Journal of Pharmaceutical and Biomedical Analysis 124 (2016) 57–66

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Determination of benzophenones in lipophilic extract of Brazilian red propolis, nanotechnology-based product and porcine skin and mucosa: Analytical and bioanalytical assays

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ARTICLE INFO

Article history:
Received 28 December 2015
Received in revised form 12 February 2016
Accepted 13 February 2016
Available online 16 February 2016

Keywords:
Brazilian red propolis
Polyprenylated benzophenones
HPLC-UV
Validation
Garcinol

ABSTRACT

Lipophilic compounds of Brazilian Red Propolis (BRP) have received increasing attention due to some interesting findings regarding their biological activities. This study was first aimed at evaluating the chemical composition of BRP *n*-hexane extract (HEXred) by UPLC–MS-PDA. Chemical investigation mainly resulted in the identification of polyprenylated benzophenones (PPBs) in this extract, named oblongifolin A, guttiferone E, and/or xanthochymol. After that, an isocratic HPLC-UV method was validated for the determination of total content of PPBs (at 260 nm) expressed as garcinol, a commercially available guttiferone E diastereoisomer. The method showed to be specific, precise, accurate, and linear (0.1–10 μg/mL) for the determination of PPBs in HEXred, BRP-loaded nanoemulsions, as well as, in porcine skin and mucosa samples after permeation/retention studies. The matrix effect was determined for all complex matrices, demonstrating low effect during the analysis. The stability-indicating method was verified by submitting HEXred to acidic, alkaline, oxidative, and thermal stress conditions. No interference of degradation products was detected during analysis. Therefore, the proposed analytical and bioanalytical methods proved to be simple and reliable for the determination of PPBs in the presence of different matrices.

1. Introduction

Propolis is a resinous product that contains beeswax, enzymes, sugar, and plant exudates collected by *Apis mellifera* bees from different plant sources. Various parameters may have effect on the chemical composition of propolis being the vegetation at the place of collection the most relevant [1–3]. Brazilian propolis was classified by Park et al. [4] in twelve major groups according to the physicochemical properties and the geographic locations. Brazilian Red propolis (BRP), a new group classified as Group 13, received this name due to its strong red color [5].

Various biological and pharmacological properties have been described for BRP [5–8]. A well-documented literature has demonstrated the antimicrobial activity of this propolis, usually as ethanol preparations, against bacterial and fungal pathogens, including *Candida* species [9–14]. Recently, a pronounced antifungal activity of the *n*-hexane extract of the BRP (HEXred) against non-*albicans Candida* (NAC) species – *Candida krusei*, *Candida glabrata*, *Candida tropicalis* and *Candida parapsilosis* – was demonstrated [15]. The yeast damage of this extract was also confirmed by MTT assay.

Previous literature has demonstrated the presence of the polyprenylated benzophenones (PPBs) guttiferone E, xanthochymol and oblongifolin A in the lipophilic extract (*n*-hexane) of BRP. The chemical structures of these PPBs are shown in Fig. 1. However, the complete separation of these PPBs was not achieved even by high-throughput ultra-fast liquid chromatography. In fact, the isomers guttiferone E and xanthochymol have been considered as an inseparable mixture [1,8,16]. Gustafson et al. [17] had already reported the extraction of guttiferone E and xanthochymol from *Garcinia* and *Clusia* species as a mixture.

Despite the promising results concerning the biological activities of lipophilic compounds from BRP preparations, analytical studies have yet to be performed. Pharmaceutical analysis of propolis is a difficult task once it is often composed of a complex mixture of compounds. In this study, we first aimed to characterize the chemical composition of the HEXred by UPLC–MS-PDA. After that, a stability-indicating HPLC/UV method to determine

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http://dx.doi.org/10.1016/j.jpba.2016.02.018
0731-7085/

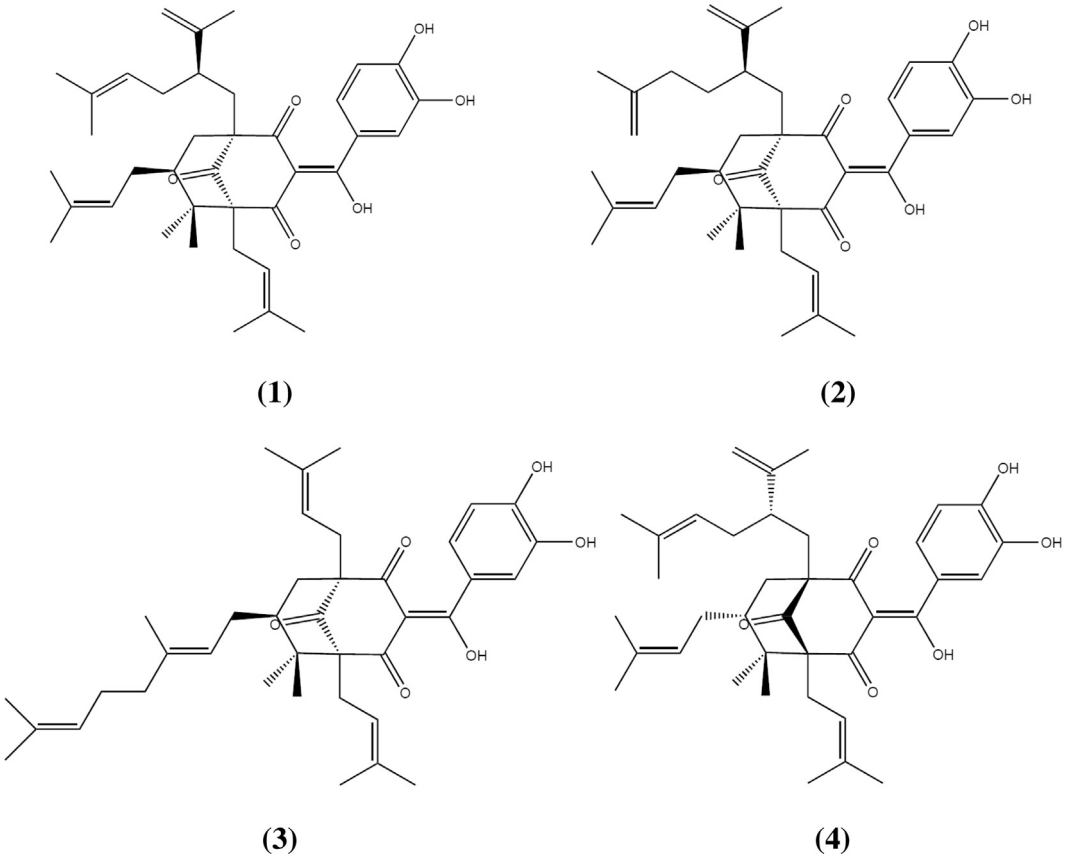

Fig. 1. Chemical structure of guttiferone E (1), xanthochymol (2), oblongifolin A (3) and garcinol (4).

the PPBs content in HEXred, nanotechnology-based product and porcine skin/mucosa samples of permeation/retention studies was validated. PPBs content was expressed as garcinol, a commercially available PPB guttiferone E diastereoisomer. The matrix effect for all of these applications was assessed to demonstrate the versatility and reliability of the method.

2. Material and methods

2.1. Materials

Garcinol (purity ≥95%) was purchased from Cayman Chemical (Ann Arbor, Michigan, USA). Methanol and acetonitrile were HPLC grade and all other reagents used were analytical grade. Red propolis was purchased from Natucentro® (Bambuí, MG, Brazil). Porcine skin and porcine esophageal mucosa were purchased from Cooperativa Ouro do Sul (Harmonia, RS, Brazil). Egg-lecithin (Lipoid E-80) and medium chain triglycerides (MCT) were purchased from Lipoid GmbH (Ludwigshafen, Germany).

2.2. Preparation of n-hexane BRP extract (HEXred)

The BRP was ground and the powdered material was successively extracted by maceration with *n*-hexane over 72 h (3 times) and sample:solvent ratio of 1:10 (w/v). The extracts were combined and evaporated to dryness under reduced pressure using a rotary evaporator at 40 °C. The residue was treated with cold acetone to obtain an insoluble fatty residue that was filtered through paper filter. The acetone-soluble fraction was evaporated to dryness and used in the further experiments.

2.3. UPLC–MS-PDA analysis of HEXred

The HEXred was analyzed by UPLC-MS-PDA on a Micromass-LCT Premier Time of Flight (TOF) mass spectrometer (Waters, MA, USA) with an electrospray interface and coupled with an Acquity UPLC system (Waters, MA, USA). A C_{18} reversed-phase column (Hypersil Gold 10 mm × 2.1 mm, 1.8 μm, Thermo Scientific, Massachusetts, USA) was used, operating at 45 °C. The analyses were performed using positive ion mode electrospray ionization mass spectrometry (ESI(+)-MS). The optimized system parameters were as follows: capillary (V) 3000, sample cone (V) 30, extraction cone (V) 3.3, temperatures of source (120 °C) and desolvation (300 °C) and collision energy 4 V. Nitrogen was used as nebulizer gas and argon as collision gas. Samples were eluted using a linear gradient system and the mobile phases consisted of a mixture of water:formic acid (100:0.1, v/v) (A) and methanol (Tedia® HPLC grade, Ohio, USA) (B). The gradient profile was: 0.0–1.54 min from 40 to 65% of B, 1.54–4.38 min from 65 to 70% of B, 4.38–7.21 min from 70 to 75% of B, 7.21–8.63 min from 75 to 80% B, 8.63–11.00 min 80% of B, 11.00–11.46 min from 80 to 85% of B, 11.46–12.88 min from 85 to 88% of B, 12.88–14.29 min from 88 to 90% of B, 14.29–15.71 min from 90 to 40% of B and finally, to restore the initial conditions, 15.71–16.00 min 40% of B. The flow-rate was 0.4 mL/min and the injection volume was 2 μL. The detection was at 260 nm in the photodiode array detector. The UV spectra were recorded with a 200–400 nm range.

2.4. HPLC/UV analysis of PPBs

The HEXred was analyzed by HPLC/UV on a Shimadzu LC-10A system, equipped with a LC-10 AD pump, a CBM- 10A system controller, a SIL-10A autosampler and a SPD-20AV UV/vis detec-