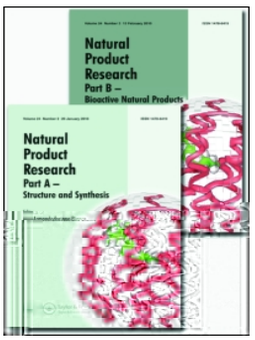

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SHORT COMMUNICATION

Artepillin C, is it a good marker for quality control of Brazilian green propolis?

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ABSTRACT

As commercialisation of Brazilian green propolis is going on, quality evaluation and authenticity are important. The result demonstrated that artepillin C found by far in Brazilian green propolis by HPLC-ESI-MS/MS analysis, while a small interferent may be mistaken as artepillin C in some propolis from China. A new HPLC quality control method as artepillin C for marker was developed, which is the primary assessment criteria for quality control of Brazilian green propolis.

1. Introduction

Brazilian green propolis, its plant origin is *Baccharis dracunculifolia* DC (Kumazawa et al. 2003), has been widely used in health-care, cosmetic and medicine products especially in Japan and China since 1990s. Contrary to poplar-type propolis, Brazilian green propolis has relatively low concentration of flavonoids while phenolic acids especially isoprenyl

It is generally accepted that artepillin C is the characteristic chemical composition (Park et al. 2004) and has become an important factor in evaluating the quality of Brazilian green propolis. However, Wang (2010) identified artepillin C in Chinese propolis from Jilin province using ESI-MS/MS. Wu et al. (2013) also detected artepillin C by HPLC in Chinese propolis from Shandong, Beijing, Jiangsu and Xijiang provinces. It is necessary to know with certainty whether artepillin C only exists in Brazilian green propolis?

This study is aimed to verify if artepillin C also exist in other propolis samples and develop a simple and selective method for evaluation of artepillin C. Samples of propolis of different geographic origins were investigated. Furthermore, we compared the content of artepillin C concentrations among different Brazilian green propolis. These results will provide scientific theoretical basis for the quality control of Brazilian green propolis.

2 Results and discussion

2.1. The determination of artepillin C in propolis

The artepillin C was analysed under HPLC conditions from poplar-type propolis, birch-type propolis, ecalyptus-type and macaranga-type propolis (Figure S2). No interfering peaks were found with retention times similar to artepillin C except propolis samples from Jilin, China. There is a small peak near where artepillin C appeared. ESI-MS/MS was carried out in both positive and negative mode. The fingerprint peak in Brazilian green propolis showed very similar ESI-MS2 spectra as the artepillin C. Major negative ion markers are those of *m/z* 299.8, 255.7 and 200.5, and major positive ion markers are those of *m/z* 301.7, 283.7, 245.6, 277.5, 177.4 and 69.2 (Figures S3a, S3b). Although the fragment with [*m/z*] 301.7 in positive ion is a common ion product for artepillin C and the unknown compounds from Chinese propolis and the other major ion markers from artepillin C were not observed in Chinese propolis (Figure S3c). Another experiment revealed that the fragment with [*m/z*] 301.7 in positive ion was absent in Chinese propolis by Agilent 1200 QTOF LC/MS 6510(Figure S4).

Propolis fraction is highly complex, misidentification is very possible. This is the case of two recent papers based on HPLC (Wu et al. 2013) and ESI-MS/MS (Wang 2010). HPLC identification of artepillin C is inadequate because it can't exclude the interfering compounds. In fact, in ESI-MS/MS the unknown compound only gives the ion product with $[m/z]^-$ 300.4, 283.9 (Wang 2010), it also can't exclude the interfering compounds without more information.

2.2. The quantitative method of artepillin C by HPLC

A rapid method based on HPLC with UV detection is presented for the quantitative determination of artepillin C in propolis. The calibration curve of artepillin C was obtained by plotting peak areas vs. six known concentrations (0.05–0.5 mg/mL). The representative linear equation obtained was: $y = 9178.5x - 36.208$ ($r^2 = 0.9993$), which showed good linear relationships between the peak areas and the concentrations. The LOD and LOQ were 0.12 and 0.4 μg/mL, respectively. The obtained values for both LOD and LOQ were low, which indicated that the method was capable of detecting and quantifying trace amounts of artepillin C in samples. The RSD of inter-and intra-batch precisions were 0.35 and 0.79%, respectively; which showed good precision of the method. The repeatability of HPLC analysis for hydroalcoholic extract was exemplified by an RSD of 0.078%. The accuracy was studied through

recovery tests and obtained in the ranges of 98.46–99.20%.These results indicated that the developed method was validated and applicable for sample analysis.

Artepillin C were analysed in 56 Brazilian green propolis samples, it can be detected in all samples varying from 0.84 to 8.23%, and the average value is 3.09% (Table S1); which is in accordance with the results reported by Park et al. (2004) but lower than the values obtained in Matsuda's study (2008). The significant difference of artepillin C content among different batches might be account of geographic origins, seasonality, and storage condition and so on (Simões-Ambrosio et al. 2010; Bueno-Silva et al. 2016; Talla et al. 2016).

3. Conclusion

Propolis samples from different geographical origins were analysed, artepillin C existed by far in Brazilian green propolis. A small interferent may be mistaken as artepillin C in some propolis from China. A HPLC method was developed for quality control over quantity of artepillin C in Brazilian green propolis.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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