XXV th International Conference on

Polyphenols

Polyphenols Communications 2010

Volume 2

Editors :
Agnès AGEORGES
Véronique CHEYNIER
Patricia LEFER
Pascale SARNI-MANCHADO

Montpellier - France, 24 th - 27 th August 2010

Propolis phenolic profile: a study in the Atlantic islands of Azores

Soraia I. Falcão[1,2], Susana M. Cardoso[3], Maria R.M. Domingues[4], Cristina Freire[1], Miguel Vilas-Boas[2]*
[1]Departamento de Química e Bioquímica, Faculdade de Ciências do Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal
[2]CIMO - Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Sta. Apolónia, Apartado 1172, 5301-855 Bragança, Portugal
[3]CERNAS-Escola Superior Agrária, Instituto Politécnico de Coimbra, Bencanta, 3040-316 Coimbra, Portugal
[4]Centro de espectrometria de massa Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal
*Corresponding author: mvboas@ipb.pt

Abstract. This work outlines a characterization of the phenolic composition of the Azorean islands propolis. For that, eleven samples from S. Miguel and Terceira islands, were extracted and characterized. According to the HPLC analysis, all samples showed a similar phenolic profile, with 37 compounds identified by ESI-MS^n analysis and UV spectra. Although the samples revealed a close phenolic composition, significant differences were found in their concentrations, probably due to variations in flora distribution around the beehive, and therefore, in resin availability for bees.

Introduction. Propolis is a natural resinous substance that honeybees (*Apis melifera*, L.) collect from buds and exudates of plants and transform in the presence of bee enzymes. This substance plays an important role in the hive as a construction and defense material due to its chemical and biological properties [1]. The propolis composition is extremely complex and dependent on its vegetal source, geographical location and climatic conditions. Typically, this natural product includes phenolic reach resins (50%), beeswax (30%), essential oils (10%), pollen (5%) and other organic compounds (5%) [2].The abundance of phenolic compounds in this natural product, together with the evidences of their biological and pharmacological properties, focused the research interests of propolis in this group of substances.

Materials and Methods
Sampling. Eleven propolis samples were obtained from local beekeepers in 2009 from *Apis mellifera* hives of S. Miguel and Terceira islands. The samples were stored at -20 °C until analysis.

Extraction of phenolic compounds. The propolis was grounded and homogenized. The samples were extracted with 80% of ethanol/water (1/10, w/v) at 70ºC for 1h, the resulting mixture was filtered and the residue was re-extract in the same conditions. The filtrated solutions were combined, concentrated, frozen at -20ºC and freeze-dried.

HPLC-DAD phenolic profile analysis. The propolis extract was analyzed and fractionated by reversed-phase high-performance liquid chromatography (HPLC) with DAD detection, according with our previous work [3]. Quantification was achieved using calibration curves for caffeic acid, ferulic acid, quercetin, pinocembrina, chrysin, and caffeic acid phenylethyl ester in the range of 0,003-2 mg/mL. When standards were not available the quantification was made in equivalents terms with a phenolic compound of the same family. All extracts were contaminated with salicylic acid as the internal standard.

Mass spectrometry analysis by ESI-MS and ESI-MS^n. The HPLC fractions were analyzed by ESI-MS and ESI-MS^n in the negative mode according with previous work [3].

Results and Discussion. The Azorean propolis samples analyzed present a typical phenolic profile from temperate zones [2] with 37 compounds identified [3] by ESI-MS^n analysis and UV spectra, including phenolic acids, flavonoids (flavones, flavanonas and dihydroflavonols) and their esters. The quantification of caffeic acid phenylethyl ester (CAPE), a common compound found in propolis from temperate zones, although identified, revealed somehow difficult due to peak overlapping with

pinobanksin-3-*O*-acetate, a major compound. The results for the most abundant compounds found in these samples are summarized in the Table 1.

Table 1. Major phenolic compounds and their content (mg/g extract) identified in the Azorean propolis samples

Compound	RT (min)	UV_{max}	$[M-H]^-$	Content interval (mg/g extract)
Caffeic acid	6,7	324	179	2,2-17,9
p-Coumaric acid	10,9	310	163	7,3-17,1
Isoferulic acid	13,2	324	193	1,7-17,1
Dimethyl caffeic acid	18,7	324	207	5,2-19,0
Pinobanksin-5-methyl-ether	22,8	290	285	1,3-12,5
Cinnamic acid	23,2	279	147	1,8-6,9
p-coumaric acid methyl ester	24,9	311	177	6,1-22,2
Pinobanksin	29	290	271	9,5-26,2
Cinnamylidenacetic acid	38,4	311	173	5,1-11,5
Caffeic acid isoprenyl ester	46,4	324	247	8,7-39,2
Caffeic acid isoprenyl ester (isomer)	47,8	324	247	12,9-42,5
Caffeic acid benzyl ester	48,2	324	269	6,7-14,7
Pinocembrin	49,9	290	255	24,8-62,8
Chrysin	51	265, 311	253	17,8-60,5
Pinobanksin-3-*O*-acetate	52,1	292	313	19,0-66,3

RT – retention time; UV_{max} wavelength at maximum absorbance; $[M-H]^-$ product ion

The propolis samples revealed a similar phenolic composition, but significant differences were found in their concentrations. The pinobanksin derivatives are the only compounds absent in some phenolic extracts: while pinobanksin-5-methyl-ether is not observed in five samples, pinobanksin -3-*O*-acetate is lacking in just one sample. Overall, the most abundant compounds were the flavonoids pinocembrin, chrysin, pinobanksin-3-*O*-acetate. Amongst the phenolic acids, esters are proved to be more abundant than simple acids, like caffeic acid isoprenyl ester, its isomer and *p*-coumaric acid methyl ester. The comparisons between islands reveal a richer phenolic propolis in Terceira islands, with the maximum for almost the flavonoids of table 1. The higher content in phenolic acid is notice in a specific sample for S. Miguel. Although the samples are from the same climate region, significant differences are found in the phenolic quantities either from the same island, addressing the phenolic profile as a fingerprint of the flora diversity around the beehive, and therefore, in resin availability for bees.

Acknowlegdement. Soraia I. Falcão thanks FCT for a PhD grant (SFRH/BD/44855/2008).
Thanks to National Federation of Portuguese Beekeepers for propolis samples.

References

[1] Sforcin J.M. (2007) *J. Ethnopharmacol.* 113: 1-14.
[2] Bankova *et al.* (2000) *Apidologie* 31: 3-15.
[3] Falcão *et al.* (2010) *Anal. Bioanal. Chem.* 396: 887-897.