Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 174–178
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Exotic flora dependence of an unusual Brazilian propolis: The pinocembrin biomarker by capillary techniques

Juliana Adelmann [a], Mauricio Passos [a], Daniel Henrique Breyer [a], Maria Helena Rocha dos Santos [a], Cesar Lenz [b], Natalício Ferreira Leite [b], Fernando Mauro Lanças [c], José Domingos Fontana [a,*]

[a] *Biomass Chemo/Biotechnology Laboratory (LQBB), Department of Pharmacy, Federal University of Parana (UFPR), R. Lothario Meissner 632, Subsede Botânico, Curitiba PR (80210-170), Brazil*
[b] *Institute of Technology of Parana (TECPAR), Brazil*
[c] *Chromatography Laboratory (CROMA), Institute of Chemistry of São Carlos (IQSC), University of São Paulo (USP), São Carlos, SP, Brazil*

Received 31 March 2006; received in revised form 22 June 2006; accepted 1 July 2006
Available online 14 August 2006

Abstract

Significant amounts of pinocembrin (>10%), a dihydroxy-flavanone, was found in the composition of an unusual brand of a subtropical Brazilian propolis. Incidentally, this sealing material was obtained from hives surrounding a large forestry site based on a single exotic flora, namely poplar (*Populus* sp.). Examination of the different botanical parts of poplar revealed the buds as the main source of the flavanone. Techniques used for the establishment of the chemical correlation between the propolis brand and the poplar buds were TLC/densitometry, capillary GC–MS in the e.i. mode, and CZE with DAD monitoring. Since color enhancement after Al^{3+} complexation applies just for more hydroxylated flavonoids, the alternative techniques herein applied were of value for pinocembrin detection and estimation. Analytical data indicated the dominance of the main phenolic pinocembrin biomarker as well as the presence of other related flavonoids in the botanical source and in the propolis derived thereof.

Keywords: Propolis; Pinocembrin; Flavonoids; CZE; GC–MS; TLC-densitometry; Chromogeny

Propolis, the beehive sealing material, is one of the most heterogeneous natural cocktail built from plant sources. Hundred(s) of components – some of them like the flavonoids and modified phenol-carboxylic acids bioactive against human pathogens – can be detected in propolis following chemical TMS-derivatization and capillary GLC inspection [1]. Other biological activities such as against inflammations, ulcers, and tumors may also correlate with the antioxidant activity of these phenols [2]. The flavonoids encompass a rather complex family of natural compounds since more than 4000 were described [3]. Naringin, a glycosylated form of the flavanone naringenin, when associated to food colorants like anthocyanin or carmin, displays a marked reduction of induced hyperlipidemia [4].

Although surrounding flora to beehives is the main source for propolis components, the chemical correlation between donor plants and propolis is a hard task since bees feed on a lot of different botanical sources to produce the sealing material. Several literature reports deal with the similarity or identity of a few plant natural substances to the same components found in propolis but no report had yet established complete identity between a defined plant source and the resulting propolis whole composition. Reason for this is simply the flora diversity found close to the hives as well as, to some extent, the biochemical ability of bees altering the native composition or adding own components to propolis (e.g., bee wax). Apiculture activity in the vicinity of a homogeneous forest can facilitate this kind of search and chemical correlation. This was the purpose of the present report considering the availability of an intense forestry on *Populus deltoides* in the mid-South geographical region of Paraná State, Brazil. The European plant was brought to Brazil by Swedish entrepreneurs about a century ago. The exotic poplar timber from *P. deltoides* (also from *P. euro-americana*, in a minor extent) is exclusively used for a local matches manufacture industrial plant.

Propolis, mainly under the form of hydroethanolic extratcts, is finding increase room in the pharmaceutical market due to the use in creams, tablets, shampoos, and toothpastes [5]. In fact more than a 10th of bioactivities are reported including inhibition of fungi, bacteria and viruses [6].

* Corresponding author. Tel.: +55 41 3604136; fax: +55 41 3604101.
E-mail address: jfontana@ufpr.br (J.D. Fontana).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.07.014

The inhibition power is very often correlated with the occurrence of flavonoids and/or phenol-carboxylic acids or their esters. For instance, a pinocembrin content of 4.1–4.5% was reported for propolis samples from five different countries [7]. Due to its antiandrogenic activity, this 5,7-dihydroxy-flavanone has potential application in cases of androgen-dependend hyperplasia of prostate, hair loss, and even cancer. Other inhibitory actions of pinocembrin were reported for testosterone reductase and sarcoplasmic reticulum Ca^{2+}-ATPase [8,9]. *Staphylococcus aureus* is involved in nosocomial infections. Concerning the flavonoid antibacterial activity against this particular pathogen, *inter alia*, pinocembrin is reported as one of the most active [10]. Pinocembrin was also indicated as one of the propolis components responsible for the inhibition of glucosyltransferases of *Streptococcus mutans*, an oral microorganism involved in the cariogenic process [11].

Gas chromatographic analysis of 10 propolis samples arising from European countries have appointed, in two cases, unexpected composition due to the enrichement in phenolic glycerides and diterpenic acids [12] instead of the expected flavanone poplar profile.

Since propolis collected from homogeneous reforest regions may be valuable for pharmaceutical formulations enriched in flavonoids, the confirmation of the chemical relationship between the flora available to bees and propolis as final product is desirable.

1. Experimental

1.1. General procedures

Spectrophotometric quantitation of total flavonoids followed the aluminum nitrate/potassium acetate procedure [13].

In order to detect flavonoids among the propolis components turned volatile under moderately high temperatures GC–MS analyses were performed with a GC 17/GC/MS QP-5000 module from Shimadzu using non-derivatized ethanol-extracted samples and a 30 m capillary column HP-5 with 0.2 mm int. diam., 30 μm film of 5% phenyl–95% poly-dimethylsiloxane from 100 °C (2 min hold time) to 300 °C (15 min hold time) at 10 °C/min and a FID detector.

Capillary electrophoresis of buffered samples of weakly ionized phenols filtered by a 0.22 μ Millipore membrane was carried out in a 65 m fused silica capillar (i.d. of 50 μ) with a sodium tetraborate (30 mM)/sodium phosphate (50 mM) buffer (pH 8.5) modified with the inclusion of 12% of methanol in order to improve flavonoids solubility. The routine voltage was 20 kV generating a current of 53 μA and the flavonoids were monitored by a DAD device at 220, 250, and 320 nm as the main absorption wavelengths for pinocembrin.

1.2. Propolis sampling and processing

Propolis samples (0.5 kg each) were collected by Breyer Ltd. Co. at the geographical region around the town of União da Vitória in the mid-South of State of Paraná, Brazil (around latitude 51° and altitude 26° 2″). Sample PP-2-WDC was from *Apis mellifera* hives close to a heterogenous native flora (mainly angyosperm) and sample PP-4-AL from a more homogeneous flora mainly composed by a forestry with exotic poplar (gymnosperm). The later was also the source for buds and other aerial botanical parts which were lyophilized just after the crop. The biological materials were extensively extracted with warm ethanol in order to provide the initial crude organosolvent extracts. These were fractionated in columns of silicic acid (or of silica gel G as a preliminary batch procedure) using a progressive polarity-gradient from hexane:ethyl acetate from 99:1 to 1:99, the final columns washing being carried out with methanol. Fractions enriched in components reacting to hot sulfuric anisaldehyde (105 °C; 3–5 min) as strong orange colors like those obtained with hexane-ethyl acetate 90:10–50:50 fraction were selected as enriched preparations in pinocembrin and related flavonoids.

1.3. Standard substances

Pinocembrin standard and related flavonoids were purchased from Extrasynthese (Genay, France) and the standards of phenol carboxylic acids were obtained from Sigma–Aldrich Co (St. Louis, MO, USA).

1.4. TLC and densitometry

Analytical TLC was made on silica gel plates (Merck, art. 1.05553) using 1.5 successive developments of the plates with hexane: ethyl acetate (3:2, v/v) as mobile phase. Densitometry with a Shimadzu CS-9301PC flying spot-densitometer of the selected TLC lanes following the hot spray with 0.5% anisaldehyde–5.0% sulfuric acid in methanol was obtained from the colored pictures taken under visible or UV (365 nm) light with a digital camera.

2. Results and discussion

2.1. Ecological aspect

Visit of bees to poplar trees was often observed and a photographic shoot was taken for *A. mellifera* feeding on poplar buds (Fig. 1).

Fig. 1. *A. mellifera* visit to a *P. deltoides* bud.