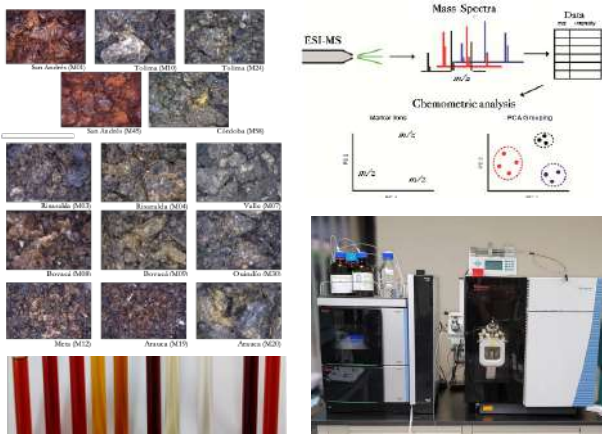


Mass spectrometry has been used in conjunction with gas chromatography (GC/MS) for many years for the analysis of the main volatile and semi-volatile components of propolis. Electrospray ionization (ESI) is a technique used in mass spectrometry to produce ions using an electrospray in which a high voltage is applied to a liquid to create an aerosol. It is especially useful in producing ions from macromolecules because it overcomes the propensity of these molecules to fragment when ionized. ESI(-)MS fingerprinting focuses on the more polar and acidic components of the sample, which is of special importance for the study of propolis.

MATERIALS AND METHODS

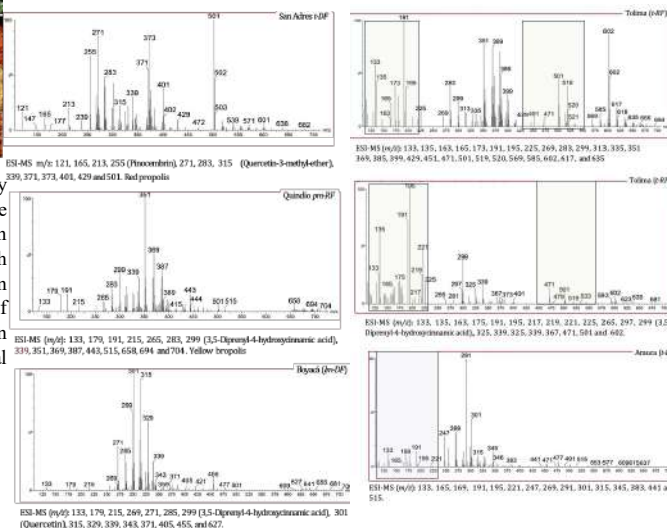
A total of 45 Colombian bee propolis were collected from different biogeographical zones of Holdridge's wild life: montan lower wet forest (*ml-WF*), premontane wet forest (*pm-WF*), Tropical rainforest (*t-RF*), Very humid low montane forest (*vh-LMF*), Premontane very humid forest (*pm-VHF*), Low montane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

Ethanol extract was obtained by cavitation process y mixing 5 g of raw propolis sample, with 25 mL of ethanol (96% v/v h/25°C). The dry propolis extracts were dissolved in a solution of 70% (v/v) chromatographic grade methanol (Tedia, Fairfield, OH, USA), 30% (v/v) deionized water and 0.1% ammonium hydroxide. The solutions of propolis were infused directly into the ESI source by means of a syringe pump (Harvard Apparatus) at a flow rate of 10 µl min⁻¹. ESI(-)MS fingerprints were obtained on a hybrid Micromass Q-TOF mass spectrometer under the following conditions: capillary and cone voltages were set to -3000 V and -40 V, respectively, with a de-solvation temperature of 100°C. Although fingerprints were acquired in the m/z 100 – 1000 range, no important ions were observed below m/z 100 or above m/z 700, therefore ESI(-)MS data is shown in this range.

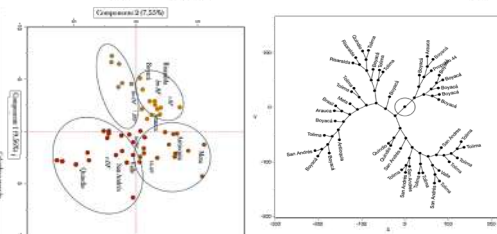


RESULTS AND DISCUSSION

Electrospray ionization mass spectrometry (ESI-MS), in either negative or positive mode, offers significant analytical potential. Ionization is achieved by applying a high electrical charge to the sample. Phenolic compounds generally exhibit higher sensitivity and better fragmentation in the negative ion mode. Since phenolic fingerprint compounds are predominantly detected in this mode, it becomes essential to apply discrimination processes that reveal associations within Colombian propolis samples. The observed fragmentation patterns are consistent with those previously reported in the literature.



Compound	m/z	Compound	m/z
Chlorogenic acid	353	Quercetin	301
Caffeic acid	179	Quercetin-3-methyl-ether	315
Diethylsuccinic acid	515	Kaempferol	285
Ellagic acid	301	Isochaerone	315
p-Coumaric acid	163	Kaempferol-methyl-ether	299
Ferulic acid	193	Kaempferol-methoxy-methyl-ether	329
Isolonic acid	195	Pinobanksin-5-methyl-ether-3-O-pentanoate	289
Tricloroacetic acid	677	Pinobanksin-7-methyl-ether-5-O-phthalocyanepropionate	292
Benzoic acid	121	Quercetin-3-methyl-ether	329
3,4-Dimethyl-coumaric acid	207	Quercetin-3-methyl-ether	350
Ellagic acid	301	Galangin-3-methyl-ether	283
Rhamnetin	167	Quercetin-3-methyl-ether	329
Chromydicarboxylic acid	173	Galangin	269
3-phenyl-p-coumaric acid	232	Kaempferol	299
Caffeic acid isopropyl ester	247	Flavonol glycosides	m/z ESI (-)
Caffeic acid benzyl ester	269	Quercetin-3-O-rutinoside	609
Caffeic acid phenethyl ester	283	Quercetin-3-O-glucuronide	477
p-Coumaric acid isopropyl ester	231	Quercetin-3-O-glucoside	465
Pinobanksin	271	Kaempferol-3-O-rutinoside	593
Caffeic acid dimethyl ester	295	Isochaerone-3-O-rutinoside	623
p-Coumaric acid dimethyl ester	279	Isochaerone-3-O-pentanoate	447
Apetillin C	299	Quercetin-3-O-rutinoside	447
3-Phenyl-4-(2-methylpropionyl-oxo)-cinnamic acid	315	Isochaerone-3-O-glucuronide	491
3-Phenyl-4-(2-hydroxypropionyl-oxo)-cinnamic acid	363	Kaempferol-methyl-ether-3-O-glucoside	461
Methoxy cinnamic acid dimethyl ester	293	Isochaerone-3-O-acetyl-rutinoside	665
p-Coumaric acid-4-hydroxyphenylethyl ester dimer	565		
Di-hydroflavonols	m/z ESI (-)		
Pinobanksin-5-methyl-ether	285	Quercetin-3-O-glucuronide	491
Pinobanksin-5-methyl-ether-3-O-acetate	327	Quercetin-3-methyl-ether-3-O-rutinoside	637
Pinobanksin-5,7-dimethyl-ether	271	Kaempferol-3-O-rutinoside	593
Pinobanksin-5,7-dimethyl-ether	299	Pinobanksin-3-O-acetate-5-O-phthalocyanepropionate	292
Pinobanksin-3-O-acetate	292	Pinobanksin-5-methyl-ether-3-O-pentanoate	289
Flavonones	m/z ESI (-)	Pinobanksin-7-methyl-ether-5-O-phthalocyanepropionate	292
Pinobanksin-5-methyl-ether	269		
Pinobanksin	255	Flavones	m/z ESI (-)
3-Hydroxy-5-methoxyflavone	289	Luteolin	285
		Apigenin	269
		Luteolin-5-methyl-ether	299
		Chrysin	313
		Acacetin	351



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