NEW INSULAR RED PROPOLIS FROM COLOMBIA: BOTANICAL ORIGIN, BIOLOGICAL AND CHEMICAL MARKERS

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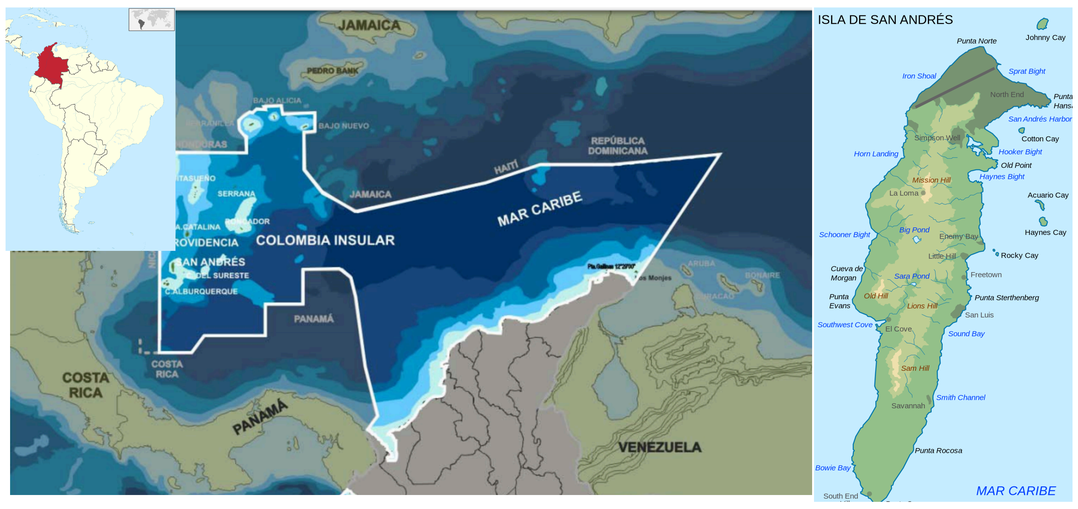
Introduction

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. Tropical propolis has chemistry quite different from that of temperate-zone propolis. Moreover, its composition is highly variable because of the richness and versatility of tropical flora. The red propolis (PR), is a particular type that has proven an important biological activity under the metabolites according to their origin. It has been report as a special product from Cuba (2001), Venezuela (Andes Trujillo State, 2004), Brazil (Mangrovea area also in Paraiba, 2007 and 2008), México (Champoton, Campeche, 2010), China (Shandong, 2012) and Nigeria (Ogun, Delta, Rivers, Cross Rivers, 2017).

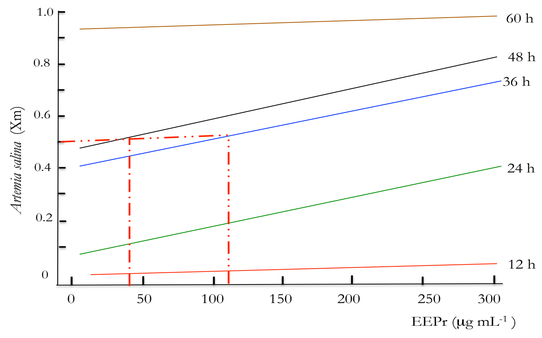
A wide spectrum of biological activities of propolis has been revealed, such as antimicrobial, anti-inflammatory, antitumor, immune-modulating and wound-healing. The interest in the medicinal and nutritional properties of propolis expanded among people from several countries. in which several markers have been identified similar chemicals. Today there are no studies respect to propolis in San Andres Isla, Colombia, their botanical origin and relationship between chemical composition.

Materials and methods

Seven (7) red propolis samples (RP) were evaluated five from insular area of San Andrés Island, (12º 35´00´´N; 81º42´00´´) and two from Marechal Deodoro, Alagoas Northeastern Brazil, that was used as a reference.



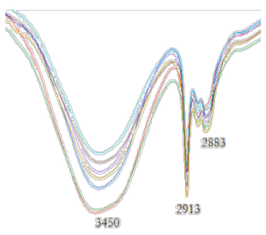
Alcoholic extracts: Ethanolic PR were obtained by mixing 5 g of raw propolis with 25 mL of ethanol (96%/72 h/25 °C). Phytochemical screening was performed for phenols, pyrogallic and phlobaphene tannins, anthocyanin, anthocyanidin, flavones, flavonols, xanthones, chalcones, aurones, flavononols, leucoanthocyanidins, catechins, flavanones, steroids, triterpenoids and saponins. HPTLC: Extracts of RP (3 µL) were applied to the 20 × 10 cm. HPTLC plates as 5 mm. Development was performed toluene-ethyl acetate-formic acid (30:12:5, P´3.39). Infrared Spectroscopy was performed using FTIR analysis was done in a Perkin Elmer Spectrum GX, 16 scans per samples in the region of (4000-650) cm^{-1}. **Phenol and flavonoids:** UV and IRFT. **Biological activity.** Cytotoxicity assay *Artemia salina* (nauplii) lethality was determined using RP extract, (50, 100, 150, 200 and 300) µg/mL Genotoxicity assay Allium sepa L. (Amarilydaceae) mitotic phase, 50 a 300 µg/mL 24-72h.



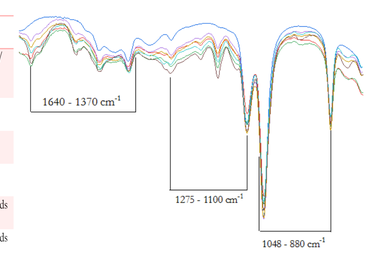
Cytotoxicity showed effects on *Artemia salina* from 12 and up to the 60 h of exposure. The reduction of 50% of the population exposed (CL_{50}) occurs at about 36 and 48 h of exposure (CL_{50})36h, 114 (g mL^{-1}) and (CL_{50})48h 50 (g mL^{-1}).

Results

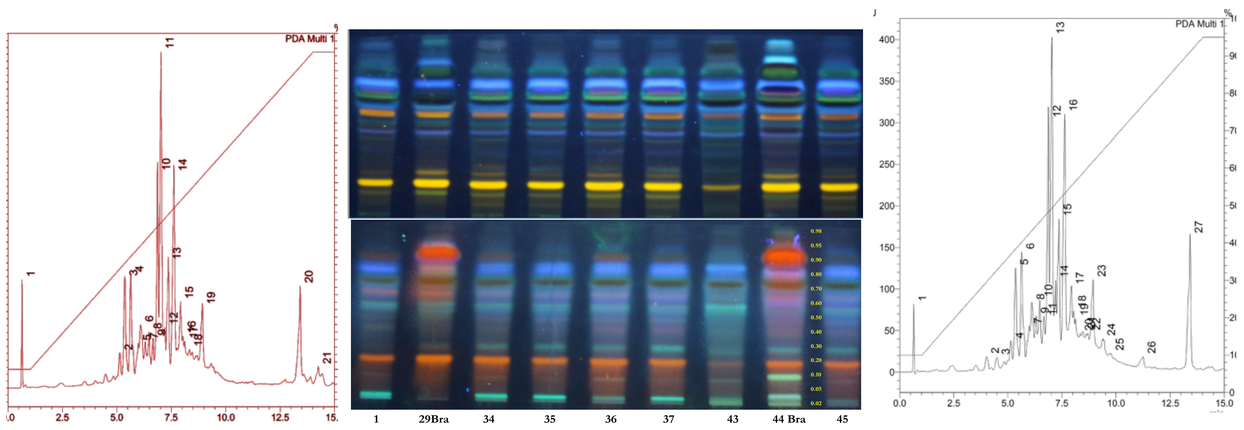
Prospection of the phytochemical components of EEP from San Andres and its fractions detected the presence of phenolic compounds (Phenols flavonoids, flavones and flavonols). Total EERP 23.7 g/100 g. T. Phenols (Folina-Ciocalteu): 233.3 mg EGA/g and Flavonoids (Aluminiun tricloride): 50 mg E.Q/g Extract. IR-FT show particular absorption 3450, 1637 and 1633 also 1275 and 1048 cm^{-1}



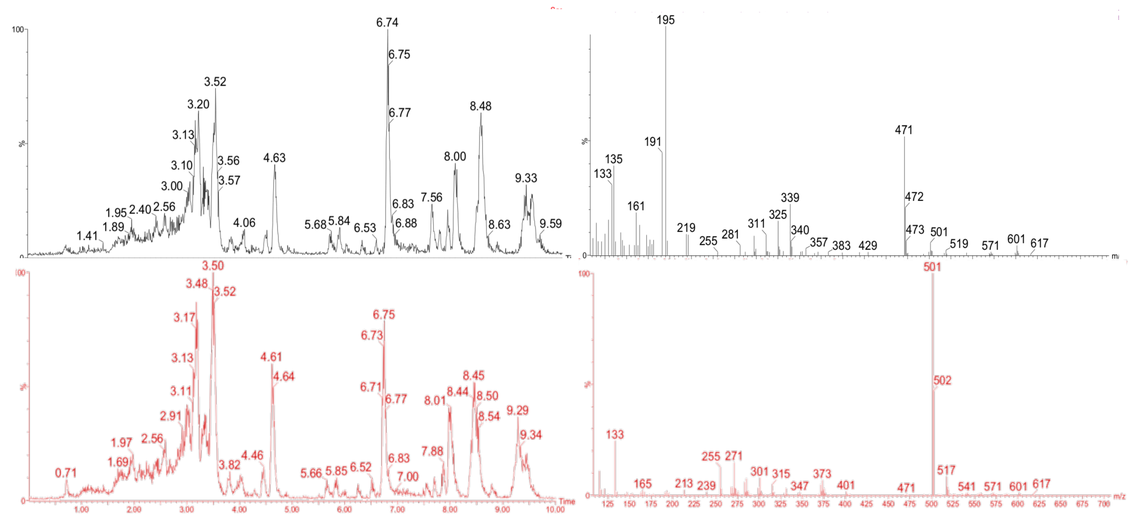
Band (cm^{-1})	Vibration	Main attibution	Band (cm^{-1})	Vibration	Main attibution
3450	ν (O-H)	OH	1496/1440	δ (C-H) ν (aromatic)	CH_3, CH_2, Flavonoids/ Isoflavonoids ring
2913	ν_{as} (C-H)	CH_2 Saturation	1375	δ (C-H)	CH_3, Flavonoids/ Isoflavonoids ring
2883	ν_s (C-H)	Hydrocarbon	1269	δ (O-H), δ_{ip} (C-CO)	
1633	ν (C=O)	Flavonoids /Isoflavonoids	1162	ν(C-O), δ (C-OH)	-OH groups
1637	ν (C=O) ν (C=C)	Flavonoids /Isoflavonoids ring	1088	ν (C-C), δ (C-OH)	Flavonoids/Isoflavonoids
1513	ν (aromatic)	Phenols/Flavonoids /Isoflavonoids ring	1047	ν (=C-O-C), ν (C-C), δ (C-OH)	Flavonoids/Isoflavonoids



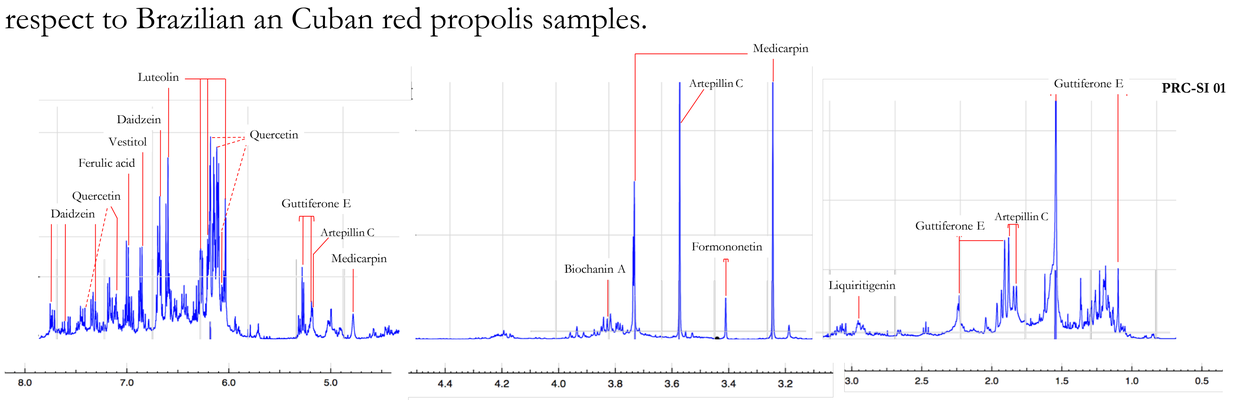
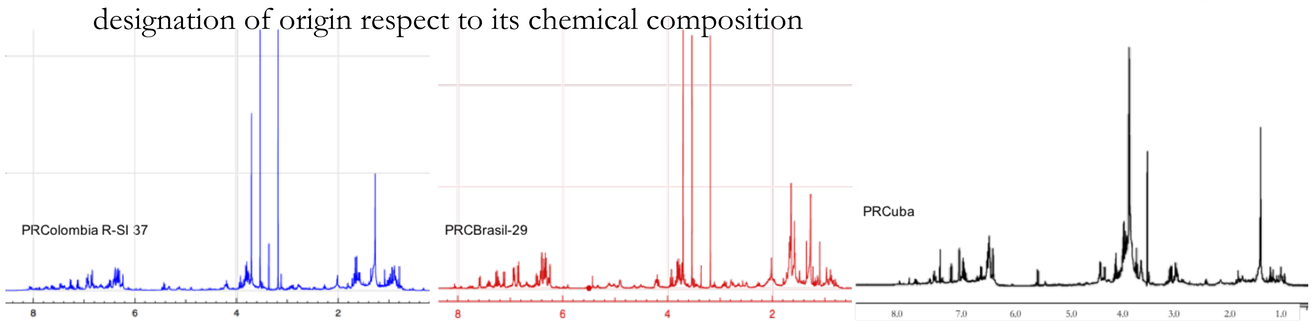
HPLC and HPTLC show 21 chemical compounds and ESI(-)-MS, and LC-ESI(-)-MS/MS of colombian red propolis, have the same chemical finger print and characteristic ion fragmentation patterm at *m/z:* 213, 255, 269, 271, 283, 313, 315, 339, 371, 373, 401, 429, 501, 502 y 503), for pinocembrine, biochanine A (283), formononetin (267), daidzein, They have also been identified in red propolis samples in the northeast of Brazil, Alagoas/Sergipe).



In addition there were signs characteristics of molecular markers coincident with liquiritigenin (m/z: 119, 135, 255), vestitol, neovestitol, artellipin C (m/z: 200, 255, 299), quercetin (m/z: 151, 169, 179, 193, 211, 213, 229, 239, 257, 273, 283), guttiferone E, *p*-coumaric acid (*m/z:* 93, 119, 163), el 3-methoxy-4-hydroxy cinnamaldehyde (*m/z:* 105, 121, 133, 134, 149, 162, 177), el 2,2-dimethyl-6-carboxyethenyl-2H-1-benzopyran (*m/z:* 146, 160, 170, 174, 185, 211, 229), á 3-prenyl-4-hydroxy cinnamic acid (*m/z:* 119, 132, 176, 187, 231), crisin (*m/z:* 107, 119, 143, 145, 208 y 253), pinocembrin (*m/z:*107, 136, 145, 151, 164, 171, 187, 213, 231 y 255), 3,5-diprenyl-4-hydroxy cinnamic acid (*m/z:* 145, 200, 244, 255, 284 y 299) and dicaffeoylquinic acid (*m/z:* 135, 155, 161, 173, 179, 191, 353 y 515).



Inspection of the ^{1}H-NMR spectra allows one to obtain information regarding chemical heterogeneity of the sample as highlighted for the discrepant respect to resonance at the aliphatic and aromatic region. Qualitative analytical pattern by ^{1}H-NMR for Colombian are different respect to Brazilian an Cuban red propolis samples.



The propolis of San Andres Island, Colombia is a new type of matrix with potential pharmaceutical interest, which could be presented as a product with a geographical designation of origin respect to its chemical composition